VALORIZATION OF JAND RESIDUE IN MUFFIN PREPARATION

by

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This dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech in Food Technology

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Approval Letter

This *dissertation* entitled ***Valorization of Jand Residue in Muffin Preparation*** presented by **Shreeka Regmi** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology**

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(Shreeka Regmi)

Abstract

Millets are small, nutrient-rich cereal grains known for their drought tolerance, short growing period, and health benefits, making them a sustainable alternative to conventional cereals. The purpose of this study was to valorize fermented millet residues, a by-product of traditional millet-based alcoholic beverage production, by incorporating them into functional muffins and evaluating their physicochemical, nutritional, and sensory qualities. The residues were dried, milled, and partially substituted with wheat flour. A simple lattice design using Design Expert version 13 generated six muffin formulations with 0%, 40%, 50%, 60%, 70%, and 80% substitution levels, coded as A, B, C, D, E, and F, respectively. Proximate analysis was done for wheat flour, finger millet flour, and fermented millet residue flour. Prepared muffin samples were subjected to sensory evaluation, and the results were statistically analyzed using one-way ANOVA at a 5% significance level. Muffins prepared from each formulation were evaluated for physical properties and sensory characteristics.

The results showed that fermented millet residue flour had higher protein, crude fiber, ash, calcium, iron, antioxidant activity, and total phenolic content compared to wheat flour. Higher substitution levels resulted in decreases in muffin weight, volume, and specific loaf volume. Sensory evaluation showed that the 40% substituted muffin (Sample B) was significantly superior among the substituted samples and was comparable to the control in taste, aroma, aftertaste, and overall acceptability. Chemical analysis indicated that the 40% fermented millet residue flour substituted muffin had higher protein, crude fiber, ash, calcium, iron, total phenolic compound, and antioxidant activity than the control muffin. Based on sensory and nutritional evaluation, the 40% fermented millet residue flour substituted muffin was selected as the best formulation. Microbiological analysis confirmed that the selected formulation remained acceptable for up to six days at room temperature in LDPE packaging of 50 μm, with no detection of coliforms, yeast, mold, or total viable count, indicating product safety without the use of artificial preservatives. This study demonstrates a sustainable approach to by-product valorization, converting fermented millet residues into nutrient rich functional bakery products, thereby contributing to waste reduction and enhanced food security.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of Variance
BSG	Brewers' spent grain
°C	Degree Centigrade
CCT	Central Campus of Technology
db	Dry basis
DFTQC	Department of Food Technology and Quality Control
DPPH	2,2-diphenyl-1-picrylhydrazyl
FMR	Fermented millet residue
GAE	Gallic acid equivalent
LAB	Lactic Acid Bacteria
m/v	Mass per volume
µm	Micrometer
ml	Milliliter
OAC	Oil absorption capacity
RF	Refined flour
rpm	Revolutions per minute
SD	Standard Deviation
TPC	Total Phenolic Content
TSS	Total Soluble Solids
v/v	Volume per volume
WAC	Water holding capacity
WF	Wheat flour
WWF	Whole wheat flour

PART I

Introduction

1.1 General introduction

Millets are small-seeded cereal crops that grow well in tropical regions with minimal water, high temperatures, and resistance to pests and diseases. Their short growing season and adaptability make them suitable for intensive farming. Being gluten-free, nonacid-forming, and easily digestible, millets are beneficial for individuals with gluten allergies or celiac disease (Shankaramurthy and Somannavar, 2019). It also serves as valuable forage and is rich in minerals such as iron, magnesium, phosphorus, and potassium, along with beneficial phytochemicals like phytic acid (Admassu *et al.*, 2009).

Finger millet (*Eleusine coracana*) holds significant importance in the production of traditional alcoholic beverages, particularly in Nepal. Jand, a sweet-sour cereal beer made from finger millet and other grains, is deeply rooted in the cultural practices of ethnic groups such as the Newar, Limbu, Tamang, and Rai. It plays a central role in rituals, marriages, hospitality, and social customs across Nepal and Tibetan cultures. Fermentation of finger millet during jand preparation involves complex biochemical and microbial interactions that transform its nutritional profile and functional quality (Karki *et al.*, 2023). After fermentation, the liquid is extracted, leaving behind a solid residue. This residue retains significant nutrients, including protein, fiber, and minerals, and thus holds potential for use as a functional food ingredient during manufacturing of bakery products, jelly foods, and thickening foods. Fermented and native finger millet flours are prospective raw materials to substitute wheat flour for gluten-free products with desirable properties (Mudau *et al.*, 2022).

Traditionally, these residues are underutilized or discarded, which represents a potential loss of valuable nutrients and contributes to agro-industrial waste. The concept of valorization i.e. transforming underutilized by-products into functional food ingredients has gained significant attention in food science and sustainability research. Valorizing cereal residues and its incorporation into food formulations is aligned with circular economy principles, reducing waste and increasing the economic and nutritional value of agricultural products (Chetrariu and Dabija, 2023). Studies on cereal by-products, such as brewer's spent grain, demonstrate that incorporation into bakery products like bread, muffins, and cookies can enhance dietary fiber, protein, and bioactive compound content, while maintaining acceptable sensory qualities (Eche *et al.*, 2025).

Muffins are popular cereal-based snacks, widely consumed as a breakfast item in the United States due to their pleasant taste and convenience. They are primarily made from flour, with gluten serving as the main protein component of wheat flour (Bhaduri, 2013). Despite their health benefits, the consumption of whole grain products remains low in many regions. Incorporating millets, particularly finger millet, into regular diets offers both nutritional and economic advantages, especially in areas where they are readily available and affordable. Using finger millet flour in bakery items such as muffins enhances their nutritional profile while retaining a familiar taste. Muffins, being convenient and ready-to-eat, provide an ideal medium for promoting the use of nutrient-rich grains in everyday diets. Integrating finger millet into such products not only supports better nutrition but also adds value to the crop, encouraging its utilization in both household and commercial food applications (Rajiv *et al.*, 2011).

1.2 Statement of the problem

The increasing global demand for food has intensified the search for alternative raw materials that are both affordable and nutritionally superior. In this context, the valorization of food-processing by-products has become a key focus of modern research. Strengthening the circular economy requires the development of biomass conversion strategies and biorefinery technologies that promote a sustainable, bio-based production system. Utilizing spent grain as an ingredient for human food is particularly promising because it enriches products with protein, fiber, vitamins, and minerals while lowering starch levels and overall caloric content. To effectively utilize food by-products, the circular economy framework must be applied in a sustainable and practical manner (Chetrariu and Dabija, 2023).

As the food sector expands and consumers increasingly seek healthier and more diverse food choices, there is a growing need for low-cost, nutrient-rich alternatives as well as efficient use of industrial by-products. Agro-industrial residues are valuable resources that can be transformed into high-quality food ingredients, reinforcing circularity in the food system. One of the central challenges in food engineering today is the sustainable management of organic waste and agri-food by-products. Spent grain, produced in large quantities and at low cost, represents a highly promising raw material. The rising interest in food products made with stable, functional ingredients derived from by-products is driving the search for new applications (Chetrariu and Dabija, 2023).

Furthermore, refined wheat flour, the mainstay of many bakery products, pose various health challenges. Its low fiber content often leads to digestive issues and weight gain. Additionally, the gluten present in wheat triggers adverse health conditions such as celiac disease and gluten intolerance (Hui *et al.*, 2007). These factors, along with the rising prevalence of obesity and related health complications, highlight the need for alternative, nutrient-rich ingredients in the baking industry (Apovian *et al.*, 2019). As a result, new ingredients are being explored, knowledge regarding their influence on baking quality, shelf-life, and consumer acceptability is limited. Establishing standards for partial wheat replacement using locally available materials can provide cost-effective, high-quality, and sustainable solutions for the food industry (Shahzadi, 2004).

Thus, the current work was focused on the development of muffins by partially substituting refined wheat flour with fermented millet dregs, a nutrient-rich by-product of traditional millet-based alcoholic beverage production, aiming to produce functional, value-added, and sustainable bakery products that meet consumer demand for healthier and environmentally responsible foods.

1.3 Objectives

1.3.1 General objective

The general objective of this work was to evaluate the potential utilization of fermented millet residues as a partial substitute for refined wheat flour in muffin production to develop a nutritious, value-added, and sustainable baked product.

1.3.2 Specific objectives

1. To prepare the flour from fermented millet residues obtained from traditional millet-based alcoholic beverage and formulate muffins by substituting refined wheat flour.
2. To perform physiochemical analysis of the refined wheat flour, millet flour and fermented millet residue flour.

3. To determine the acceptability of the best formulation through sensory analysis and to evaluate its physicochemical, functional, and sensory properties.
4. To perform microbiological stability of muffin.

1.4 Significance of the study

Utilizing fermented millet residue as a partial flour substitute in muffins directly supports circular-economy goals by reintegrating waste streams into the food production cycle, reducing organic waste, and promoting resource efficiency (Skendi *et al.*, 2020) and (Danciu *et al.*, 2023). This is particularly relevant as global efforts increase toward reducing food waste and promoting sustainable finding within the agro food sector.

From a nutritional perspective, replacing refined wheat flour which is low in fiber and micronutrients, with fermented millet residue flour can create more nutrient-dense bakery products. Fermented millets have demonstrated nutrient bioavailability and enhanced protein, mineral, and phytochemical profiles, antioxidant potential, and higher digestibility (Tomar *et al.*, 2025). Such improvements align with public-health priorities, especially in regions where refined-flour consumption contributes to rising rates of obesity and diet-related issues.

According to Onweluzo and Nwabugwu (2009), fermentation process prolongs the shelf-life of foods, increases protein content and digestibility, relative nutritive value, availability of minerals, functional properties and decreases the concentration of phytic acid while enhancing sensory properties and imparting antimicrobial properties on the food product . By converting residues into marketable ingredients, producers can generate additional income and partially recover initial investments, while simultaneously reducing disposal expenses. The overall economic viability of these technologies ultimately depends on aligning production costs with consumer demand for value-added products. Improvements in processing efficiency and product quality can further strengthen profitability and long-term sustainability (Dixit, 2025).

Overall, this research fills a clear gap by evaluating the functional use of fermented millet residues in muffin production, contributing evidence for sustainable ingredient development, healthier product formulation, and circular-economy practices in cereal-based food systems.

1.5 Limitation of work

1. Storage stability of the muffin was not studied.
2. Only one variety of millet was used.

Part II

Literature review

2.1 Muffins

Muffins are individual-sized baked goods characterized by a soft, spongy texture, a porous structure, and a relatively high volume (Martínez-Cervera *et al.*, 2012). They are popular among consumers due to their light texture and distinctive flavor (Ramya and Anitha, 2020). While similar to cupcakes, muffins are generally less sweet and are not frosted. Muffins can be either sweet or savory, with examples including cornbread or cheese muffins. In some regions outside the United Kingdom, the term "muffin" refers to a flat, round bread known as an English muffin. There are numerous varieties of muffins, ranging from low-fat options to those containing specific ingredients such as fruits, nuts, or spices, including blueberries, chocolate chips, bananas, pumpkin, almonds, and carrots. Muffins are commonly consumed at breakfast but are also suitable for tea or other meals (Limbachiya and Amin, 2015).

The top of a well-prepared muffin should be uniform and smoothly rounded, without peaks or cracks, and proportionally large compared to its weight. The surface may appear slightly pebbled or textured, with a polished look and an even golden-brown color. The interior should have a fine, evenly distributed crumb that is moist, soft, and light, with a creamy white or slightly yellow hue and no streaks. Muffins can be customized by incorporating ingredients such as fruits, nuts, herbs, cheese, meats, or spices into the batter. Consumers expect muffins to have a tender, spongy crumb that is delicate yet maintains some structural integrity (Öztürk and Mutlu, 2019).

2.1.1 The method of mixing

Muffin formulations consist of a complex combination of ingredients, including sugar, varying amounts of fat, flour, eggs, and baking powder, which together create the characteristic porous structure and high volume of muffins (Baixauli *et al.*, 2008). There are two main methods for preparing muffins: the muffin method and the conventional method. In the muffin method, the ingredients are divided into two separate mixtures: a wet mixture containing eggs, liquid or soft fat, milk, and sugar, and a dry mixture consisting of flour, baking powder, salt, and flavorings such as cocoa powder. Once the mixtures are prepared and the oven is preheated, the wet and dry components are briefly combined, swirled, and then poured into molds for baking (Miller, 1971).

2.1.2 Preparation of muffin

Initially, the ingredients were separated into dry and wet components. The dry ingredients included wheat flour, oats flour, baking powder, and sugar, while the wet ingredients consisted of eggs, water, and butter. The eggs were beaten for two minutes before adding milk and oil. In a separate bowl, all dry ingredients were thoroughly mixed. Finally, the wet and dry mixtures were combined to form a uniform muffin batter (Rahman *et al.*, 2015).

Egg
↓
Beating (2 min)
↓
Melted butter, water → Creaming
↓
Cream
↓
Flour, Sugar, Baking powder → Mixing
↓
Batter
↓
Molding
↓
Baking (215ºC for 20±3 min)
↓
Cooling (35ºC)
↓
Storage (at ambient temperature)

Fig. 2.1 Flow chart for the preparation of muffin

Source: Rahman *et al.* (2015)

2.1.3 Objective of mixing

The main goal of mixing is to obtain a homogeneous mixture, ensuring that the ingredients are evenly distributed throughout. A distinction can be made between batch and continuous mixing processes. Ideally, the concentration of each ingredient should remain uniform in the final product, consistent over time, and each portion of the mixture should be processed in the same manner (Cullen, 2009).

2.1.4 Chemical composition of a muffin

Chemical composition of muffin is shown in Table 2.1

Table 2.1 Chemical composition of muffin

Constituents	Percentage
Moisture	20.33
Protein	14.37
Fat	17.60
Carbohydrate	44.28
Total dietary fiber	2.22
Ash	1.21

Source: Rahman *et al.* (2015)

2.2 Finger millet

In Nepal, finger millet holds the second position among cereal crops in hilly regions after maize and is also grown in the inner Terai on unirrigated lands. Its drought resistance and adaptability to varying soil and climatic conditions have made it increasingly important, especially with irregular monsoon patterns. Cultivation typically takes place during July–August, often in intercropping systems with maize, with harvesting occurring in December–January. Locally, finger millet is known as Kodo in the hills and Maduwa in the Terai, while the term *Ragi* is more common in southern India (Shrestha, 2068).

Globally, finger millet is widely cultivated in Africa and Asia, with India being the largest producer. In Nepal, it is considered the fourth most important cereal crop, particularly vital in the mid-hills where it is often grown alongside maize or as a monocrop. Its resilience, long storage life, and broad adaptability have contributed to its widespread cultivation and importance in food security (Shrestha, 2068).

Fig 2.2 Finger Millet (*Elusine coracana*)

Source: Kaur *et al.* (2024)

2.2.1 Taxonomic classification of finger millet

Taxonomic classification of finger millet is shown in Table 2.2

Table 2.2 Taxonomic classification of finger millet

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae
Genus	*Eleusine* Gaertn.
Species	*Eleusine coracana* (L.) Gaertn.

Source:Harb *et al.* (2024)

2.2.2 Proximate composition of finger millet

Proximate composition of finger millet is shown in Table 2.3

Table 2.3 Proximate composition of finger millet

Component	Range (on dry basis)
Protein	6.8 – 7.3%
Crude Fat	1.3 – 1.7%
Crude Fiber	2.8 – 3.3%
Total Ash	1.9 – 2.2%
Moisture Content	13.2 – 14.5%
Starch	59.8 – 60.3%

Source: Shrestha and Karki (2019)

2.2.3 Mineral composition of finger millet

Mineral composition of finger millet is shown in Table 2.4

Table 2.4 Mineral composition of finger millet

Mineral	Range (mg/100g dry matter)
Calcium	300 – 400
Phosphorus	230 – 285
Iron	4.0 – 7.3
Zinc	1.7 – 2.7
Sodium	0.6 – 0.95
Potassium	620 – 1140

Source: Shrestha and Karki (2019)

2.2.4 Anti-nutritional factors before and after fermentation

Anti nutritional factors of finger millet before and after fermentation is shown in Table 2.5

Table 2.5 Anti nutritional factors of finger millet before and after fermentation

Anti-nutritional factor	Finger millet (mg/100g dry matter)	After Fermentation (mg/100g dry matter)	% Reduction (approx.)
Phytate	0.85	0.20	≈ 76 % ↓
Tannins	3.23	1.54	≈ 52 % ↓
Oxalate	1.59	0.88	≈ 45 % ↓
Trypsin inhibitor activity	6.23	2.73	≈ 56 % ↓

Source:Abioye *et al.* (2021)

2.2.5 Value addition for functional food applications

Finger millet is rich in dietary fiber and polyphenols, which contribute to multiple health benefits such as antidiabetic, hypocholesterolemic, antioxidant, and antimicrobial effects, as well as protection against diet-related chronic diseases. The application of suitable processing techniques can enhance the bioavailability of its micronutrients and improve the overall nutritional quality of finger millet-based diets. Proper processing and value addition can increase the consumption of finger millet, bridging its use from rural to urban areas. Due to its well-balanced protein profile and gluten-free nature, finger millet is suitable for developing various value-added food products. Moreover, promoting finger millet cultivation and its processed products can boost rural income, profitability for farmers, and generate employment opportunities (Rathore *et al.*, 2019).

2.2.6 Traditional method of preparing finger millet jand

Millet

↓

Dehusking and Winnowing in Nanglo

↓

Washing with water

↓

Cooking

↓

Spreading in tray for cooling

↓

Addition of Murcha (approx. 0.5-1%)

↓

erobic fermentation at room temp. for 2-3 days

↓

Filling to fermentation jar and plugging

↓

Anaerobic fermentation of 12-15 days (room temperature)

↓

Addition of 1.5 volume of water

↓

Jand

Fig 2.3 Traditional method of preparing finger millet jand

Source:Karki (2013)

.

2.2.7 Chemical characteristics of millet jand

Chemical characteristics of millet jand is shown in Table 2.6

Table 2.6 Chemical characteristics of millet jand

Parameters	Values (Millet Jand)
pH	3.84
Total acidity as lactic acid (% m/v)	1.11
Fixed acidity as lactic acid (% m/v)	0.51
Volatile acidity as acetic acid (% m/v)	0.20
Alcohol (% v/v)	7.15
Methanol (g/L alc)	2.797
Total aldehydes as acetaldehyde (mg/L alc)	8.5
Esters as ethyl acetate (mg/L alc)	98.0
TSS (°Bx)	6.00
Dry matter (% m/v)	4.91
Reducing sugar as dextrose (% m/v)	0.48
Total ash (% m/v)	0.21
Total nitrogen (% m/v)	0.38

Source:Upadhyaya (2005)

2.2.8 Chemical and nutritional changes during alcoholic fermentation

2.2.8.1 Enzymatic and microbial contributions

The microorganisms participating in finger millet fermentation collectively drive its nutritional improvement. Mucoraceous molds are responsible for producing amylases, which catalyze the saccharification of starch to simple sugars. This conversion not only provides fermentable substrates for yeasts but also contributes to mild sweetness and improved mouthfeel in fermented millet products. Yeasts, particularly Saccharomyces cerevisiae, act as the primary alcohol-producing agents. Beyond ethanol, they synthesize a variety of secondary metabolites like esters, aldehydes, and higher alcohols which impart desirable aroma and flavor. Yeasts are also a source of B-complex vitamins and essential amino acids, enriching the final product. Lactic acid bacteria (LAB) generate lactic and acetic acids, which reduce the pH to around 3.8–4.2. This acidification enhances microbial safety and extends shelf life. LAB activity also contributes to partial degradation of phytate complexes, freeing up minerals and improving digestibility (Karki, 2013).

The synergistic action of these microbial groups leads to both nutritional enhancement and sensory refinement of the product. The development of mixed microflora mimics controlled fermentation systems and demonstrates that traditional murcha starters act as natural composite inocula containing yeasts, molds, and LAB, each contributing distinct biochemical roles (Karki, 2013).

2.2.8.2 Mineral and micronutrient enhancement

One of the most important nutritional outcomes of finger millet fermentation is the significant improvement in mineral bioavailability. In the unfermented grain, much of the phosphorus, zinc, calcium, and iron exist in bound forms as phytate–mineral complexes, which are poorly soluble and not easily absorbed. During fermentation, phytase enzymes secreted by molds and bacteria hydrolyze phytic acid into inositol phosphate and free inorganic phosphate, thereby releasing these bound minerals. Quantitative analysis from the thesis indicates that phosphorus (P), manganese (Mn), zinc (Zn), sodium (Na), and potassium (K) levels all showed measurable increases in the soluble fraction of fermented millet. This enhancement was attributed to the breakdown of complex carbohydrate and protein matrices and the concentration effect caused by carbohydrate depletion during fermentation. Iron content remained largely unchanged, possibly due to its persistent binding with other organic ligands (Karki, 2013).

2.3 Fermented finger millet residue

The global alcoholic beverage industry produces a wide range of fermented drinks such as beer, wine, and spirits each shaped by distinct cultural traditions. Fermentation relies on microorganisms like yeast and bacteria, which convert grains and fruits into alcohol. This process leaves behind residue, a mixture of dead yeast cells and residual solids. Although often treated as waste, these residues are increasingly recognized for their nutritional and functional potential. Additionally, repurposing millet residues contributes to sustainable waste management by transforming fermentation by-products into valuable, functional ingredients (Selvaraj *et al.*, 2025).

Fermented millet residues (also known as millet lees or spent solids) represent a nutritionally valuable by-product generated after the production of traditional millet-based alcoholic beverages such as jand and tongba. During the preparation of jand, fermented cereal solids are squeezed and strained to separate the liquid beverage (Karki and Kharel, 2010).

2.3.1 Fermented millet residue flour

The fermented finger millet residue flour was prepared by modifying the traditional jand (fermented finger millet beverage) processing method and finger millet flour production approach described by (Karki, 2013) and (Mudau *et al.*, 2022). The leftover fermented millet residue obtained after jand extraction was collected carefully and used for the preparation of fermented millet residue flour. The residue was spread evenly on stainless steel trays and oven-dried at 65°C until a constant weight was achieved. The dried material was then pulverized using a mixer grinder and sieved through a 60 μm mesh sieve to obtain a fine, uniform flour. The resulting flour was stored in airtight polyethylene pouches until further use for muffin formulation and analysis.

2.3.2 Compositional characteristics of the flour

Fermentation induces several biochemical modifications in the finger-millet matrix, and these transformations directly influence the nutritional and functional composition of the fermented millet residue flour obtained after filtration.

1. Reduction of antinutrients and improved mineral bio accessibility: A major compositional improvement associated with fermentation is the substantial reduction of antinutrients, chiefly phytic acid and condensed tannins. These compounds naturally chelate minerals and limit their bioavailability. Controlled studies on finger millet demonstrate significant decreases in phytic acid and tannin levels, accompanied by measurable improvements in the in vitro bio accessibility of minerals such as iron, zinc and calcium (Endalew *et al.*, 2024).

2. Protein hydrolysis and improved digestible fractions: During fermentation, microbial enzymes particularly proteases break down millet proteins into smaller peptides and free amino acids, thereby increasing the digestibility and solubility of the protein fractions. Studies on fermented finger-millet flours report enhanced in vitro protein digestibility and greater proportions of soluble nitrogen, reflecting partial hydrolysis of storage proteins and modification of the starch–protein network (Narayanasamy, 2021).
3. Carbohydrate consumption and relative concentration shifts: Microbial saccharification and fermentation consume portions of the starch and simple sugars, which lead to a relative concentration increase in non-consumed constituents i.e. ash/minerals, fiber, and some protein fractions. Analytical studies report decreases in total starch or carbohydrate and corresponding relative increases in measured ash or mineral content after fermentation (Jan *et al.*, 2022).

These compositional changes enhance the nutritional density and functional behavior of the flour, making it suitable for incorporation into functional bakery products.

2.3.3 Functional properties of the flour

Fermentation has long been recognized as one of the most effective bio-processing techniques for enhancing both the nutritional and functional attributes of cereal flours, including finger millet (*Eleusine coracana*). It not only enhances the nutritional quality of finger millet flour but also profoundly influences its functional, thermal, and pasting properties that determine its performance in baked products. Functional properties like bulk density, water absorption capacity (WAC), and oil absorption capacity (OAC) are critical indicators of the flour's behavior during mixing and baking. High WAC and OAC contribute to better batter viscosity, softer crumb texture, and improved moisture retention, all of which are desirable attributes for utilization during manufacturing of bakery products, jelly foods, and thickening foods. Fermented and native finger millet flours are prospective raw materials to substitute wheat flour for gluten-free products with desirable properties. Fermentation also modifies the microstructure of finger millet flour by loosening the starch–protein network and increasing porosity. These changes promote better hydration and gas retention during baking, leading to improved rise, crumb texture, and overall product uniformity. The enzymatic degradation during fermentation also create compounds that enhance Maillard browning, contributing to an appealing golden color and aroma in bakery goods (Mudau *et al.*, 2022).

2.3.4 Valorization potential of fermented millet residue

The food industry generates significant amounts of by-products, which not only contribute to environmental burden but also signal inefficiencies in resource utilization. A growing trend, however, is to repurpose these by-products into human food, thereby enhancing sustainability and value (Saberian *et al.*, 2024). Recent studies on millet fermentation by-products have expanded scientific understanding of the nutritional and functional value of millet residue. The superior proximate composition, higher total phenolic and flavonoid contents, and significantly enhanced antioxidant activity observed in the fermented samples are attributed to fermentation induced biochemical transformations that increase bioactive compounds and reduce antinutritional factors (Selvaraj *et al.*, 2025).

Although fermented millet residues are rich in proteins, fibers, vitamins, minerals, and various bioactive compounds such as polyphenols and dietary fiber, most existing research has focused primarily on their use in animal feed. They are sustainable and healthy feed ingredient, demonstrating that the residue possesses considerable nutritive value rather than being mere waste material. The bioactive components offer notable health benefits, including protection against oxidative stress, enhanced immune function, and reduced inflammation. However, the findings hold strong relevance for human food applications particularly in the development of functional foods because higher phenolic content, greater antioxidant capacity, and improved nutrient bioavailability are key attributes desired in health promoting formulations. Additionally, upcycling fermented millet residues supports sustainability by aligning with circular food system principles, reducing processing waste, and generating nutrient-dense, value-added functional ingredients (Selvaraj *et al.*, 2025).

Analogous cereal processing residues offer valuable insights into the valorization potential of fermented millet by-products. Among them, brewers' spent grain (BSG) is one of the most well documented and widely utilized industrial co-products. As a close analogue to fermented millet residue, BSG is recognized as a fiber and protein rich substrate containing substantial amounts of cellulose, hemicellulose, and phenolic compounds. It has been successfully incorporated into various bakery formulations including bread, muffins, pasta, and other cereal-based products not only to enhance nutritional quality but also to support product fortification and enrichment. Numerous studies have demonstrated that BSG supplementation can elevate dietary fiber and protein content while maintaining acceptable technological and sensory attributes in cereal foods. With increasing consumer interest in sustainable, health-promoting foods, BSG valorization has emerged as a promising innovation pathway aligned with global circular-economy and sustainability goals (Eche *et al.*, 2025). The success of BSG valorization therefore provides a strong conceptual and practical foundation for developing fermented millet residue as a novel ingredient in sustainable bakery applications.

2.4 Wheat

Wheat (*Triticum spp.*), a member of the Gramineae or "grass" family, is one of the most important cereal crops used in bakery and food industries. The two main species cultivated for food purposes are common wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*). For commercial use, common wheat is categorized as hard or soft, red or white, and as spring or winter types. Hard wheat flour, due to its high protein content, is mainly used for yeast-leavened products such as breads, rolls, muffins, and doughnuts, whereas soft wheat flour with lower protein content is preferred for biscuits, pastries, cakes, and breakfast cereals (Hoseney, 1989).

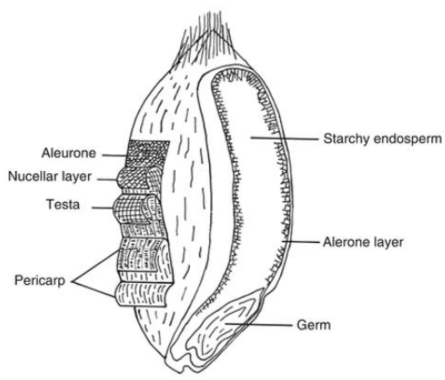
Wheat flour used in muffin production is obtained from the endosperm and finely ground to pass through a 100-mesh sieve (Kent-Jones and Amos, 1947). Its unique characteristic lies in its ability to form an elastic dough when mixed with water, a property attributed to gluten proteins. Gluten comprises two major components glutenin and gliadin which together make up about 75–80% of the total protein in wheat flour (Mukhopadhyay, 1990). For soft-textured baked products like muffins and cakes, flour with approximately 7.5% gluten is considered ideal for achieving a tender and crumbly structure (Lauterbach and Albrecht, 1994).

The quality and strength of flour proteins significantly affect the texture of baked goods. Strong flours contain long protein chains with fewer bonds, while weak flours possess shorter chains with multiple bonds, making them more extensible and suitable for cakes and muffins (Kim and Kim, 1999). Moreover, the functional properties of flour can be modified through treatments. For instance, Sulphur dioxide treatment weakens flour strength, while heat treatment enhances it. The use of improvers can also modify gluten behavior, allowing it to function similarly to that found in stronger flours during fermentation (Kent-Jones and Amos, 1947).

2.4.1 Structure of wheat

From a botanical perspective, wheat grain is a single-seeded fruit known as a caryopsis, commonly referred to as a kernel. It comprises a pericarp (fruit coat) tightly adhering to the seed coat, enclosing the seed, which contains an embryo (germ) and endosperm surrounded by a nucellar epidermis and seed coat. The caryopsis develops within modified leaves called glumes, which are removed during threshing, resulting in a "naked" grain. Wheat grains vary in color from light buff or yellow to red-brown, depending on the presence of red pigments in the seed coat, which is genetically determined (Kent and Evers, 1994).

Structurally, the wheat grain can be divided into three main parts: the bran, germ, and endosperm. The bran constitutes about 13% of the grain and comprises six principal layers. The outer three layers form the pericarp: epidermis (0.5%), epicarp (1%), and endocarp (1.5%). The inner layers form the seed coat, which includes the testa (2%) containing the pigment that classifies wheat as red, white, or yellow, the nucellar layer (1%), and the aleurone layer (7%).The germ, accounting for roughly 2% of the grain, is the wheat embryo and consists of the plumule (developing shoot), radicle (root), and scutellum, which stores most of the grain's vitamins. The germ also contains a high proportion of diastatic enzymes that facilitate the conversion of starch into sugar (Kent and Evers, 1994).



Source: Cauvain (2015)

Fig 2.4 General structure of wheat

2.4.2 Chemical composition and nutritive value of wheat

Wheat is widely recognized as a valuable source of protein, calories, vitamins, and minerals, as summarized in Table 2.8. Its nutrient profile is comparable to that of other cereals, with a higher protein content than rice, maize, and sorghum, and roughly similar levels to other grains. Wheat forms a significant part of the diet for billions of people worldwide, making the nutritional value of its proteins especially important, particularly in developing countries where wheat-based products like bread and cakes constitute a large portion of daily food intake. Wheat contributes approximately 55% of dietary carbohydrates and 20% of total food calories (Kumar and Jhariya, 2013).

The nutritional value of wheat flour depends on the degree of extraction. Flours with lower extraction rates, such as white flour, have reduced amounts of bran, germ, and outer endosperm, which are rich in protein, minerals, and vitamins. Consequently, these flours differ nutritionally from whole wheat flour, which retains most of these components (Kent-Jones and Amos, 1947).

Chemical composition of wheat flour (whole and refined) is shown in Table 2.8

Table 2.7 Chemical composition of wheat flour (whole and refined)

Parameters	WF	WWF
Moisture (g)	13.3	12.2
Protein (g)	11	12.1
Fat (g)	0.9	1.7
Carbohydrate (g)	73.9	69.4
Minerals (g)	0.6	2.7
Fiber (g)	0.3	1.9
Energy (Kcal)	348	341
Calcium (mg)	23	48
Phosphorous (mg)	121	355
Iron (mg)	2.7	4.9
Carotene (μg)	25	29
Vitamin C (mg)	0	0
Thiamine (mg)	0.12	0.49
Riboflavin (mg)	0.07	0.17
Niacin (mg)	2.4	4.3

Source: DFTQC. (2017)

2.5 Ingredient and their role in muffin making

2.5.1 Wheat Flour

Wheat (Triticum spp.) is believed to have originated in the eastern Mediterranean, Near East, and Middle East, and it is well-suited to cool, dry, and temperate climates. Whole wheat flour (WWF) retains all anatomical components of the grain i.e. endosperm, bran, and germ in the same proportions as in the intact grain. As a result, WWF contains higher levels of fiber, vitamins, minerals, and phytochemicals compared to RF, making it a rich source of nutritional and functional compounds associated with health benefits, including reduced risks of diabetes, cardiovascular diseases, obesity, and cancer (Liu, 2007).

Despite these advantages, WWF can alter the structural and sensory properties of food, potentially lowering consumer acceptance. Achieving the same functionality and quality as refined grain products remains challenging. WWF also affects dough characteristics and requires adjustments in processing parameters. Particle size of WWF significantly influences the flour's quality and performance in baking (Kihlberg *et al.*, 2004). For instance, reducing coarse bran particle size slightly improved bread volume (MODER JR *et al.*, 1984), but smaller bran particles have also been reported to negatively impact baking quality, causing reduced bread volume and increased starch retrogradation during storage (Cai *et al.*, 2014) and (Noort *et al.*, 2010).

2.5.1.1 Requirements of flour characteristic

The flour should possess a free-flowing texture, feel dry to the touch, and exhibit a creamy appearance without any visible bran particles. It should have a pleasant, characteristic flavor and be free from musty or rancid odors. The required flour characteristics are presented in table 2.9

Table 2.8 Requirements of flour characteristic

Characteristics	Requirements
Moisture content	13.0% max
Gluten content in dry basis	7.5% min
Total ash on dry basis	0.5% max
Acid insoluble ash on dry basis	0.05% max
Protein (N×7.5) on dry basis	9%
Alcohol acidity as H2SO4 in 90% alcohol	0.1%
Water absorption	5%
Sedimentation	22%
Granularity	To satisfy the taste

Source: Kaur *et al.* (2015)

2.5.2 Fat

Fats, present naturally in many animals and plant-based diets, contribute important qualities to baked goods, including flavor, tenderness, mouthfeel, and nutritional value. Common bakery fats include butter, shortenings, and hydrogenated fats, and they are widely used in products such as muffins, puff pastry, bread, biscuits, scones, and pie crusts (Figoni, 2010) and (Kamel and Stauffer, 2013).

Fats are crucial in pastry making, ranking as the third most expensive ingredient after wheat and sugar. During mixing, water and fat compete with flour particles and other ingredients. Gluten develops when water interacts with flour proteins to form an extensible network, but the addition of fat interrupts this bonding, resulting in softer, crispier baked products (Renzyaeva, 2013).

In bakery products, fats serve several key functions:

1. Flavor enhancement – especially animal fats, which provide richness and distinctive taste.

2. Texture improvement – producing softer, less firm products.

3. Moistness and mouthfeel – by coating the tongue and reducing surface grittiness.

4. Leavening support – helping to incorporate air during creaming and baking.

5. Staling retardation – by interfering with starch gelatinization, extending freshness.

Source:(Figoni, 2010)

2.5.3 Leavening agent

Leavening in baked goods refers to the process by which carbon dioxide gas, produced through yeast fermentation, chemical reactions, or the effect of heat, causes the expansion of air bubbles incorporated into the dough or batter during mixing. This expansion increases the volume and develops the cell structure, thereby improving the texture and palatability of the final product. The primary leavening gases are air, steam, and carbon dioxide. Air is incorporated during mixing, steam forms when water in the dough or batter evaporates during baking, and carbon dioxide is released from chemical leavening agents such as sodium bicarbonate, potassium bicarbonate, and ammonium bicarbonate. For instance, ammonium bicarbonate decomposes during baking to produce ammonia, carbon dioxide, and water, all of which contribute to leavening (Neeharika *et al.*, 2020).

The chemical reactions for common soda-acid combinations are:

- $NaHCO_3 + H^+ \longrightarrow Na^+ + CO_2 + H_2O$
- $NH_4HCO_3 \xrightarrow[\blacktriangle]{} NH_3 + CO_2 + H_2O$

Source:(Neeharika *et al.*, 2020)

Ammonium bicarbonate is advantageous because it does not form residual salts that could affect dough rheology. From a practical standpoint, the selection of chemical leavening agents depends on several factors:

1. The ability to produce an adequate amount of CO_2.

2. Minimal formation of residual salts that may negatively impact product quality.

3. Safety and non-toxicity.

4. Stability, ensuring that reactions occur primarily during baking rather than mixing.

2.5.4 Sweeting agents

Sugar is one of the most commonly used ingredients in muffins due to its multifunctional properties. Being hygroscopic, sugar attracts and retains water molecules, which helps maintain moisture in baked products. It is an essential component in almost all baked goods, ranging from chemically leavened treats to yeast-based pastries. In muffin preparation, sugar serves multiple roles:

1. Acts as a sweetener.

2. Participates in fermentation, where yeast produces alcohol and carbon dioxide for leavening.

3. Extends shelf life by lowering water activity through moisture retention.

4. Provides moisture retention due to its hygroscopic nature.

5. Functions as a tenderizer and aerator, improving texture in batter systems.

6. Enhances mouthfeel and overall texture.

7. Contributes to color and flavor via Maillard and caramelization reactions.

8. Acts as a foaming agent in sponge-type muffins when combined with egg whites.

9. Lowers the freezing point, useful in certain frozen bakery products.

10. Serves as a bulking agent, adding volume to the batter.

Source:(Hui *et al.*, 2008)

2.5.5 Whole Egg

Eggs are essential for maintaining both the nutritional and structural properties of muffins. Their proteins interact with other ingredients to form the muffin's structure, while emulsifiers in the yolk help blend ingredients that would normally separate, such as water and oil. During baking, these proteins contribute to a golden-brown crust, and the lipids in the yolk enhance flavor (Lin *et al.*, 2017).

Eggs also act as foam stabilizers, supporting the expansion of air bubbles formed during mixing. The carbon dioxide released by baking powder serves as nuclei for these bubbles, and the egg solids, with egg whites to a lesser extent, help prevent bubble coalescence, contributing to a soft, aerated texture (Singh *et al.*, 2017).

2.5.6 Water

Water, though a simple chemical compound, is as essential as flour in baking. It serves multiple functions, some not fully understood, but most are critical for achieving the desired properties of the dough and final product. Water used in baking must be potable, and its mineral composition can influence dough characteristics. Key aspects of water quality to consider include flavor, chemical composition, and mineral content (Sinani *et al.*, 2014).

The main functions of water in baking include:

1. Providing moisture to the product.
2. Hydrating and combining dry ingredients to form a uniform batter or dough.
3. Contributing to the structure of baked goods.
4. Regulating dough and batter temperature, which can enhance storage stability.
5. Controlling consistency, which directly affects the volume, texture, and overall quality of muffins.

2.6 Baking Profile

Baking is a critical step in muffin preparation, as improper baking can negatively affect the product's quality, texture, flavor, and appearance. During baking, the raw dough is transformed into an edible muffin through cooking, flavor development, and color formation, with the primary purpose being the removal of excess moisture via controlled heating (Bloksma, 1990).

Heat transfer during baking occurs through conduction, convection, and radiation, with radiation providing the majority of heat to the dough, while convection contributes more significantly only when air velocity exceeds five feet per second. High-frequency heating can also be employed for faster moisture removal (Smith, 1972).

Ovens typically consist of four main components:

1. A heat source
2. A heated base (sole or hearth) on which the dough rests
3. A cover or chamber that retains heat
4. A closable opening for inserting and removing the dough

During baking, the dough undergoes physical and chemical changes. Physical changes include:

1. Formation of a crust
2. Melting of fats
3. Expansion due to gas release
4. Conversion of water into steam
5. Escape of carbon dioxide and other gases

Chemical changes include:

1. Generation of gases
2. Starch gelatinization
3. Protein denaturation and coagulation
4. Caramelization of sugars
5. Dextrinization

Temperature in the baking oven has different effect on the raw dough, which is shown in Table 2.10.

Table 2.10 Temperature related changes in muffin during baking

Temperature (˚F)	Changes occurred
90-100	Skin development on the top crust (Evaporation of surface moisture).
90-120	CO_2 evolution within crumb (Less solubility of CO_2).
90-150	CO_2 evolution within crumb (Less solubility of CO_2).
90-210	Gelatinization of starch (Muffin structure).
125-210	Protein coagulation (Irreversible).
170-250	The process of dextrinization (surface gloss).
370-400	Skin development on the top crust (Evaporation of surface moisture).

Source:Mukhopadhyay (1990)

Steam plays a key role in baking, as the moisture generated from the dough and fuel combustion is often insufficient. Injecting steam at the beginning of baking helps form a shiny crust, prevent cracks, increase volume, and improve heat circulation. Rapid air circulation via fans can sometimes replace steam injection. Oven dampers are essential for managing pressure and moisture, particularly in high-moisture baked goods (Smith, 1972).

Part-III

Materials and methods

3.1 Raw Materials

3.1.1 Wheat flour

'Fortune' wheat flour (*Triticum asetivum)* was brought from local market of Dharan.

3.1.2 Finger Millet

Finger millet (*Eleusine coracana*) was brought from local market of Dharan. It was later fermented and converted into flour in dry pilot plant of CCT, Dharan.

3.1.3 Murcha

Murcha required for the inoculation of fermentation culture was brought from the local market of Dharan.

3.1.4 Sugar

Sugar in the form of powdered sugar was used and brought from the local market of Dharan.

3.1.5 Fat

Butter, produced by Dairy Development Corporation, Biratnagar, was brought from local market of Dharan.

3.1.6 Egg

Eggs were brought from local market in Dharan.

3.1.7 Baking powder

'Weikfied baking powder double action' manufactured and packed by Weikfied food Pvt. Ltd., Pune, India was brought from supermarket of Dharan.

3.1.8 Water

Potable water was provided in Dharan's CCT.

3.1.9 Plastic jar and muffin cups

Plastic jar manufactured by Sujal Plastics Pvt. Ltd was used to carry out overall fermentation process. Muffin cups were brought from local market of Dharan.

3.1.10 Equipment and chemicals

The necessary apparatus and chemicals were obtained from the CCT laboratory, Dharan and are listed in Appendix B.

3.2 Methods

3.2.1 Experimental procedure

An experimental plan for response-surface analysis was generated using Design-Expert® v13.0.1.0. A D-optimal design was used to study two mixture components (Component A = wheat flour, Component B = fermented millet residue flour). Component proportions are reported as parts (by weight) for each run.

Table 3.1 Experiment plan for response surface analysis (Format generated by Design Expert® v13.0.1.0)

Standard	Run	Component A A: Wheat flour %	Component B B: Fermented millet residue flour
6	10	100	0
1	9	100	0
9	8	100	0
5	7	40	60
8	6	60	40
2	5	20	80
7	4	20	80
4	3	80	20
10	2	20	80
3	1	60	40

3.2.2 Formulation of recipe

The recipe for fermented millet residue flour substituted muffins were carried out as per table 3.2. The amount given is on different basis:

Table 3.2 Recipe formulation for muffins

Ingredients	A	B	C	D	E	F
Wheat flour	100	60	50	40	30	20
Fermented millet residue flour	0	40	50	60	70	80
Sugar	60	60	60	60	60	60
Fat	65	65	65	65	65	65
Baking powder	1.42	1.42	1.42	1.42	1.42	1.42
Egg	57	57	57	57	57	57
Water	31	31	31	31	31	31

The muffin was made as per the recipe formulation and coded A, B, C, D, E and F.

3.2.1.2 Preparation of jand

Jand was prepared following the procedures given by (Karki, 2013). For the preparation of jand, finger millet grains (Eleusine *coracana*) collected from the local market of Dharan were first cleaned to remove foreign materials such as stones and soil. The cleaned grains were then dehusked manually and winnowed. The dehusked grains were washed thoroughly with clean water and cooked until the grains became soft, ruptured and partially gelatinized. This cooking step improves starch availability for microbial fermentation. The cooked millet was then spread on a clean tray to cool at room temperature.

After cooling, approximately 0.5–1.0% of murcha (traditional starter culture) was uniformly mixed with the cooked grains. The inoculated mixture was transferred to a plastic jar and covered with a piece of muslin cloth subjected to aerobic fermentation at room temperature (25–28 °C) for initial 2–3 days in order to ensure the proper microbial biomass build up and partial saccharification. After the initial aerobic phase, the lid was tightly closed and allowed to undergo anaerobic fermentation for 12–15 days at room temperature. This stage promotes ethanol production and flavor development in the fermenting mass. After fermentation, 1.5 times the volume of clean water was added to the fermented mass, the contents were mashed thoroughly and filtered through a muslin cloth to separate the liquid and solid fractions. The liquid extract was collected as jand, while the solid residue (by-product) was retained for further processing to prepare fermented millet flour. Method of preparation of jand followed in this experiment is shown in Fig. 3.1.

Millet

↓

Dehusking and Winnowing

↓

Washing with water

↓

Cooking (30 min at 100ºC)

↓

Spreading in tray for cooling

↓

Inoculating with murcha (0.5%)

↓

Primary fermentation for 3 days (aerobic)

↓

Filling to fermentation jar and lidding

↓

Secondary fermentation of 14 days (anaerobic)

↓

Addition of 1.5 volume of water

↓

Jand

Fig 3.1 Finger millet jand preparation process

Source:(Karki, 2013)

3.2.1.3 Preparation of fermented millet residue flour

The fermented millet residue flour was prepared following procedure given by (Mudau *et al.*, 2022) with some modification. The leftover fermented millet solid residue was mashed and washed three times with water for maximum jand extraction and collected carefully for the preparation of fermented finger millet flour. The residue was spread evenly on stainless steel trays and oven-dried at 65°C hours until a constant weight was achieved. The dried material was then pulverized using a mixer grinder and sieved through a 60 µm mesh sieve to obtain a fine, uniform flour. The resulting flour was stored in airtight polyethylene pouches until further use for muffin formulation and analysis. This modification allowed for the utilization of a traditionally discarded by-product, contributing to sustainable processing and nutrient recovery. Method of preparation of fermented millet residue flour followed in this experiment is shown in Fig. 3.2.

Leftover fermented millet solid residue

↓

Mashing and washing (3 times)

↓

Collection of solid residues

↓

Spreading evenly on stainless steel trays

↓

Oven drying until constant weight (65°C for 12 hrs)

↓

Pulverizing using mixer grinder

↓

Sieving (60 µm)

↓

Fine fermented millet reside flour

↓

Packaging

Fig 3.2 Preparation of fermented millet residue flour

Source: Mudau *et al.* (2022)

During oven drying (65°C, thin layers, forced air) residual ethanol is expected to volatilize because ethanol has substantial vapor pressure at temperatures well below its boiling point; evaporation is promoted by temperature, surface area and airflow. Literature on volatile loss during drying and baking confirms that fermentation-derived volatiles including ethanol are readily lost during thermal processing, and studies of cooking/thermal processing report near-complete removal under typical drying and baking conditions (Pico *et al.*, 2020).Similarly, (Karki, 2013) confirmed that the alcoholic fraction is mainly present in the filtrate, with negligible amounts in the solid residue.

Thus, the dried fermented millet residue flour used for muffin preparation was entirely non-alcoholic. Furthermore, any trace volatiles would be completely eliminated during baking, ensuring a safe and alcohol-free product.

3.2.1.4 Preparation of fermented millet residue flour substituted muffins

In this study, six different muffin formulations were developed. As shown in Table 3.2 one formulation served as the control without fermented millet residue flour, while the remaining five formulations contained varying levels of fermented millet residue flour suggested by Design Expert 13. The preparation process began with the separation of dry and wet ingredients. The dry ingredients included wheat flour, fermented millet residue flour, baking powder, and sugar, whereas the wet ingredients consisted of egg, water, and butter. The egg was whisked thoroughly for about two minutes before adding water and butter. Subsequently, all dry ingredients were mixed uniformly in a separate bowl. Finally, the wet and dry mixtures were combined to produce a homogeneous muffin batter ready for baking.

Egg Beating (2 min)

↓

Melted butter, water ⟶ Creaming

↓

Cream

↓

Wheat Flour, Baking powder, Sugar
+ Fermented millet residue flour ⟶ Mixing

↓

Batter

↓

Molding and Panning

↓

Baking (215ºC for 20±3 min)

↓

Cooling (35ºC)

↓

Storage (at ambient temperature)

Fig. 3.3 Flow chart for the preparation of muffins

Source:Rahman *et al.* (2015)

3.2.1.5 Muffin baking process

3.2.1.5.1 Mixing

The main goal of mixing is to obtain a uniform and homogeneous batter, ensuring that all ingredients are evenly distributed throughout the mixture. Mixing can be carried out using either a batch or a continuous process. In an ideal mix, the concentration of each ingredient remains consistent throughout the entire batter, and all portions undergo the same degree of processing (Cullen, 2009).

3.2.1.5.2 Baking

After mixing, the batter should be poured into molds and baked immediately. If immediate baking is not possible, the batter must be tightly covered with plastic wrap and stored in a refrigerator until baking. Once baking powder dissolves in the mixture, carbon dioxide gas begins to form. Allowing the batter to stand for an extended period causes some gas to escape, resulting in muffins with a coarse and uneven texture (Trimbo and Miller, 1966).

3.2.1.5.3 Cooling

After baking, muffins should be cooled to room temperature before packaging. During the cooling process, moisture migrates from the center of the muffin toward the crust and eventually into the surrounding air. If excessive moisture accumulates in the crust, it can become leathery and lose the desirable crispness of freshly baked muffins. Conversely, over-drying during cooling can lead to weight loss and an undesirable crumb texture. Therefore, the purpose of cooling is to reduce temperature efficiently while minimizing moisture loss. Additionally, the sugar content in muffins contributes to structural firmness and overall product stability as the muffins cool (Kent and Evers, 1994).

3.3 Analytical procedure

3.3.1 Moisture content

The moisture content of both raw materials and finished products was determined using a hot air oven, following the method described by Ranganna (1986).

3.3.2 Crude fat

Crude fat was determined using Soxhlet extraction with petroleum ether as the solvent as described by Ranganna (1986).

3.3.3 Crude protein

Crude protein content ($N \times 6.25$) was determined using the micro-Kjeldahl method as described by Ranganna (1986).

3.3.4 Crude fiber

Crude fiber was measured by treating the sample with boiling dilute sulfuric acid and sodium hydroxide, followed by alcohol, according to Ranganna (1986).

3.3.5 Total Ash

Ash content was determined using the dry ashing method, according to Ranganna (1986).

3.3.6 Carbohydrate

Total carbohydrate content was determined by the difference method as described by Ranganna (1986).

3.3.7 Energy value

Energy computed as followed for all the samples as described by Ranganna (1986)

Energy (kcal/100 g) = [Protein (g) x 4] + [Carbohydrate (g) x 4] + [Fat (g) x 9]

3.3.8 pH and TSS

pH was determined by a digital pH meter and TSS by a handheld refractometer according to Committee (2000). Ten grams of each flour sample was mixed with 100 ml of distilled water and allowed to stand at room temperature for 30 minutes. The pH of the resulting mixture was then measured using a pre-calibrated digital pH meter. The instrument was calibrated prior to use with standard buffer solutions of pH 4.00, 7.00, and 9.00 to ensure accurate readings. Similarly, for the determination of TSS approximately four drops of extract were placed onto the refractometer prism for reading. After each measurement, the refractometer surface was thoroughly rinsed with distilled water to remove any residual sample before the next reading.

3.3.9 Reducing sugar

Reducing sugar was determined by Lane and Eynon method as described by Ranganna (1986). 25 g of neutralized sample was clarified and maintained with distilled water in 250 ml volumetric flask. 10 ml of such sample was titrated with standard Fehling mix solution on hot plate till brick red color was developed. After adding few drops of methylene blue, the same was again titrated till permanent brick red color was obtained.

% reducing sugar was determined using the following equation:

$$\% \text{ Reducing sugar} = \frac{Dilution \times Fehling\ factor}{Aliqout\ titer \times Sample\ wt(g)} \times 100$$

3.3.10 Sample preparation

The extraction of sample was carried out using methanol as a solvent as (Singh *et al.*, 2010). One gram of sample was mixed with 30ml methanol, followed by centrifugation at 4000 rpm for 15min at room temperature. The supernatant was filtered using Whatman's filter paper. The volume was made up to 50ml with 80% methanol which was used further for the analysis of antioxidant activity. The antioxidant activity of extracts was carried out using the following method.

3.3.10.1 DPPH

Radical scavenging activity was measured using the method described by Hawa *et al.* (2018) with slightly variation. Different concentrations of extracts (1:5, 1.5:5, 2:5) were added to 80% methanol. 0.5 ml extract was mixed with 4 ml of 0.1 mM DPPH solution and left in dark for 30 min and absorbance was measured at 517 nm in UV-Visible spectrophotometer. Blank samples were prepared with methanol (0.5 ml) and DPPH (4 ml) only.

% scavenging activity was expressed in percentage using the equation:

$$\% \text{ scavenging activity} = \frac{Ab - As}{Ab} \times 100$$

Where, A_b is the absorbance of the control, A_s is the absorbance of the test sample.

3.3.10.2 Phenolics Content

The total phenolic content of the sample was determined using the Folin-Ciocalteu method. An aliquot of 0.2 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent and 0.8 ml of saturated sodium carbonate solution. The reaction mixture was allowed to stand at room temperature for 30 minutes, after which the absorbance was measured at 765 nm using a spectrophotometer The total phenolic content was expressed as micrograms of gallic acid equivalent (GAE) per milliliter of solution. The results were calculated based on the calibration curve prepared using standard gallic acid solutions (Vaher *et al.*, 2010).

3.3.11 Calcium

The calcium content of the sample was analyzed using a flame photometer at 768 nm as per Kc and Rai (2007) .

3.3.12 Iron

The iron content of the muffin was determined colorimetrically. All the iron present in the sample was first converted into the ferric form by using suitable oxidizing agents such as potassium persulfate ($K_2S_2O_8$) or hydrogen peroxide (H_2O_2). The resulting ferric ions were then treated with potassium thiocyanate (KSCN) to form a red-colored ferric thiocyanate complex. The intensity of the developed color was measured colorimetrically at 480 nm, and the iron concentration in the muffin sample was determined by comparison with a standard calibration curve prepared using known iron concentrations (Kc and Rai, 2007).

3.3.13 Volume

The volume of the muffin loaf was measured using the mustard seed displacement method. A box of known volume was filled with mustard seeds, which were then poured over the sample to determine the volume displaced Committee (2000).

3.3.14 Weight of Muffin

The weight of each muffin was measured using a standard weighing balance available at the Central Campus of Technology, Dharan (Committee, 2000).

3.3.15 Specific Loaf Volume

Specific loaf volume was calculated by dividing the volume of the muffin by its weight (Committee, 2000).

Specific loaf volume $=\frac{weight\ of\ muffin}{volume\ of\ muffin}$

3.4 Sensory analysis

Sensory evaluation was conducted by semi-trained panelists, including faculty and students from the Central Campus of Technology. The parameters assessed included, appearance, taste, aroma, mouthfeel, and overall acceptability. Evaluations were carried out using a 9-point Hedonic Scale.

3.5 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA)medium (incubated at 30ºC/48h). Coliform Count was determined by pour plate technique on MaConkey Medium (incubated at 37ºC/48 hrs) (Kodaka *et al.*, 2005).

3.6 Statistical analysis

The experimental design was performed using Design-Expert® version 13 (Stat-Ease, Inc., Minneapolis, MN, USA). Data from proximate analysis and sensory evaluation were analyzed using IBM SPSS Statistics 25 (Copyright 2017, IBM Corp., Armonk, NY, USA) for Analysis of Variance (ANOVA) at a 5% level of significance. Tukey's HSD test was performed to determine significant difference between the sample means. Microsoft office 2021 was used for graph and table construction.

Part IV

Results and Discussion

The prepared fermented millet residue flour and wheat flour were both analyzed for their proximate composition to compare their nutritional characteristics. Muffins were formulated using the muffin mixing method by substituting wheat flour with fermented millet residue flour in different proportions (100:0, 60:40, 50:50, 40:60, 30:70, and 20:80), coded as samples A, B, C, D, E, and F respectively. The muffins were evaluated for their physical properties (such as volume, weight, and specific loaf volume) and subjected to sensory analysis to assess attributes like appearance, taste, aroma, mouthfeel, after taste, and overall acceptability.

4.1 Analysis of materials

In the preparation of fermented millet residue flour substituted muffin, fermented millet residue flour, finger millet and wheat flour were the major ingredients. They were analyzed for their composition. The proximate composition of wheat flour, millet flour and fermented millet residue flour is presented in Table 4.1.

Table 4.1 The proximate composition of wheat flour, millet flour and fermented millet residue flour.

Parameters (db)	Wheat Flour	Millet Flour	Fermented Millet Residue Flour
Moisture (%)	11.37±0.26	12.20±0.66	10.10± 0.55
Protein*	11.54±0.09	11.2±0.2	12.72±0.28
Fat*	1.2±0.43	1.58±0.09	1.85±0.07
Crude fiber*	0.47±0.25	4.16±0.62	5.02±0.58
Ash content*	0.59±0.03	2.96±0.44	3.39±0.39
Carbohydrate*	86.17±0.57	80.07±1.03	77.29±0.94
Energy values (kcal/100 g)	401.64±4.51	379.3±4.28	376.69±3.98
Reducing sugar (mg/g)	0.96±0.38	0.82 ± 0.21	1.46±0.37
TPC (mg GAE/g)	0.68± 0.04	0.89±0.96	1.32±0.91
Antioxidant (%)	5.35± 0.39	68.42±0.13	72.48±0.31
Calcium (mg/100g)	18.42±1.9	332±0.11	368±13
Iron (mg/100g)	2.11±0.47	3.9±0.2	5.1±0.3

[*represents values in dry basis, values following ± represents standard deviation]

4.1.1 Chemical composition of wheat flour

The moisture content, protein, fat, crude fiber, carbohydrate, calcium and iron of wheat flour were determined to be 11.37, 11.54, 1.2, 0.47, 86.17, 18.42 and 2.11. The reported proximate composition values for moisture, protein, fat, fiber carbohydrate, calcium and iron were 13.3%, 11%, 0.9%, 0.3%, 73.9%, 23 mg/100g and 2.7 mg/100g according to (DFTQC., 2017). The value for ash content was determined to be 0.59. In a study by (Mãrculescu *et al.*, 2022) it was found to be 0.51–0.55%. The value for reducing sugar was determined to be 0.96. According to (Ojha, 2024) it was reported to be 1.11%-1.33%. Similarly, the TPC and antioxidant activity was determined to be 0.68 and 5.35 whereas that were reported to be 1.92 ± 0.02 mg GAE/g and 7.01 ± 0.13 μmol Trolox eq/g according to (Vaher *et al.*, 2010). The variations observed between the standard reference values and the experimentally determined results may be attributed to several factors such as varietal differences, climatic conditions, soil characteristics, maturity stage of the grains, fertility status, and other environmental influences.

4.1.2 Chemical composition of finger millet flour

The moisture content, protein, fat, crude fiber, ash, carbohydrate, calcium and iron of finger millet flour was found out to be 12.20, 11.2, 1.58, 4.16, 2.96, 80.07, 332 and 3.9.According to (Takale, 2025) the reported proximate composition values for moisture, protein, fat, fiber, ash, carbohydrate, calcium and iron were reported to be 13.22, 8.03, 1.6, 5.58, 3.10, 74.07, 345.07mg and 4.36mg.The value for reducing sugar was determined to be 0.82. In a study it was reported to be 0.68-0.78% according to (Ojha, 2024). Similarly, the energy value, TPC and antioxidant activity was determined to be 379.30, 0.89 and 68.42. In a study it was found to be 346.50±3.50 kcal/100 g, 1.07±0.07 mg GAE/g and 74.35±0.97% according to (Jan *et al.*, 2022).A disparity was observed between the standard reference values and the experimentally obtained results. Such variations in proximate composition can be attributed to several factors, including varietal differences, climatic conditions, soil characteristics, grain maturity, fertility levels, and other environmental factors that influence the nutritional profile of finger millet.

4.1.3 Chemical composition of fermented millet residue flour

The moisture content, protein, fat, crude fiber, ash, carbohydrate, calcium, and iron of the prepared fermented millet residue flour were found to be 10.10, 12.72, 1.85,5.02,3.39,77.29, 368 and 5.1. According to (Takale, 2025) the reported proximate composition values for moisture, protein, fat, crude fiber, ash, carbohydrate, calcium and iron were 14.03-13.53, 9.54-9.66, 1.72-1.9, 5.47-5.67, 3.63-3.53, 70.72-72.44, 349.17-348.99mg and 4.87-4.56mg. The energy value, TPC and antioxidant activity was determined to be 336.42, 1.32 and 72.48.According to (Jan *et al.*, 2022) the energy value, TPC and antioxidant activity was found to be 376.69±3.08 kcal/100 g, 1.95±0.46 mg GAE/g and 81.66±0.37%. Similarly, the reducing sugar content was reported to be increased substantially during fermentation according to (Karki *et al.*, 2023). A noticeable variation was observed between the standard reference values and the experimentally determined results. Since no papers that report proximate composition of fermented finger millet residue flour was found. Therefore, the best available values were taken from the studies of fermented finger millet flours. Along with these such differences in proximate composition may be attributed to several factors, including microbial population dynamics, fermentation time and temperature, and processing conditions before and after fermentation, climatic conditions and other environmental factors that influence the overall nutritional composition of fermented finger millet residue flour.

Fermentation improved the nutritional and functional properties of millet residues. Protein content increased due to enhanced bioavailability and microbial synthesis of amino acids, along with reduced antinutritional factors such as tannins and phytates (Sripriya *et al.*, 1997). Fat content increased because of moisture reduction and the release of bound lipids following cell-wall disruption (Nyathi, 2021). Crude fiber was concentrated as fermentation retained fiber while soluble components like sugars were utilized by microbes (Malleshi and Desikachar, 1985; Mudau *et al.*, 2022). Ash content improved due to higher mineral contribution and better mineral extractability following phytate reduction Sripriya *et al.* (1997), while carbohydrates decreased because microbes metabolized starch and sugars (Nyathi, 2021). Energy values increased as a result of higher protein and fiber content (Mudau *et al.*, 2022). Antioxidant activity and total phenolic content were enhanced owing to the release of bound phenolics and generation of bioactive metabolites (Mudau *et al.*, 2022; Nyathi, 2021). Reducing sugars increased due to microbial starch degradation and enzymatic hydrolysis (Sripriya *et al.*, 1997). Finally, mineral availability, including calcium and iron, improved as phytate breakdown during fermentation enhanced bio accessibility (Sripriya *et al.*, 1997).

4.2 Fermented millet residue flour impact on physical characteristics of muffin

4.2.1 Effect on weight

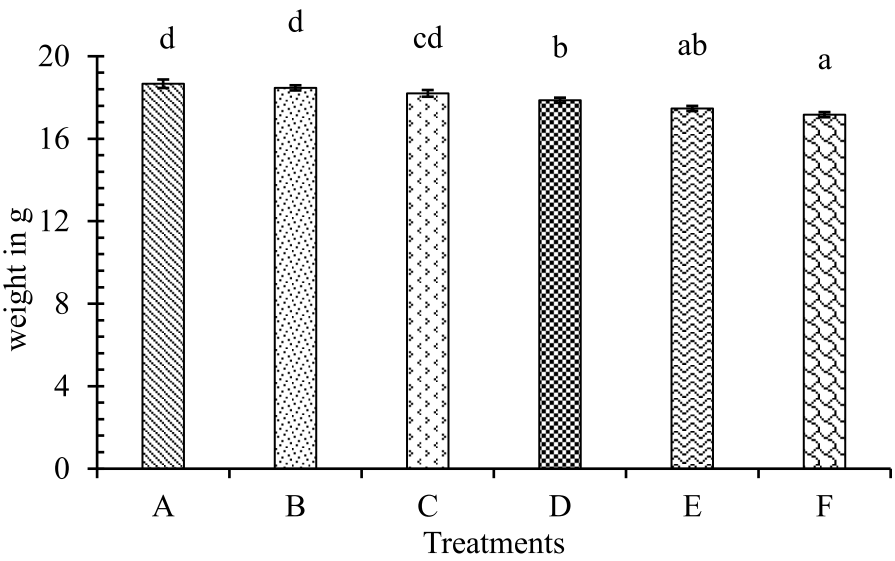


Fig. 4.1 Change in weight during baking at different proportions of fermented millet residue flour

In Fig. 4.1, the obtained mean values are presented as a bar diagram, and the data indicate a significant difference in muffin weights among treatments ($p \leq 0.05$), as confirmed by ANOVA in Appendix E table E.1**.** The alphabets above the bars denote significant differences, while error bars represent the standard deviation of triplicates.

The control muffin (sample A) showed the highest mean weight (18.6± 0.67 g), followed by sample B (18.4± 0.67 g), C (18.2± 0.01 g), D (17.8± 0.67 g), E (17.4± 0.67g), and F (17.1 ± 0.67g). The weight of muffins decreased gradually with the increase in fermented millet residue flour substitution level. The lowest weight was recorded for sample F.

The reduction in muffin weight with increased incorporation of fermented millet residue flour is attributed due to the higher fiber content and lower gluten-forming protein in millet flour. The higher fiber content binds water but reduces its availability for gluten development, which restricts dough expansion and water retention during baking, resulting in lower mass after baking similar to the findings of (Goswami *et al.*, 2015).

However, although ANOVA indicated overall significant differences among treatments, some samples were not significantly different from each other, as denoted by superscript letters. This may be due to the relatively small incremental substitution levels between certain treatments (e.g., 40% and 50%), which might not have caused sufficient structural or compositional variation to produce statistically distinct weight differences.

4.2.2 Effect on volume

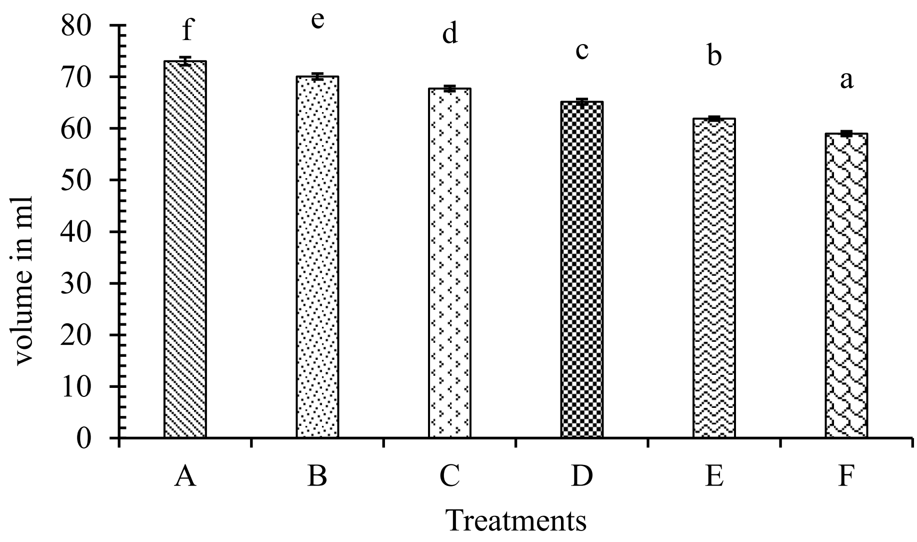


Fig. 4.2 Change in volume during baking at different proportions of fermented millet residue flour

In Fig. 4.2, the obtained mean values for muffin volume are presented as a bar diagram. There is a significant difference ($p \leq 0.05$) in muffin volume among the samples, as indicated by the ANOVA Appendix E Table E.2**.** The alphabets above the bars denote significant differences, while error bars represent the standard deviation of triplicate measurements.

The control muffin (sample A) showed the highest volume (73± 0.33 ml), followed by sample B (70± 0.67ml), C (67.7± 0.33ml), D (65.1± 0.67ml), E (61.9± 0.01 ml), and F (59± 0.01ml). A gradual decrease in muffin volume was observed with increasing levels of fermented millet residue flour.

The decline in muffin volume is attributed to the dilution of gluten-forming proteins, which weakens the structural matrix required for gas entrapment during baking. Fermented millet residue flour also introduces more insoluble fiber, which disrupts gluten film formation and reduces air retention similar to the finding of (Devani *et al.*, 2016).

4.2.3 Effect on specific loaf volume

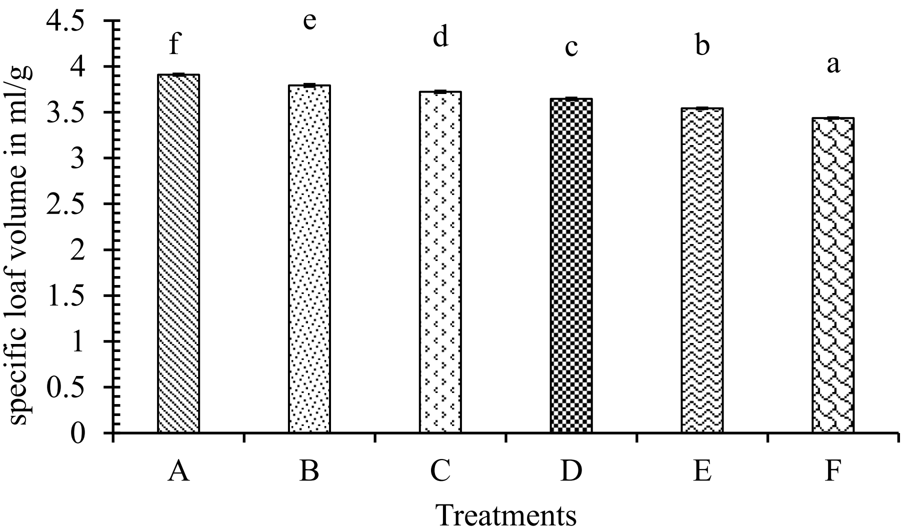


Fig. 4.3 Change in specific loaf volume during baking at different proportions of fermented millet residue flour

In Fig. 4.3, the obtained mean values for specific loaf volume are shown as a bar diagram. The ANOVA results Appendix E table E.3 revealed a significant difference ($p \leq 0.05$) among the samples. The alphabets above the bars represent significant differences, and the error bars indicate the standard deviation of triplicates.

The control muffin (sample A), showed the highest specific loaf volume (3.9± 0.10), followed by sample B (3.7± 0.93), C (3.7± 0.23), D (3.6± 0.47), E (3.5± 0.43), and F (3.4± 0.37). The reduction in specific loaf volume corresponded with the increasing substitution of fermented millet residue flour in the muffin formulation was observed.

This decreasing trend suggests that higher fermented millet residue flour substitution reduces gas retention and expansion during baking due to limited gluten development and increased fiber interference. This trend is similar to the findings of (Goswami *et al.*, 2015). Furthermore, fermented millet residue flour is rich in dietary fiber and non-gluten proteins, which interfere with gluten continuity and disrupt bubble stability in the batter system (Sabanis *et al.*, 2009). Dietary fiber competes for water, limiting gluten hydration and elasticity, and increases batter viscosity, thereby restricting expansion during baking (Rosell *et al.*, 2001).

4.3 Sensory evaluation of muffin

Sensory evaluation of muffins prepared with varying proportions of wheat flour and fermented millet residue flour, as per the experimental design, was carried out. The sensory attributes evaluated included color, aroma, appearance, texture, taste, and overall acceptability, using a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely) as described by Rangana (1986). A panel of thirteen trained judges participated in the evaluation. The panelists were instructed on the evaluation procedure and guided to rate each muffin sample based on their perception of the specified sensory parameters. Scores were recorded on structured score sheets prepared for the study**.** Appendix D presents the ANOVA results for the sensory evaluation of muffins prepared with different proportions of wheat flour and fermented millet residue flour.

Here, A = muffin made with 100% wheat flour (0% fermented millet residue flour), B = muffin made with 60% wheat flour and 40% fermented millet residue flour, C = muffin made with 50% wheat flour and 50% fermented millet residue flour, D = muffin made with 40% wheat flour and 60% fermented millet residue flour, E = muffin made with 30% wheat flour and 70% fermented millet residue flour, and F= muffin made with 20% wheat flour and 80% fermented millet residue flour.

4.3.1 Appearance

The mean scores for appearance were 7.9, 6.8, 6.2, 6.0, 5.3, and 5.3 for samples A, B, C, D, E, and F, respectively. The obtained mean values are represented as bar diagrams in Fig. 4.4. There was a significant difference ($p \leq 0.05$) in appearance among the muffin samples, as confirmed by ANOVA (Appendix D). Based on mean scores, the ranking order for appearance was A > B > C > D > E = F.

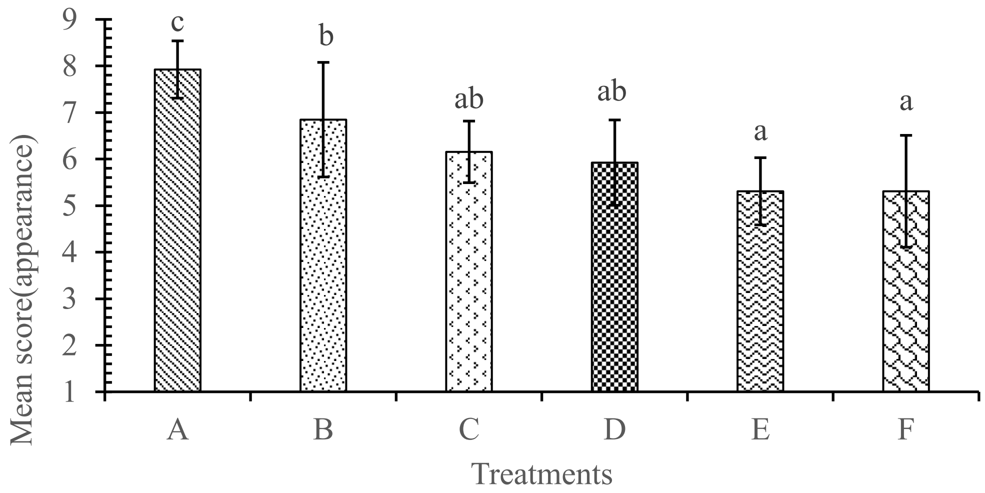


Fig: 4.4 Characteristics of product with respect to appearance

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis showed that sample A differed significantly from the samples B, C, D, E and F. Samples B, C, and D were similar to each other but significantly different from A, while samples E and F showed no significant difference among themselves or with C and D, yet both differed significantly from A and B which is shown in the figure 4.4.1. Muffin A had the highest appearance score, indicating the most appealing surface color and uniformity, while F received the lowest score. Muffins containing higher proportions of fermented millet residue flour exhibited a darker, coarser surface due to the inherent color and fiber content of millet, which might have reduced visual appeal and softness of the crumb.

Color in baked goods comes from two sources: intrinsic color imparted by individual ingredients and developed color resulting from interaction of ingredients (Acosta *et al.*, 2011). Maillard browning results from the interaction of free amino groups with reducing sugars, and compared with amylose, amylopectin possesses a higher number of reducing ends. The observed dark color may also be attributed to the intrinsic dark brown color of fingermillet. These results are in accordance with previous findings reported by (Bhaduri, 2013).

4.3.2 Taste

The mean scores for taste were 7.6, 7.6, 7.2, 6.2, 5.5, and 4.8 for samples A, B, C, D, E, and F, respectively. The obtained mean values are represented as bar diagrams in Fig. 4.5. There was a significant difference ($p \leq 0.05$) in taste among the muffin samples, as confirmed by

ANOVA (Appendix D). Based on mean scores, the ranking order for appearance was A = B > C > D = E > F.

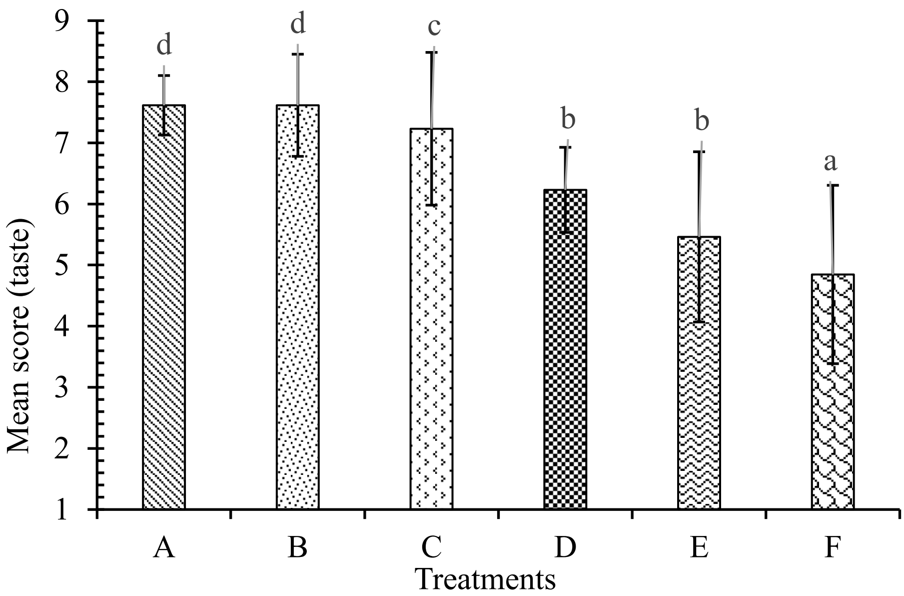


Fig: 4.5 Characteristics of product with respect to taste

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis showed that samples A and B were not significantly different from each other but differed significantly from C, D, E, and F. Sample C differed significantly from A, B, D, E and F, while samples D and E were not significantly different from each other. Sample F was significantly different from all other samples. Muffin sample A and B had the most acceptable taste, whereas sample F was least preferred. The decline in taste scores with increasing levels of fermented millet residue flour is attributed to the millet's characteristic earthy and slightly bitter flavor. Additionally, millet lacks the sweetness and caramelization potential of wheat flour, reducing the overall flavor development during baking.

4.3.3 Aroma

The mean scores for aroma were 8.0, 7.3, 6.8, 5.9, 5.1, and 4.5 for samples A, B, C, D, E, and F, respectively. The obtained mean values are presented as bar diagrams in Fig. 4.6. There was a significant difference ($p \leq 0.05$) in aroma among the muffin samples, as confirmed by ANOVA (Appendix D). Based on mean scores, the ranking order for aroma was A > B > C > D > E > F.

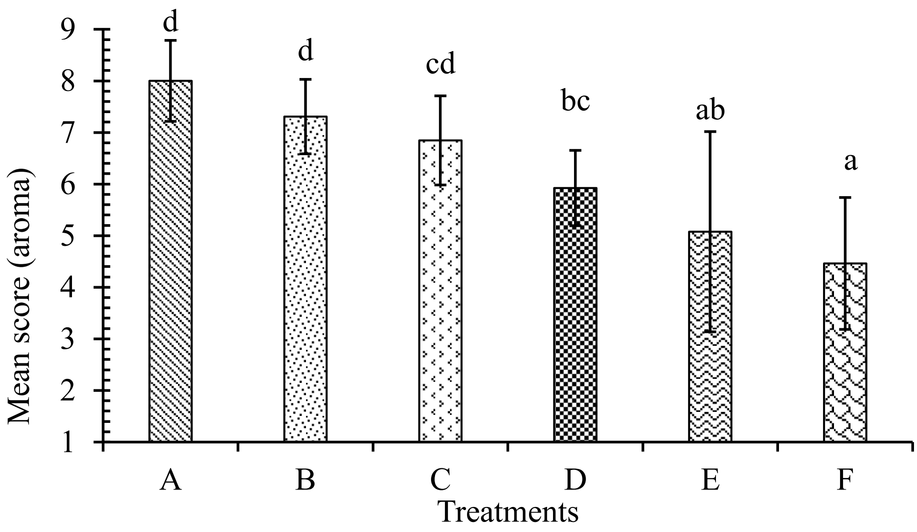


Fig: 4.6 Characteristics of product with respect to aroma

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis showed that samples A, B, and C were not significantly different from each other but differed significantly from samples D, E, and F. Sample D was similar to C and E but differed from A, B, and F. Samples E and F were not significantly different between themselves but were significantly different from A, B, and C. Muffins sample A and B were rated highest in aroma, likely due to the development of typical baked notes through Maillard reactions. Increasing fermented millet residue substitution introduced an earthy and slightly grassy odor characteristic of millet grain, while the lower wheat proportion reduced volatile compounds responsible for pleasant sweet-baked aroma, resulting in decreased sensory scores.

4.3.4 Mouthfeel

The mean scores for mouthfeel were 7.5, 7.0, 6.3, 6.1, 4.7, and 4.7 for samples A, B, C, D, E, and F, respectively. The obtained mean values are presented as bar diagrams in Fig. 4.7. There was a significant difference ($p \leq 0.05$) in mouthfeel among the muffin samples, as confirmed by ANOVA (Appendix D). Based on mean scores, the ranking order for mouthfeel was A > B > C > D > E = F.

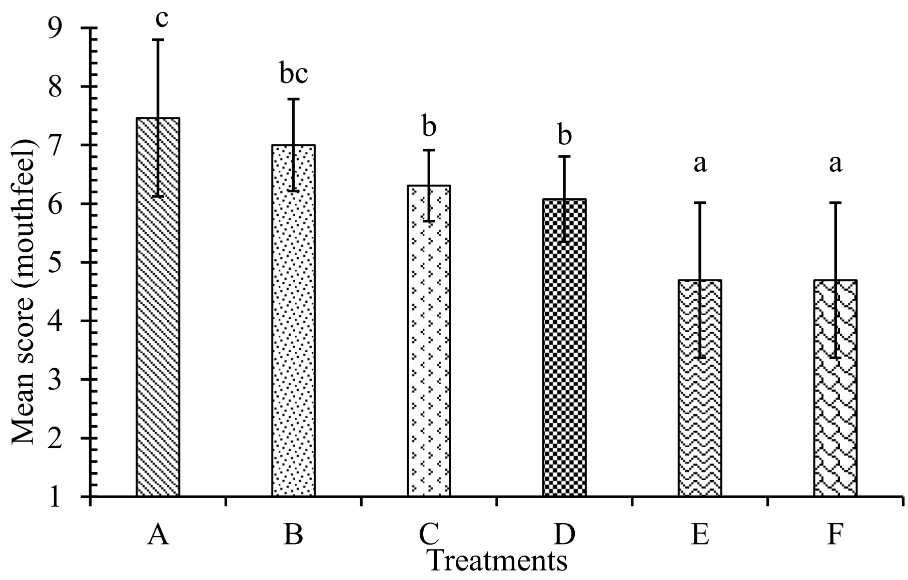


Fig: 4.7 Characteristics of product with respect to mouthfeel

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis showed that sample A was not significantly different from B but differed significantly from C, D, E, and F. Samples B, C, and D were similar to each other, while E and F were not significantly different between themselves but were significantly different from A, B, C, and D. Muffin A had the best mouthfeel, with a soft, spongy texture. Muffins containing higher levels of fermented millet residue flour exhibited a coarse and slightly dry mouthfeel. This is attributed due to millet's high fiber and low gluten content, which limit gas retention and reduce crumb softness, leading to a denser texture.

The addition of fermented millet residues made the muffins denser yet more cohesive, with higher fiber and protein boosting water absorption and retention. This yielded a softer bite and greater chewiness compared to the control (Rosell *et al.*, 2009). The enhanced mouthfeel observed in the present study aligns with these reports, indicating that the inclusion of fermented millet residues not only improves the nutritional profile but also positively affects sensory quality by modulating texture and palatability.

4.3.5 After taste

The mean scores for aftertaste were 7.2, 7.2, 6.8, 6.2, 4.8, and 4.6 for samples A, B, C, D, E, and F, respectively. The obtained mean values are presented as bar diagrams in Fig. 4.8.

There was a significant difference ($p \leq 0.05$) in aftertaste among the muffin samples, as confirmed by ANOVA (Appendix D). Based on mean scores, the ranking order for aftertaste was A = B > C > D > E> F.

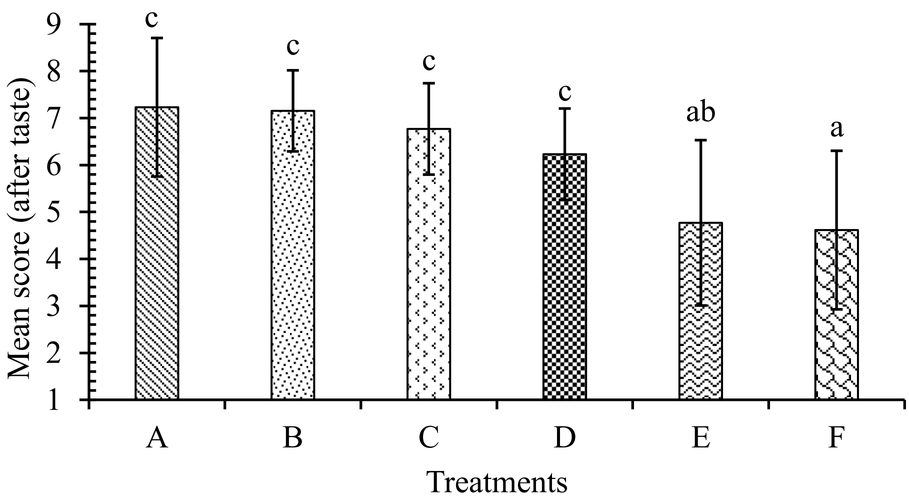


Fig: 4.8 Characteristics of product with respect to after taste

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis revealed that samples A, B, C, and D were not significantly different from each other but differed significantly from samples E and F. Samples E and F were similar to each other but significantly different from all other samples. Muffins with higher fermented millet residue flour had lower aftertaste scores, due to the inherit millet's gritty texture and astringent sensation that lingers after swallowing.

4.3.6 Overall acceptance

The mean scores for overall acceptability were 7.8, 7.4, 6.6, 5.9, 4.8, and 4.2 for samples A, B, C, D, E, and F, respectively. The obtained mean values are presented as bar diagrams in Fig. 4.9. There was a significant difference ($p \leq 0.05$) in overall acceptability among the muffin samples, as confirmed by ANOVA (Appendix D). Based on mean scores, the ranking order for overall acceptability was A > B > C > D > E > F.

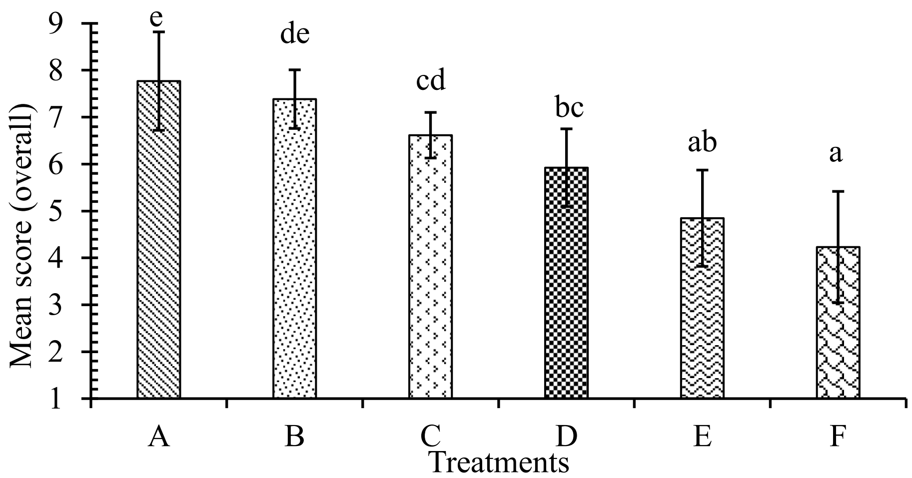


Fig: 4.9 Characteristics of product with respect to overall acceptance

Similarly, the alphabet above the bar denotes significant difference. Statistical analysis showed that sample A was not significantly different from B but differed from C, D, E, and F. Sample B was similar to C but different from A and D, E and F. Samples D and E were not significantly different from each other, while F was similar to E but differed from all other samples. Muffin A was the most preferred, followed by B. The decline in acceptability with increasing fermented millet residue content can be linked to darker color, coarse crumb, and characteristic earthy flavor, gritty texture of finger millet, which together reduced the preference.

4.4 Chemical composition of muffin

The proximate compositions of control muffin and best sample (40 % fermented millet residue flour substituted muffin) were analyzed and obtained results are given in Table 4.2.

Table 4.2 Proximate composition of control and best product

Parameters(db)	Control muffin (%)	Best product (%)
Moisture	14.3 ±2.68^{a}	11.04±0.6^{a}
Protein*	6.01 ±0.42^{a}	7.55±0.37^{b}
Fat*	15.32 ±0.09^{a}	17.9±0.12^{b}
Crude fiber*	1.05 ±0.22^{a}	2.13±0.18^{b}
Ash content*	1.30 ±0.003^{a}	1.96±0.05^{b}
Carbohydrate*	62.02±2.72^{a}	59.42 ± 0.74^{a}
Energy values (kcal/100 g)	409.99±11.04^{a}	428.98±3.48^{a}
Antioxidant (%)	18.6±1.9^{a}	36.9±2.8^{b}
Reducing sugar (mg/g)	1.92±0.31^{a}	2.35±0.44^{a}
Total phenolic content (mg GAE/100 g)	6.04±0.21^{a}	11.92±0.47^{b}
Calcium (mg/100g)	30.5 ±1.2^{a}	145.22±0.32^{b}
Iron (mg/100g)	4.03±0.18^{a}	6.76±0.47^{b}

[*represents values in dry basis, values following ± represents standard deviation]

The values revealed notable differences between the two formulations due to the incorporation of fermented millet residue flour and the compositional changes occurring during fermentation. The fermented millet residue muffin exhibited notable improvements in its nutritional and functional properties compared to the control.

Overall, the incorporation of fermented millet residue flour resulted in a nutritionally superior muffin with higher protein, fiber, minerals, phenolic compounds, and antioxidant activity compared to the control. These enhancements demonstrate the potential of fermented millet residue as a functional ingredient capable of improving both nutritional value and health promoting properties of baked products.

4.5 Microbiological analysis

No coliform colonies were detected in the samples. Similarly, yeast and mold growth was absent up to the sixth day of storage, and the total plate count remained nil during this period. The absence of microbial growth may be attributed to the high temperature applied during baking, which effectively destroyed microorganisms. Furthermore, the use of LDPE of 50 μm thickness packaging likely helped prevent post-baking contamination and maintained the microbiological safety of the muffins during storage.

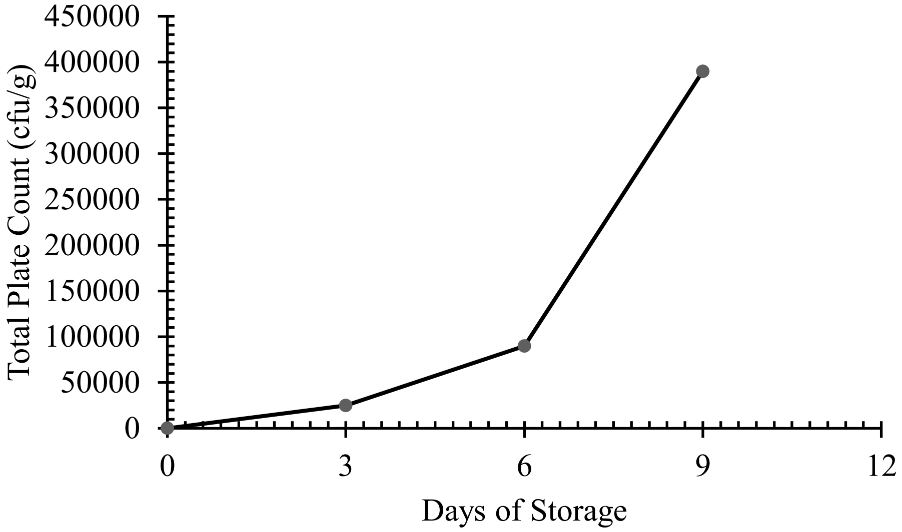


Fig. 4.10 Changes in TPC with respect to number of days of storage

4.6 Cost evaluation

The overall cost associated with the best muffin (sample B) was determined, and the cost of fermented millet residue flour incorporated muffin was NRs.48.25. Calculation is shown in the Appendix C.

Part V

Conclusion and recommendations

5.1 Conclusions

1. Fermented millet residue flour was successfully incorporated as a partial substitute for wheat flour in muffin preparation, demonstrating its potential for value addition and utilization in functional muffin production.
2. Fermented millet residue flour had higher protein, fiber, minerals, phenolics, and antioxidant activity compared to wheat flour.
3. Increasing fermented millet residue flour levels reduced muffin weight, volume, and specific loaf volume.
4. The 40% fermented millet residue flour substituted muffin (sample B) showed the best balance of physical and sensory properties among all substituted samples.
5. Chemical analysis of the 40% muffin confirmed improved nutritional value, particularly in protein, fiber, calcium, iron, TPC, and antioxidant activity.
6. Microbiological analysis of product showed acceptability of FMR muffin up to six days at room temperature in LDPE package without any artificial preservatives.
7. The cost of fermented millet residue flour substituted muffin was found to be slightly higher than that of the control muffin.

5.2 Recommendations

1. Chemical stability of fermented millet residue flour incorporated muffins are recommended.
2. Substitution with other nutrient rich products can be done to develop functional bakery products with multiple health benefits.

Part VI

Summary

The present study aims to prepare and evaluate muffins incorporated with FMR flour and determine the optimum substitution level that provides acceptable sensory and nutritional quality. Fermented millet residues were oven-dried at 65°C until constant weight, ground, and sieved to 60 µm to obtain fine flour. The millet flour, fermented millet residue flour and wheat flour were analyzed for moisture, protein, fat, crude fiber, total ash, carbohydrate, energy values, reducing sugar, TPC, antioxidant activity, calcium and iron. The values were found to be 12.20%, 9.84%, 1.39%, 3.66%, 2.60%, 70.31%, 333.11kcal/100g, 0.82%, 0.89 mg GAE/g, 68.42%, 332 mg/100g, 3.9 mg/100g for millet flour, 10.10%, 11.43%, 1.67%, 4.52%, 3.05%, 69.49%, 336.42kccal/100g, 1.46%, 1.32mg GAE/g, 72.48%, 368 mg/100g and 5.1 mg/100g for fermented millet residue flour and 11.37%, 10.23%, 1.07%, 0.42%, 0.53%, 76.38%, 356.07kcal/100g, 0.96%, 0.68 mg GAE/g, 5.35%, 18.42 mg/100g and 2.11mg/100g for wheat flour respectively.

Using a simple lattice design in Design Expert Version 13, six muffin formulations were prepared with 0%, 40%, 50%, 60%, 70%, and 80% substitution of FMR flour. All the other ingredients such as sugar (60 parts), fat (65 parts), baking powder (1.42 parts), egg (57 parts), and water (31 parts) were kept constant. Muffins were baked using the standard muffin method at 215°C for 20 ± 3 minutes. The finished samples were evaluated for sensory qualities (appearance, taste, aroma, aftertaste, mouthfeel, and overall acceptability) and physical parameters (weight, volume, and specific loaf volume), followed by chemical and nutritional analysis of the best formulation. The data were statistically analyzed using one-way ANOVA at 5% level of significance.

As substitution levels increased, muffin's physical and sensory parameter decreased and it turned darker color, increased hardness, and slight bitterness. Sensory evaluation revealed that the 40% substitution level scored highest among all FMR formulations. The best scored muffin was chemically analyzed and moisture, protein, fat, crude fiber, total ash, carbohydrate, energy values, reducing sugar, TPC, antioxidant activity, calcium and iron were found to be 11.04%, 7.55%, 17.9%, 2.13%, 1.96%, 59.42%, 428.98kcal/100g, 36.9%, 2.35 mg GAE/g, 11.92%, 145.22mg/100g and 6.76mg/100g respectively. Microbiological analysis indicated that the FMR muffin remained acceptable for up to six days at room

temperature when packaged in LDPE, without the use of artificial preservatives. Thus, a sustainable approach to reducing waste and promoting circular economy principles while creating a functional, value-added bakery product was done.

Reference

Abioye, V., Adejuyitan, J., Adeoye, A. and Gbadegesin, I. (2021). Effect of natural fermentation period on nutritional, anti nutritional, I total phenols, flavonoids and antioxidant contents of finger millet flour. *Eur. J. Nutr. Food Saf.* **13** (3), 74-82. [doi:10.9734/EJNFS/2021/v13i330392].

Acosta, K., Cavender, G. and Kerr, W. L. (2011). Sensory and physical properties of muffins made with waxy whole wheat flour. *J. Food Qual.* **34** (5), 343-351.

Admassu, S., Teamir, M. and Alemu, D. (2009). Chemical composition of local and improved finger Millet [Eleusine Corocana (L.) Gaetrtin] varieties grown in Ethiopia. *Ethiop. J. Health Sci.* **19** (1).

Apovian, C. M., Okemah, J. and O'Neil, P. M. (2019). Body weight considerations in the management of type 2 diabetes. *Adv. Ther.* **36** (1), 44-58. [doi:10.1007/s12325- 018-0824-8].

Baixauli, R., Sanz, T., Salvador, A. and Fiszman, S. (2008). Muffins with resistant starch: Baking performance in relation to the rheological properties of the batter. *J. Cereal Sci.* **47** (3), 502-509. [doi:10.1016/j.jcs.2007.06.015].

Bhaduri, S. (2013). A comprehensive study on physical properties of two gluten-free flour fortified muffins. *J. Food Process. Technol.* **4** (7), 1-4. [doi: 10.4172/2157-7110.1000251].

Bloksma, A. (1990). Dough structure, dough rheology, and baking quality. *Cereal Foods World*. **35** (2), 237-244.

Cai, L., Choi, I., Hyun, J. N., Jeong, Y. K. and Baik, B. K. (2014). Influence of bran particle size on bread-baking quality of whole grain wheat flour and starch retrogradation. *Cereal Chem.* **91** (1), 65-71.

Cauvain, S. (2015). Wheat milling and flour testing. *In:* "Technology of Breadmaking".). pp. 339-375. Springer.

Chetrariu, A. and Dabija, A. (2023). Spent grain: A functional ingredient for food applications. *Foods.* **12** (7), 1533. [doi:10.3390/foods12071533].

Committee, A. A. o. C. C. A. M. (2000). "Approved methods of the American association of cereal chemists". Vol. 1. American Association of Cereal Chemists. [1891127128].

Cullen, P. J. (2009). "Food mixing: Principles and applications". John Wiley & Sons. [1444309889].

Danciu, C.-A., Tulbure, A., Stanciu, M.-A., Antonie, I., Capatana, C., Zerbeș, M. V., Giurea, R. and Rada, E. C. (2023). Overview of the sustainable valorization of using waste and by-products in grain processing. *Foods.* **12** (20), 3770. [doi:10.3390/foods12203770].

Devani, B. M., Jani, B. L., Kapopara, M. B., Vyas, D. M. and Ningthoujam, M. D. (2016). Study on quality of white bread enriched with finger millet flour. *Int. J. Agric. Environ. Biotechnol.* **9** (5), 903. [doi: 10.5958/2230-732X.2016.00116.9].

DFTQC. (2017). "Nepalese Food Composition Table". Department of Food Technology and Quality Control (Ministry of Agricultural Development)". p. 8. Retrieved from http://www.dftqc.gov.np/downloadfile/Food%20Composition%20Table%202017_1572781821_1590383994.pdf. .

Dixit, R. R. a. P. (2025). Valorization of Cereal and Millet Food Processing Waste: A Review of Value-Added Products and Technologies. *Journal of Cereal Research*. **17** (2), 124-134. [doi:10.25174/2582-2675/2025/158043].

Eche, V., Emenike, C. and Rupasinghe, H. V. (2025). Nutritional Value of Brewer's Spent Grain and Consumer Acceptance of Its Value-Added Food Products. *Foods.* **14** (16), 2900. [doi:10.3390/foods14162900].

Endalew, H. W., Atlabachew, M., Karavoltsos, S., Sakellari, A., Aslam, M. F., Allen, L., Griffiths, H., Zoumpoulakis, P., Kanellou, A. and Yehuala, T. F. (2024). Effect of fermentation on nutrient composition, antinutrients, and mineral bioaccessibility of finger millet based Injera: A traditional Ethiopian food. *Food Res. Int.* **190**, 114635. [doi:10.1016/j.foodres.2024.114635].

Figoni, P. I. (2010). "How baking works: exploring the fundamentals of baking science". John Wiley & Sons. [0470392673].

Goswami, D., Gupta, R., Mridula, D., Sharma, M. and Tyagi, S. (2015). Barnyard millet based muffins: Physical, textural and sensory properties. *LWT – Food Sci. Technol.* **64** (1), 374-380. [doi:10.1016/j.lwt.2015.05.060].

Harb, H. E., El-Tabakh, M. A., Khattab, A. M., Mohamed, Y. A., Saleh, A. M. and El-Abeid, S. E. (2024). Recent advances of using innovative strategies in management of millet plant pathogens. *Genet. Improv. Small Millets*. 297-328. [doi:10.1007/978-981-99-7232-6_13]

Hawa, A., Satheesh, N. and Kumela, D. (2018). Nutritional and anti-nutritional evaluation of cookies prepared from okara, red teff and wheat flours. **25** (5).

Hoseney, R. (1989). The interactions that produce unique products from wheat flour. 595-606.

Hui, Y. H., Chandan, R. C., Clark, S., Cross, N. A., Dobbs, J. C., Hurst, W. J., Nollet, L. M., Shimoni, E., Sinha, N. K. and Smith, E. B. (2007). "Handbook of Food Products Manufacturing, Volume 2: Health, Meat, Milk, Poultry, Seafood, and Vegetables". Vol. 2. John Wiley & Sons. [047012525X].

Hui, Y. H., Corke, H., De Leyn, I., Nip, W.-K. and Cross, N. A. (2008). "Bakery products: science and technology". John Wiley & Sons. [0470276320].

Jan, S., Kumar, K., Yadav, A. N., Ahmed, N., Thakur, P., Chauhan, D., Rizvi, Q. and Dhaliwal, H. S. (2022). Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (Eleusine coracana L.). *J. Appl. Biol. Biotechnol.* **10** (1), 46-52. [doi:10.7324/JABB.2022.10s107].

Kamel, B. S. and Stauffer, C. E. (2013). "Advances in baking technology". Springer. [1489972560].

Karki, D. B. (2013). Improvement on traditional millet fermentation process and its brewing quality assessment. Ph.D Dissertation. Faculty of Food Technology, Nepal.

Karki, D. B. and Kharel, G. P. (2010). Solid versus semi-solid fermentation of finger millet (Eleusine coracana L.). *J. Food Sci. Technol. Nepal*. **6**, 31-35.

Karki, R., Karki, D. B., Ojha, P., Shrestha, R., Basnet, P., Maharjan, S., Manandhar, U. and Karki, K. (2023). Traditional Fermentation Practices and Brewing Dynamics of Nepalese Millet-Based Alcoholic Beverages. 538-565.

Kaur, M., Sandhu, K. S., Arora, A. and Sharma, A. (2015). Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: Physicochemical and sensory properties. *LWT – Food Sci. Technol.* **62** (1), 628-632.

Kaur, S., Kumari, A., Seem, K., Kaur, G., Kumar, D., Verma, S., Singh, N., Kumar, A., Kumar, M. and Jaiswal, S. (2024). Finger millet (Eleusine coracana L.): from staple to superfood—a comprehensive review on nutritional, bioactive, industrial, and climate resilience potential. *Planta.* **260** (3), 75. [doi:10.1007/s00425-024-04502-2].

Kc, J. and Rai, B. (2007). Basic food analysis handbook.

Kent-Jones, D. W. and Amos, A. J. (1947). Modern cereal chemistry.

Kent, N. L. and Evers, A. (1994). "Technology of cereals: an introduction for students of food science and agriculture". Woodhead Publishing. [0080408346].

Kihlberg, I., Johansson, L., Kohler, A. and Risvik, E. (2004). Sensory qualities of whole wheat pan bread—influence of farming system, milling and baking technique. *J. Cereal Sci.* **39** (1), 67-84.

Kim, K. C. and Kim, I. (1999). Ganoderma lucidum extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. *Int. J. Mol. Med.* **4** (3), 273-280.

Kodaka, H., Mizuochi, S., Teramura, H. and Nirazuka, T. (2005). Comparison of the compact dry TC method with the standard pour plate method (AOAC Official Method 966.23) for determining aerobic colony counts in food samples: performance-tested methodSM. *J. AOAC Int.* **88** (6), 1702-1713. [doi:10.1093/jaoac/88.6.1702].

Kumar, D. and Jhariya, A. N. (2013). Nutritional, medicinal and economical importance of corn: A mini review. **2319**, 555X.

Lauterbach, S. and Albrecht, J. A. (1994). Functions of baking ingredients.

Limbachiya, C. and Amin, B. (2015). Development of multigrain product. *Off. J. Int. Inst. Food Nutr. Sci.* **4** (5).

Lin, M., Tay, S. H., Yang, H., Yang, B. and Li, H. (2017). Replacement of eggs with soybean protein isolates and polysaccharides to prepare yellow cakes suitable for vegetarians. *Food Chem.* **229**, 663-673.

Liu, R. H. (2007). Whole grain phytochemicals and health. *J. Cereal Sci.* **46** (3), 207-219.

Malleshi, N. and Desikachar, H. (1985). Milling, popping and malting characteristics of some minor millets. *J. Food Sci. Technol.* **22** (6), 400-403.

Mãrculescu, O., Serbancea, C., Gradea, E. C. and Semenescu, A. (2022). The influence of temperature on the stability of reference materials.

Martínez-Cervera, S., Sanz, T., Salvador, A. and Fiszman, S. (2012). Rheological, textural and sensorial properties of low-sucrose muffins reformulated with sucralose/polydextrose. *LWT – Food Sci. Technol.* **45** (2), 213-220. [doi:10.1016/j.lwt.2011.08.001].

Miller, E. (1971). Product evaluation study guide for doughs and batters. *M. Tech Thesis. Kansas State Univ., USA*.

MODER JR, G., Finney, K., Bruinsma, B., Ponte, J. and Bolte, L. (1984). Bread-making potential of straight-grade and whole-wheat flours of Triumph and Eage-Plainsman V hard red winter wheats. *Cereal Chem.* **61** (4), 269-273.

Mudau, M., Ramashia, S. E. and Mashau, M. E. (2022). Mineral content, functional, thermo-pasting, and microstructural properties of spontaneously fermented finger millet flours. *Foods.* **11** (16), 2474. [doi:10.3390/foods11162474].

Mukhopadhyay, M. (1990). A seminar paper on process of manufacturing quality biscuits and new product development.

Narayanasamy, S. (2021). Efficacy of fermentation parameters on protein quality and microstructural properties of processed finger millet flour. *J. Food Sci. Technol.* **58** (8), 3223-3234.

Neeharika, B., Suneetha, W. J., Kumari, B. A. and Tejashree, M. (2020). Leavening agents for food industry. *Int. J. Curr. Microbiol. Appl. Sci.* **9**, 1812-1817.

Noort, M. W., van Haaster, D., Hemery, Y., Schols, H. A. and Hamer, R. J. (2010). The effect of particle size of wheat bran fractions on bread quality–Evidence for fibre–protein interactions. *J. Cereal Sci.* **52** (1), 59-64.

Nyathi, L. A. (2021). Nutritional, antioxidant and microbiological properties of finger millet-based beverages as influenced by starch source during lactic acid fermentation. University of Pretoria,

Ojha, P. K., Roman & Pandey, Luma & Bokkhim, Huma & Koirala, Pramod & Mote, Meena & Manandhar, Utshah & Upadhyaya, Sanju & Shrestha, Ranjan & Karki, Kabina & Basyal, Arusha & Rai, Dewaki. (2024). " Nepal Food Composition Database" (1st ed.). National Food Research Centre, Nepal Agricultural Research Council Retrieved from https://www.researchgate.net/publication/385945654_Nepal_Food_Composition_Database.

Onweluzo, J. and Nwabugwu, C. (2009). Fermentation of millet (Pennisetum americanum) and pigeon pea (Cajanus cajan) seeds for flour production: Effects on composition and selected functional properties. *Pak. J. Nutr.* **8** (6), 737-744. [doi:10.3923/pjn.2009.737.744].

Öztürk, S. and Mutlu, S. (2019). Physicochemical properties, modifications, and applications of resistant starches. *In:* "Starches for food application".). pp. 297-332. Elsevier.

Pico, J., Khomenko, I., Capozzi, V., Navarini, L. and Biasioli, F. (2020). Real-time monitoring of volatile compounds losses in the oven during baking and toasting of gluten-free bread doughs: A PTR-MS evidence. **9** (10), 1498. [doi:10.3390/foods9101498].

Rahman, R., Hiregoudar, S., Veeranagouda, M., Ramachandra, C., Kammar, M., Nidoni, U. and Roopa, R. (2015). Physico-chemical, textural and sensory properties of muffins fortified with wheat grass powder. *Karnataka J. Agric. Sci.* **28** (1), 79-82.

Rajiv, J., Soumya, C., Indrani, D. and Venkateswara Rao, G. (2011). Effect of replacement of wheat flour with finger millet flour (Eleusine corcana) on the batter microscopy, rheology and quality characteristics of muffins. *J. Texture Stud.* **42** (6), 478-489. [doi:10.1111/j.1745-4603.2011.00309.x].

Ramya, H. and Anitha, S. (2020). Development of muffins from wheat flour and coconut flour using honey as a sweetener. *Int. J. Curr. Microbiol. Appl. Sci.* **9** (7), 2231-2240. [doi:10.20546/ijcmas.2020.907.260].

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products". Tata McGraw-Hill Education. [0074518518].

Rathore, T., Singh, R., Kamble, D. B., Upadhyay, A. and Thangalakshmi, S. (2019). Review on finger millet: Processing and value addition. *Pharma Innov. J.* **8** (4), 283-291.

Renzyaeva, T. (2013). On the role of fats in baked flour goods. *Foods Raw Mater.* **1** (1), 19-25.

Rosell, C. M., Rojas, J. A. and De Barber, C. B. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocoll.* **15** (1), 75-81. [doi:10.1016/S0268-005X(00)00054-0].

Rosell, C. M., Santos, E. and Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: A comparative approach. *Food Res. Int.* **42** (1), 176-184. [doi:10.1016/j.foodres.2008.10.003].

Sabanis, D., Lebesi, D. and Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *LWT–Food Sci. Technol.* **42** (8), 1380-1389. [doi:10.1016/j.lwt.2009.03.010].

Saberian, H., Yazdi, A. P. G., Nejatian, M., Bazsefidpar, N., Mohammadian, A. H., Rahmati, M., Assadpour, E. and Jafari, S. M. (2024). Brewers' spent grain as a functional ingredient in bakery, pasta, and cereal-based products. *Future Foods.* **10**, 100479. [doi:10.1016/j.fufo.2024.100479].

Selvaraj, R., Annamalai, A., Kalakandan, S., Thamburaj, S. and Narayana, G. P. (2025). Valorisation of Fermented Millet Beverage Lees: A Viable Supplementation Alternative for Animal Feed Formulation. [doi:10.21203/rs.3.rs-6263796/v1].

Shahzadi, N. (2004). Use of composite lours for chapati preparation in relation to serum profile. UNIVERSITY OF AGRICULTURE, FAISALABAD,

Shankaramurthy, K. and Somannavar, M. S. (2019). Moisture, carbohydrate, protein, fat, calcium, and zinc content in finger, foxtail, pearl, and proso millets. *Indian J. Health Sci. Biomed. Res. KLEU*. **12** (3), 228-232. [doi:10.4103/kleuhsj.kleuhsj_32_19].

Shrestha, K. (2068). Evaluation of Nutritional Composition of Nepalese Finger Millet(Elusine coracana L.). B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Shrestha, K. and Karki, D. (2019). Evaluation of nutritional composition of Nepalese finger millet (elusine coracana L.). *Acta Sci. Nutr. Health*. **3** (1), 70-74.

Sinani, V., Sana, M., Seferi, E. and Sinani, A. (2014). The impact of natural water quality on baking products in Albania. *Water Resour. Prot.* **6** (18), 1659-1665.

Singh, B., Singh, A. K., Raju, P. and Rani, R. (2017). Nutritional value and physical properties of eggless muffin. *Int. J. Sci. Environ. Technol.* **6** (2), 1309-1314.

Singh, G., Kapoor, I., Singh, P., de Heluani, C. S., de Lampasona, M. P. and Catalan, C. A. (2010). Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (Curcuma longa Linn.). **48** (4), 1026-1031.

Skendi, A., Zinoviadou, K. G., Papageorgiou, M. and Rocha, J. M. (2020). Advances on the valorisation and functionalization of by-products and wastes from cereal-based processing industry. *Foods.* **9** (9), 1243. [doi:10.3390/foods9091243].

Smith, W. H. (1972). Biscuits, crackers and cookies.

Sripriya, G., Antony, U. and Chandra, T. (1997). Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (Eleusine coracana). *Food Chem.* **58** (4), 345-350. [doi:10.1016/S0308-8146(96)00206-3].

Takale, R. M., Neha & Deshmukh, Ajinkya. (2025). Effect of different fermentation on nutritional and anti-nutritional properties on finger millet cake. *International Journal of Agriculture and Food Science*. **7** (4), 34-37. [doi:10.33545/2664844X.2025.v7.i4a.331].

Tomar, T., Sachdeva, A., Dutta, J., Al Tawaha, A. R. M., Karnwal, A., Malik, T. and Selvaraj, M. (2025). Fermentation dynamics of millet beverages: Microbial interactions, nutritional enhancements, and health implications. *Food Chem.: X.*, 102199. [doi:10.1016/j.fochx.2025.102199].

Trimbo, H. B. and Miller, B. (1966). Batter flow and ring formation in cake baking. **40** (1), 40-42.

Upadhyaya, A. (2005). Effect of raw materials on the quality of Jand.

Vaher, M., Matso, K., Levandi, T., Helmja, K. and Kaljurand, M. (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chem.* **2** (1), 76-82. [doi:10.1016/j.proche.2009.12.013].

Appendices

Appendix A

Specimen card for sensory evaluation

Name: ……………………………………   **Date:** …………..

Product: Fermented millet flour incorporated muffin

Observe the product by testing. Use appropriate scale to show your attitude, by checking at the points, best described your feeling of products. An honest expression of your personnel feeling is warmly welcomed.

Sample	Appearance	Taste	Aroma	Mouthfeel	After taste	Overall Acceptance
A						
B						
C						
D						
E						
F						

9. Like extremely
8. Like very much
7. Like moderately
6. Like slightly
5. Neither like nor dislike
4. Dislike slightly
3. Dislike moderately
2. Dislike very much
1. Dislike extremely

Any comments

Signature

Appendix B

Table B.1 Chemicals Required

Chemicals	**Manufacture by**
Sodium Hydroxide pellets	Sisco Research Laboratories Pvt. Ltd.
Conc. Sulphuric acid	Research-Lab Fine-Chem Industries
Boric acid	Qualigens Fine Chemicals
Hydrochloric acid	Merch Lite Science Pvt. Ltd.
Distilled water	Central Drug House Pvt. Ltd
Petroleum ether	Central Drug House Pvt. Ltd
Sodium bicarbonate	Central Drug House Pvt. Ltd
Indicators (Methyl red, Phenolphthalein)	British Drug House

Table B.2 Apparatus Required

Equipments	Manufacturers
Cabinet dryer	Bharat Scientific
Glassware	Borosilicate
Grinder	Crompton
Hot air oven	Y.P. Scientific Industries
Magnetic stirrer	Faithful Instrument Co. Ltd.
Muffle furnace	Accumax India
pH meter	Esico

Refractometer	Trans Instruments
Refrigerator	Electrolax
Thermometer	Aceteq
Vacuum pump	Accumax India
Water bath	Mvtex Science Industries
Weighing machine	Prince Scale Industries

Appendix C

Table C.1 Cost analysis of the best muffin (sensory) prepared by incorporation of fermented millet flour

Particulars	Cost (NRs/1kg)	Weight in a lot (g)	Cost (NRs)
Wheat Flour	110	60	6.6
Fermented millet flour	220	40	8.8
Sugar	120	60	7.2
Fat	1000	65	65
Egg	580	57	33.06
Water	0	31	0
Baking powder	500	1.42	0.71
Raw material cost			121.37
Processing			12.137
Profit (15%)			18.2055
Grand total			151.7125
Total weight of muffin		314.42	
Total cost of muffin/100 g			48.25154252

Appendix D

ANOVA for sensory analysis of samples

Table D.1 Sensory analysis of appearance

Table D.1.1 Test of homogeneity of variances for appearance

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Appearance	Based on Mean	4.609	5	72	0.001
	Based on Median	3.381	5	72	0.008
	Based on Median and with adjusted df	3.381	5	56.979	0.010
	Based on trimmed mean	4.429	5	72	0.001

Table D.1.2 One-way ANOVA (No blocking) for appearance

ANOVA

Appearance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.603	5	13.121	14.148	0.000
Within Groups	66.769	72	0.927		
Total	132.372	77			

Table D.1.3 Homogeneous subsets for appearance

Homogeneous Subsets

Appearance

Tukey HSD_a

Sample	N	Subset for alpha = 0.05		
		1	2	3
E	13	5.31		
F	13	5.31		
D	13	5.92	5.92	
C	13	6.15	6.15	
B	13		6.85	6.85
A	13			7.92
Sig.		0.233	0.155	0.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Table D.2 Sensory analysis of taste

Table D.2.1 Test of homogeneity of variances for taste

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Taste	Based on Mean	3.478	5	72	0.007
	Based on Median	1.588	5	72	0.174
	Based on Median and with adjusted df	1.588	5	52.200	0.180
	Based on trimmed mean	3.122	5	72	0.013

Table D.2.2 One-way ANOVA (No blocking) for taste

ANOVA

Taste

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	89.808	5	17.962	14.104	0.000
Within Groups	91.692	72	1.274		
Total	181.500	77			

Table D.2.3 Homogeneous subsets for taste

Homogeneous Subsets

Taste

Tukey HSD[a]

Sample	N	Subset for alpha = 0.05			
		1	2	3	4
F	13	4.85			
E	13	5.46	5.46		
D	13		6.23	6.23	
C	13			7.23	7.23
A	13				7.62
B	13				7.62
Sig.		0.733	0.512	0.224	0.953

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Table D.3 Sensory analysis of aroma

Table D.3.1 Test of homogeneity of variances for aroma

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Aroma	Based on Mean	4.387	5	72	0.002
	Based on Median	1.062	5	72	0.389
	Based on Median and with adjusted df	1.062	5	27.255	0.403
	Based on trimmed mean	3.747	5	72	0.005

Table D.3.2 One-way ANOVA (No blocking) for aroma

ANOVA

Aroma

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	119.808	5	23.962	16.991	0.000
Within Groups	101.538	72	1.410		
Total	221.346	77			

Table D.3.3 Homogeneous subsets for aroma

Homogeneous Subsets

Aroma

Tukey HSD$_a$

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
F	13	4.46			
E	13	5.08	5.08		
D	13		5.92	5.92	
C	13			6.85	6.85
B	13				7.31
A	13				8.00
Sig.		0.772	0.462	0.363	0.145

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Table D.4 Sensory analysis of mouthfeel

Table D.4.1 Test of homogeneity of variances for mouthfeel

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Mouthfeel	Based on Mean	2.534	5	72	0.036
	Based on Median	1.451	5	72	0.217
	Based on Median and with adjusted df	1.451	5	56.189	0.220
	Based on trimmed mean	2.180	5	72	0.066

Table D.4.2 One-way ANOVA (No blocking) for mouthfeel

ANOVA

Mouthfeel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	86.423	5	17.285	14.068	0.000
Within Groups	88.462	72	1.229		
Total	174.885	77			

Table D.4.3 Homogeneous subsets for mouthfeel

Homogeneous Subsets

Mouthfeel

Tukey HSD$_a$

		Subset for alpha = 0.05		
Sample	N	1	2	3
E	13	4.69		
F	13	4.69		
D	13		6.08	
C	13		6.31	6.31
B	13		7.00	7.00
A	13			7.46
Sig.		1.000	0.287	0.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Table D.5 Sensory analysis of aftertaste

Table D.5.1 Test of homogeneity of variances for aftertaste

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
After Taste	Based on Mean	2.730	5	72	0.026
	Based on Median	1.300	5	72	0.274
	Based on Median and with adjusted df	1.300	5	38.119	0.285
	Based on trimmed mean	2.455	5	72	0.041

Table D.5.2 One-way ANOVA (No blocking) for aftertaste

ANOVA

After Taste

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	88.718	5	17.744	9.125	0.000
Within Groups	140.000	72	1.944		
Total	228.718	77			

Table D.5.3 Homogeneous subsets for aftertaste

Homogeneous Subsets

After Taste

Tukey HSD$_a$

		Subset for alpha = 0.05		
Sample	N	1	2	3
F	13	4.62		
E	13	4.77	4.77	
D	13		6.23	6.23
C	13			6.77
B	13			7.15
A	13			7.23
Sig.		1.000	0.094	0.454

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Table D.6 Sensory analysis of overall acceptance

Table D.6.1 Test of homogeneity of variances for overall acceptance

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Overall acceptance	Based on Mean	1.511	5	72	0.197
	Based on Median	1.038	5	72	0.402
	Based on Median and with adjusted df	1.038	5	58.651	0.404
	Based on trimmed mean	1.460	5	72	0.214

Table D.6.2 One-way ANOVA (No blocking) for overall acceptance

ANOVA					
Overall acceptance					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	127.333	5	25.467	28.928	0.000
Within Groups	63.385	72	0.880		
Total	190.718	77			

Table D.6.3 Homogeneous subsets for overall acceptance

Homogeneous Subsets

Overall acceptance

Tukey HSD$_a$

Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
F	13	4.23				
E	13	4.85	4.85			
D	13		5.92	5.92		
C	13			6.62	6.62	
B	13				7.38	7.38
A	13					7.77
Sig.		0.554	0.050	0.422	0.304	0.901

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 13.000.

Appendix E

ANOVA for physical characteristics of muffin

E.1 Physical characteristics of muffin in terms of weight

Table E.1.1 Test of homogeneity of variances for weight

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Weight	Based on Mean	0.269	5	12	0.922
	Based on Median	0.183	5	12	0.964
	Based on Median and with adjusted df	0.183	5	10.475	0.963
	Based on trimmed mean	0.263	5	12	0.925

Table E.1.2 One-way ANOVA (No blocking) for weight

ANOVA Weight					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.083	5	1.017	31.014	0.000
Within Groups	0.393	12	0.033		
Total	5.476	17			

Table E.1.3 Homogeneous subsets for weight

Homogeneous Subsets

Weight

Tukey HSDa

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
F	3	17.166667			
E	3	17.466667	17.466667		
D	3		17.866667	17.866667	
C	3			18.200000	18.200000
B	3				18.466667
A	3				18.666667
Sig.		0.381	0.145	0.283	0.070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

E.2 Physical characteristics of muffin in terms of volume

Table E.2.1 Test of homogeneity of variances for volume

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Volume	Based on Mean	0.269	5	12	0.922
	Based on Median	0.241	5	12	0.937
	Based on Median and with adjusted df	0.241	5	10.153	0.935
	Based on trimmed mean	0.267	5	12	0.922

Table E.2.2 One-way ANOVA (No blocking) for volume

ANOVA Volume					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	406.138	5	81.228	180.283	0.000
Within Groups	5.407	12	0.451		
Total	411.545	17			

Table E.2.3 Homogeneous subsets for volume

Homogeneous Subsets

Volume

Tukey HSD$_a$

		Subset for alpha = 0.05					
Sample	N	1	2	3	4	5	6
F	3	59.00000					
E	3		61.90000				
D	3			65.16667			
C	3				67.73333		
B	3					70.06667	
A	3						73.03333
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

E.3 Physical characteristics of muffin in terms of loaf volume

Table E.3.1 Test of homogeneity of variances for loaf volume

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Specific loaf volume	Based on Mean	1.100	5	12	0.410
	Based on Median	0.243	5	12	0.936
	Based on Median and with adjusted df	0.243	5	8.909	0.933
	Based on trimmed mean	0.996	5	12	0.460

Table E.3.2 One-way ANOVA (No blocking) for loaf volume

ANOVA Specific loaf volume					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.440	5	0.088	791.120	0.000
Within Groups	0.001	12	0.000		
Total	0.441	17			

Table E.3.3 Homogeneous subsets for loaf volume

Homogeneous Subsets

Specific loaf volume

Tukey HSD_a

		Subset for alpha = 0.05					
Sample	N	1	2	3	4	5	6
F	3	3.4367					
E	3		3.5433				
D	3			3.6467			
C	3				3.7233		
B	3					3.7933	
A	3						3.9100
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Color Plates

Plate 1: Baking of muffin

Plate 2: Fermented residue and raw millet flour

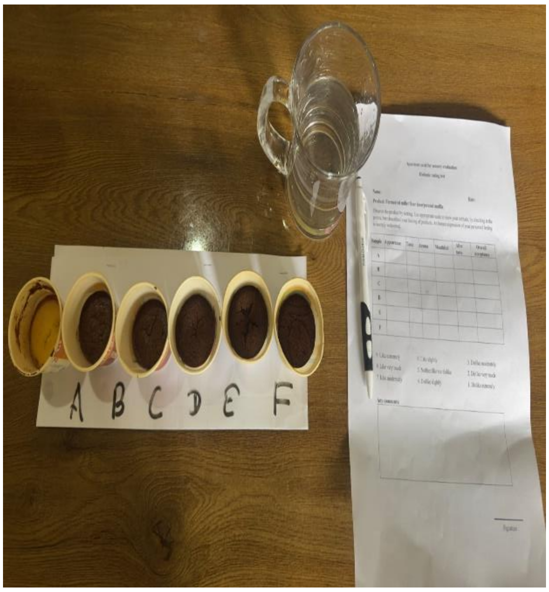


Plate 3: Ready for sensory

Plate 4: Sensory analysis by panelist

Plate 5: Analysis of sample