EFFECT OF BITTER LEAF ON NUTRITIONAL COMPOSITION AND SHELF LIFE OF OGI

By

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CERTIFICATION

This is to certify that this project was carried out by OGUNJOBI, Muminat Yetunde with matriculation number HND/23/SLT/FT/0488 submitted to the Department of Science Laboratory Technology, Microbiology Unit, Institute of Applied Science (IAS), Kwara State Polytechnic, Ilorin, in partial fulfillment for the requirements of the award of Higher National Diploma (HND) in Science Laboratory Technology (SLT).

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DEDICATION

This project is dedicated to the Almighty God for His guidance and strength throughout my academic journey. I also dedicate it to my loving parents and guardians for their constant support, prayers, and sacrifices.

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First and foremost, I give all glory to the Almighty God for His grace, strength, and wisdom throughout the course of this project and my academic journey.

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ABSTRACT

This study investigated the effect of Vernonia amygdalina (bitter leaf) extract on the nutritional composition, microbial stability, and shelf life of wet-milled sorghum under ambient conditions. The research was motivated by the high perishability of wet-milled sorghum and the health concerns associated with synthetic preservatives. An experimental design was employed where wet-milled sorghum samples were treated with varying concentrations (0.5g, 1.0g, and 1.25g) of bitter leaf extract, while untreated samples served as control. Proximate analysis, microbial count, Total Titratable Acidity (TTA), and sensory evaluation were conducted over a 28-day storage period. The results showed that the treated samples had improved protein and fiber content, significantly lower microbial loads, and slower increases in TTA compared to the control. Sensory attributes such as appearance, aroma, and texture were better preserved in the treated samples, although higher concentrations slightly affected taste. The study concludes that bitter leaf extract is an effective natural preservative that can enhance the nutritional and microbiological stability of wet-milled sorghum. It is recommended as a safe, affordable, and locally available alternative to chemical preservatives, particularly in regions with limited refrigeration.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Food preservation is a major concern in developing countries, where post-harvest losses of perishable agricultural products are high due to microbial contamination, enzymatic activities, and poor storage conditions (Adejumo et al., 2019). Sorghum (*Sorghum bicolor*), a widely cultivated cereal crop, plays a vital role in food security, particularly in Africa and Asia. It is processed into various traditional foods, including wet-milled products such as porridge, kunu, and burukutu. However, the high moisture content of wet-milled sorghum makes it susceptible to rapid spoilage, limiting its shelf life and nutritional quality (FAO, 2020).

Food spoilage is a major concern in the food industry, as it leads to nutritional losses, economic waste, and health hazards. Conventional preservation methods, such as refrigeration and the use of synthetic chemical preservatives, have been employed to extend the shelf life of food products (Adepoju & Akinyemi, 2019). However, these methods have limitations: refrigeration is expensive and requires stable electricity, while synthetic preservatives have been linked to potential health risks such as allergies, carcinogenic effects, and toxicity. As a result, there is a growing demand for natural, cost-effective, and safe alternatives to synthetic preservatives in food preservation (Eze & Onwuka, 2020).

The use of synthetic preservatives to extend the shelf life of food products has raised health and safety concerns, leading to increased interest in natural preservatives.

Vernonia amygdalina, commonly known as bitter leaf, has been recognized for its medicinal and antimicrobial properties. It contains bioactive compounds such as flavonoids, alkaloids, saponins, and tannins, which have been shown to exhibit antibacterial and antifungal effects. These properties suggest that bitter leaf extract could serve as a natural preservative to inhibit microbial growth and extend the shelf life of wet-milled sorghum without compromising its nutritional value (Oloyede et al., 2022). Sorghum (*Sorghum bicolor*) is a highly valuable cereal crop, especially in Africa, where it serves as a staple food for millions of people. It is rich in carbohydrates, fiber, protein, vitamins, and minerals, making it a crucial component of diets in many developing countries (FAO, 2020).

Sorghum (*Sorghum bicolor*) is one of the most important cereal crops in the world, ranking fifth after wheat, rice, maize, and barley in terms of production. It is widely cultivated in sub-Saharan Africa and Asia due to its ability to thrive in drought-prone regions. In Nigeria, sorghum is a staple food, used in the preparation of various traditional foods and beverages, such as ogi (fermented porridge), kunu (a non-alcoholic beverage), and burukutu (a traditional alcoholic drink) (Adebiyi et al., 2017). Despite its widespread consumption, one of the major challenges associated with sorghum processing is the short shelf life of its wet-milled form, which is highly perishable due to microbial activity, enzymatic degradation, and oxidation (Okafor et al., 2021).

One major challenge facing the food industry is the preservation of wet-milled products without compromising their nutritional quality. Traditional preservatives, such as chemical additives, are often used to extend shelf life, but concerns about their potential

health risks have driven interest in natural alternatives. Furthermore research reveal that the traditional preservation method of consistence removal of con steep liquor reduces nutritional quality of wet-milled corn leading to malnutrition. (Dykes & Rooney, 2020). Plants with bioactive compounds, such as bitter leaf (*Vernonia amygdalina*), have gained attention for their antimicrobial and antioxidant properties (Ezekwesili et al., 2020).

Bitter leaf is widely known for its medicinal value and is commonly used in traditional African medicine for treating infections, diabetes, and digestive disorders. Studies have shown that it contains phytochemicals such as flavonoids, alkaloids, saponins, and tannins, which possess antimicrobial properties that may help control microbial growth in food products. The incorporation of bitter leaf extract in wet-milled sorghum could potentially improve its shelf life while maintaining its nutritional value (Oloyede et al., 2022).

Traditionally, bitter leaf has been used for the treatment of malaria, diabetes, and gastrointestinal disorders, and recent studies suggest that it has significant antibacterial and antifungal effects against food spoilage organisms (Oloyede et al., 2022). These properties make it a promising candidate for enhancing the shelf life of perishable food products like wet-milled sorghum.

The use of plant-based preservatives aligns with the global trend toward sustainable and eco-friendly food preservation techniques. If bitter leaf extract proves effective in preserving wet-milled sorghum while maintaining its nutritional composition, it could provide a viable alternative to chemical preservatives, benefiting both food processors and consumers.

This study aims to investigate the effect of bitter leaf on the nutritional composition and shelf life of wet-milled sorghum. By exploring the use of bitter leaf as a natural preservative, this research seeks to provide an alternative solution to reduce nutritional losses and ensuring food safety in the processing of sorghum-based products in traditional method of preservation.

1.2 STATEMENT OF THE PROBLEM

Wet-milled sorghum is highly susceptible to spoilage due to microbial growth, enzymatic reactions, and environmental factors, leading to rapid deterioration in quality. Conventional preservatives used in food preservation often contain synthetic chemicals, some of which pose health risks to consumers (Gyawali & Ibrahim, 2018).

Additionally, there is limited research on the application of natural plant extracts, such as bitter leaf, in extending the shelf life of wet-milled sorghum while preserving its nutritional value (Atawodi, 2020). This study seeks to address this gap by evaluating the effectiveness of bitter leaf extract as a natural preservative and its impact on the nutritional composition of sorghum.

1.3 JUSTIFICATION OF THE STUDY

By exploring the effect of bitter leaf on the nutritional composition and shelf life of wet-milled sorghum, this study aims to provide a natural and practical solution to food preservation challenges, benefiting both the food industry and consumers.

The findings from this study will contribute to the development of natural food preservation techniques that can replace synthetic additives and traditional preservation technique. It will provide a scientific basis for the use of bitter leaf extract as a natural

preservative in wet-milled sorghum. The study may benefit food processors, nutritionists, and consumers by offering a cost-effective and healthier method of improving the shelf life of wet-milled sorghum. (Adebiyi et al., 2017). The research aligns with sustainable food processing methods by exploring plant-based preservatives that are eco-friendly and safe for human consumption.

1.4 AIM OF THE STUDY

The aim of this study is to evaluate the effect of bitter leaf (*Vernonia amygdalina*) on the nutritional composition and shelf life of wet-milled sorghum.

1.5 OBJECTIVES OF THE STUDY

The specific objectives of this study are to:

1. Determine the nutritional composition of wet-milled sorghum treated with and without bitter leaf (*Vernonia amygdalina*) extract.
2. Assess the effect of bitter leaf extract on the microbial load of wet-milled sorghum during storage.
3. Evaluate the impact of bitter leaf extract on the shelf life and storage stability of wet-milled sorghum under ambient conditions.
4. Measure the Total Titratable Acidity (TTA) of treated and untreated wet-milled sorghum samples as an indicator of fermentation or spoilage.
5. Compare the preservative effectiveness of bitter leaf supplement with traditional method of preservation methods in maintaining sorghum quality.

1.6 LITERATURE REVIEW

A literature review provides an overview of existing research and theories relevant to a study. This chapter examines previous findings on sorghum, bitter leaf (*Vernonia amygdalina*), and their effects on nutritional composition and shelf life in food preservation.

1.7 OVERVIEW OF SORGHUM AND ITS USES

Sorghum (*Sorghum bicolor*) is one of the most important cereal crops globally, ranking fifth in production after wheat, rice, maize, and barley. It is widely cultivated in Africa, Asia, and parts of the Americas, primarily due to its adaptability to harsh environmental conditions such as drought and poor soil fertility. In Nigeria and other West African countries, sorghum is a staple food crop, playing a crucial role in food security and economic development (Adebiyi et al., 2017).

Sorghum has various cultivated varieties, including:

- Red sorghum: Rich in antioxidants and used for brewing.
- White sorghum: Commonly used in food production.
- Brown sorghum: Known for its high tannin content.

It is believed to have originated in northeastern Africa, especially Ethiopia and Sudan.

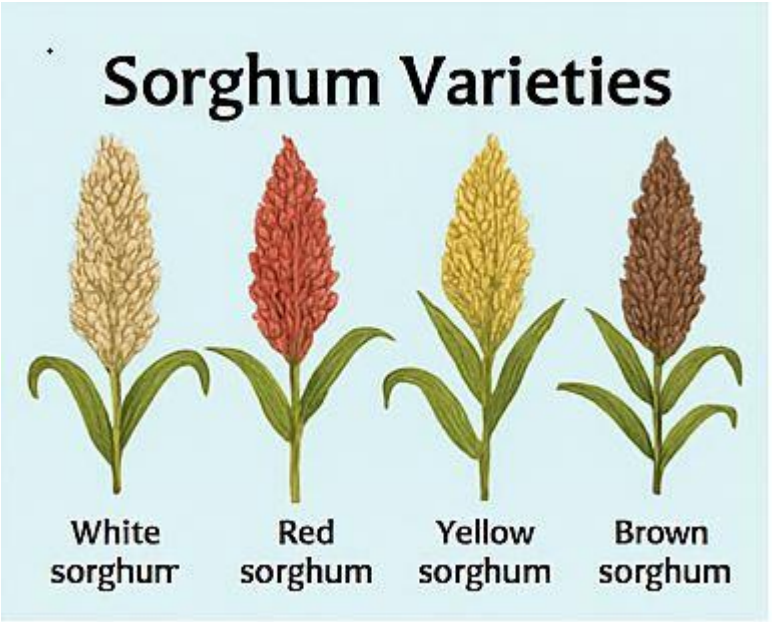

Fig. 2.1: Sorghum varieties.

1.7.1 Traditional and Industrial Uses of Sorghum

Sorghum has diverse applications in food, beverages, and industrial processing.

A. Food Uses

1. **Traditional Foods**: Sorghum is widely used in preparing local foods such as:
   - **Ogi (fermented porridge)** – a common breakfast meal in Nigeria.
   - **Tuwon dawa (sorghum paste)** – a popular staple in Northern Nigeria.
   - **Kunu (fermented sorghum drink)** – a non-alcoholic beverage.
   - **Burukutu and pito** – traditional alcoholic beverages made from fermented sorghum.
2. **Bakery and Confectionery**: Sorghum flour is used as a gluten-free alternative in bread, cakes, and pastries, making it beneficial for individuals with gluten intolerance or celiac disease (Taylor & Kruger, 2020).

3. **Fortified Food Products**: Due to its high protein and mineral content, sorghum is used in complementary foods for infants and in fortified foods for malnourished populations (Obilana & Manyasa, 2019).

B. Industrial and Non-Food Uses

i. **Beverage Production**: Sorghum serves as a key ingredient in brewing alcoholic and non-alcoholic beverages. In Africa, local breweries use sorghum for beer production, while in some Western countries, sorghum is used in gluten-free beer.

ii. **Biofuel and Ethanol Production**: Sorghum starch is fermented to produce bioethanol, a renewable energy source (Ratnavathi et al., 2018). iii. **Animal Feed**: Sorghum grains and by-products from processing are used as livestock feed due to their high energy content (Reddy et al., 2019).

1.7.2 Challenges Associated with Sorghum Processing

Despite its benefits, sorghum faces several challenges during processing and storage:

i. **Short Shelf Life**: Wet-milled sorghum is highly perishable due to microbial contamination and enzymatic activities.

ii. **Antinutritional Factors**: Sorghum contains tannins and phytic acid, which can reduce nutrient bioavailability (Dlamini et al., 2020).

iii. **Processing Difficulties**: The absence of gluten makes sorghum-based bakery products less elastic and difficult to process compared to wheat-based products (Elkhalifa & Bernhardt, 2019).

The need for effective preservation methods, such as the use of natural preservatives like bitter leaf extract, is crucial to overcoming these challenges and ensuring the long-term stability of wet-milled sorghum.

1.8 NUTRITIONAL COMPOSITION OF SORGHUM

Sorghum (*Sorghum bicolor*) is a highly nutritious cereal grain that serves as a staple food in many parts of the world. It is particularly valued for its balanced composition of macronutrients and micronutrients. On average, sorghum contains approximately 70–75% carbohydrates, 8–12% protein, 2–5% fat, and 1–3% dietary fiber. It is also a good source of essential minerals such as iron, zinc, phosphorus, and magnesium, and provides significant amounts of B-vitamins, including niacin (B3), thiamine (B1), and riboflavin (B2) (Rooney & Waniska, 2019).

In addition to these nutrients, sorghum contains a wide array of bioactive compounds such as phenolic acids, flavonoids, and tannins, which contribute to its antioxidant activity and health-promoting properties (Dykes & Rooney, 2020).

Table 1.1: Nutritional Composition of Sorghum

Nutrient	Typical Range (%) or mg/100g	Function / Significance
Carbohydrates	70–75%	Primary energy source
Protein	8–12%	Supports growth and tissue repair
Fat	2–5%	Provides essential fatty acids and energy
Dietary Fiber	1–3%	Aids digestion and improves gut health
Iron	4.1 mg	Essential for oxygen transport in the blood
Zinc	1.9 mg	Supports immune function
Phosphorus	287 mg	Important for bone health and energy metabolism
Magnesium	165 mg	Regulates muscle and nerve function
Thiamine (Vitamin B1)	0.38 mg	Aids in energy metabolism
Riboflavin (Vitamin B2)	0.14 mg	Helps in cell function and energy production
Niacin (Vitamin B3)	2.9 mg	Supports skin health and nervous system function
Phenolic Compounds (e.g. tannins, flavonoids)	Varies by variety	Antioxidant and anti-inflammatory properties

1.8.1 Macronutrient Composition

Sorghum is primarily composed of carbohydrates, proteins, lipids, and dietary fiber, which contribute to its role as an energy-dense food.

1. Carbohydrates: Sorghum is rich in carbohydrates, primarily in the form of starch, which accounts for 70-75% of its dry weight. The starch composition includes both amylose and amylopectin, with variations influencing digestibility and functional properties (Rooney & Waniska, 2019). Sorghum has a lower glycemic index than some other cereals, making it suitable for diabetic diets (Oladiran et al., 2020).

2. Protein: The protein content of sorghum ranges from 8-12%, depending on the variety and environmental factors (Dykes & Rooney, 2020). Sorghum proteins are mainly composed of kafirins, glutelins, and albumins, with kafirins being the dominant storage protein (Obilana & Manyasa, 2019). However, sorghum proteins have lower digestibility compared to wheat or maize due to the presence of cross-linked prolamins, which reduce protein bioavailability (Elkhalifa & Bernhardt, 2019).

3. Lipids (Fats): Sorghum contains 2-5% lipids, including essential fatty acids such as linoleic acid and oleic acid. The lipid content contributes to the energy value of sorghum and affects its shelf life due to lipid oxidation (Adebo et al., 2021).

4. Dietary Fiber: Sorghum is a good source of dietary fiber, particularly insoluble fiber, which aids in digestion and promotes gut health. The fiber content ranges from 1-3%, with whole-grain sorghum having higher fiber levels than refined sorghum flour (Taylor

& Kruger, 2020).

1.8.2 Micronutrient Composition

Sorghum provides essential vitamins and minerals that are important for overall health.

1. Minerals: Sorghum is rich in minerals such as:

i. **Iron (Fe):** Essential for oxygen transport in the blood and prevention of anemia.

ii. **Zinc (Zn):** Important for immune function and enzymatic activities. iii. **Phosphorus (P):** Plays a role in bone formation and energy metabolism. iv. **Magnesium (Mg):** Supports muscle and nerve function.

v. **Calcium (Ca):** Important for bone health, though present in lower amounts compared to other cereals (Dlamini et al., 2020).

However, the bioavailability of these minerals is often reduced due to the presence of phytic acid, which can inhibit mineral absorption (Elkhalifa & Bernhardt, 2019).

Processing methods such as fermentation and malting can help reduce phytic acid levels and improve mineral availability (Obilana & Manyasa, 2019).

2. Vitamins: Sorghum contains various B-complex vitamins, including:

i. **Niacin (B3):** Supports metabolism and skin health.

ii. **Thiamine (B1):** Important for energy production and nervous system function.

iii. **Riboflavin (B2):** Plays a role in growth and cellular function (Adebo et al., 2021).

1.8.3 Bioactive Compounds and Antioxidants

Sorghum is unique among cereals due to its high content of bioactive compounds and antioxidants, which contribute to its health benefits.

1. Phenolic Compounds: Sorghum contains a high concentration of polyphenols, flavonoids, and tannins, which have been linked to antioxidant, anti-inflammatory, and anti-cancer properties (Dykes & Rooney, 2020). These compounds help protect cells from oxidative damage and reduce the risk of chronic diseases such as diabetes and cardiovascular disorders.

2. Tannins and Their Effects: Some varieties of sorghum contain significant amounts of tannins, which can act as natural preservatives due to their antimicrobial properties. However, tannins can also reduce protein digestibility by binding to proteins and inhibiting enzyme activity (Oladiran et al., 2020). Low-tannin sorghum varieties are

often preferred for human consumption, while high-tannin varieties are used for animal feed and brewing.

1.8.4 Effect of Processing on Nutritional Composition

Different processing methods can alter the nutritional composition of sorghum:

i. **Milling:** Reduces fiber and micronutrient content but improves digestibility.
ii. **Fermentation:** Enhances bioavailability of minerals by reducing phytic acid content.
iii. **Germination and Malting:** Increase vitamin content, particularly vitamin C and B-complex vitamins (Obilana & Manyasa, 2019).
iv. **Cooking and Extrusion:** Improve protein digestibility but may reduce heatsensitive vitamins.

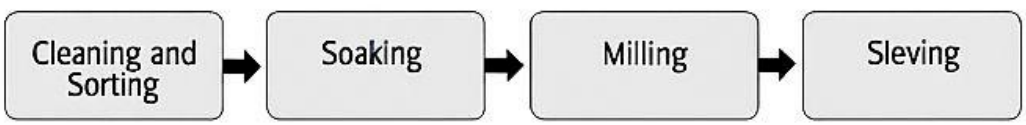

Fig. 1.2: Flowchart on wet-milled sorghum production

1.8.5 Comparison of Sorghum with Other Cereal Grains

Compared to other grains like maize, wheat, and rice, sorghum offers several advantages:

i. Higher antioxidant content than maize and rice (Dykes & Rooney, 2020).
ii. Gluten-free nature, making it suitable for people with celiac disease.
iii. Drought resistance, making it a more sustainable crop in arid regions.

However, sorghum also has limitations such as lower protein digestibility and higher levels of anti-nutritional factors compared to wheat and maize.

1.9 BITTER LEAF (*VERNONIA AMYGDALINA*): COMPOSITION AND HEALTH BENEFITS

Bitter leaf (*Vernonia amygdalina*) is a widely consumed leafy vegetable and medicinal plant in Africa, particularly in Nigeria and other West African countries. It belongs to the Asteraceae family and is known for its characteristic bitter taste, which is attributed to its rich phytochemical content. Bitter leaf is traditionally used in food preparation, herbal medicine, and as a natural preservative due to its antimicrobial and antioxidant properties (Omoregie & Osagie, 2020).

Fig. 1.3: *Vernonia Amygdalina* (Bitter Leaf) (Omoregie & Osagie, 2020).

1.9.1 Phytochemical Composition of Bitter Leaf

Bitter leaf contains a wide range of bioactive compounds that contribute to its medicinal and preservative effects. These include:

1. Alkaloids: Alkaloids are nitrogen-containing compounds known for their antimicrobial and anti-inflammatory properties. In bitter leaf, they play a role in inhibiting bacterial and fungal growth, making the plant useful in food preservation (Egharevba & Kunle, 2019).

2. Flavonoids: Flavonoids are powerful antioxidants that protect cells from oxidative stress and free radicals. Studies suggest that bitter leaf flavonoids can help improve immune function, reduce inflammation, and enhance the shelf life of perishable foods (Akinmoladun et al., 2021).

3. Saponins: Saponins are natural surfactants with antimicrobial, antifungal, and antiinflammatory properties. They have been found to inhibit the growth of spoilage microorganisms, which supports the hypothesis that bitter leaf extract may extend the shelf life of wet-milled sorghum (Udochukwu et al., 2020).

4. Tannins: Tannins are polyphenolic compounds with strong antimicrobial effects. They work by binding to bacterial and fungal proteins, preventing microbial growth and food spoilage (Ogunmoyole et al., 2018). Tannins also contribute to the bitterness of the leaf, which may impact the sensory qualities of sorghum when used as a preservative.

5. Terpenoids: Terpenoids, such as sesquiterpene lactones, are responsible for the bitter taste of *Vernonia amygdalina*. They have antimicrobial, anticancer, and

antiinflammatory properties, making them valuable in both medicine and food preservation (Oboh et al., 2022).

6. **Phenolic Compounds:** Bitter leaf is rich in phenolic acids and polyphenols, which act as natural antioxidants. These compounds help delay lipid oxidation in food, thereby improving shelf stability and preventing rancidity (Adebayo-Tayo et al., 2019).

1.9.2 Nutritional Composition of Bitter Leaf

Bitter leaf is a nutrient-dense plant that provides essential vitamins and minerals.

1. Macronutrients

- **Protein (4-6%)**: Contains essential amino acids needed for body function.
- **Carbohydrates (8-12%)**: Provides energy.
- **Dietary fiber (10-15%)**: Aids digestion and promotes gut health.
- **Fats (2-4%)**: Includes beneficial unsaturated fats (Ogunmoyole et al., 2018).

2. Micronutrients

- **Vitamin A**: Supports vision and immune function.
- **Vitamin C**: Acts as an antioxidant and boosts immunity.
- **Vitamin E**: Protects cells from oxidative damage.
- **Iron (Fe)**: Essential for red blood cell formation.
- **Calcium (Ca)**: Supports bone health.
- **Magnesium (Mg)**: Helps with muscle and nerve function (Akinmoladun et al., 2021).

1.9.3 Health Benefits of Bitter Leaf

1. Antimicrobial Properties: Several studies have shown that bitter leaf extract has strong antibacterial and antifungal activities against foodborne pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* (Egharevba & Kunle, 2019). This suggests its potential use as a natural food preservative.

2. Antioxidant Activity: The high polyphenol and flavonoid content of bitter leaf helps neutralize free radicals, reducing oxidative stress and preventing chronic diseases such as cancer and cardiovascular diseases (Oboh et al., 2022).

3. Blood Sugar Regulation: Bitter leaf has been widely used in traditional medicine for managing diabetes. It contains saponins and alkaloids, which help reduce blood glucose levels by enhancing insulin sensitivity (Udochukwu et al., 2020).

4. Anti-inflammatory and Immune-Boosting Effects: Bitter leaf has anti-inflammatory properties, making it useful in treating conditions such as arthritis, fever, and infections. Its immune-boosting effects are linked to its high vitamin and flavonoid content (Adebayo-Tayo et al., 2019).

5. Liver and Kidney Protection: Bitter leaf has been found to protect the liver and kidneys from damage caused by toxins. This hepatoprotective effect is attributed to its high antioxidant and detoxifying compounds (Akinmoladun et al., 2021).

1.9.4 Potential Use of Bitter Leaf as a Natural Preservative

Due to its antimicrobial, antioxidant, and antifungal properties, bitter leaf extract has been proposed as a natural preservative for food products. Studies suggest that it can:

- Reduce microbial spoilage in perishable foods.

- Enhance the shelf life of wet-milled sorghum by preventing fermentation and bacterial contamination.
- Improve food safety by reducing the presence of foodborne pathogens (Oboh et al., 2022).

1.9.5 Challenges in Using Bitter Leaf in Food Preservation

While bitter leaf has strong preservative potential, there are some challenges associated with its application:

1. **Bitterness**: The high concentration of alkaloids and terpenoids may affect the taste and acceptability of treated food products.
2. **Stability of Bioactive Compounds**: Processing methods such as drying and heat treatment can degrade some of the beneficial compounds.
3. **Standardization of Extracts**: The concentration of active compounds may vary depending on the method of extraction and plant source.
4. **Interaction with Food Components**: Some bioactive compounds may interact with proteins and carbohydrates, affecting food texture and nutritional quality (Adebayo-Tayo et al., 2019).

1.10 EFFECTS OF BITTER LEAF ON FOOD PRESERVATION AND NUTRITIONAL ENHANCEMENT

Bitter leaf (*Vernonia amygdalina*) has gained attention in food science due to its preservative properties and nutritional benefits. Its rich phytochemical composition, including antioxidants, antimicrobials, and bioactive compounds, makes it a promising

natural additive for extending shelf life and enhancing the nutritional value of food products (Adebayo-Tayo et al., 2019).

1.10.1 Bitter Leaf as a Natural Food Preservative

The preservation of food products is a critical concern in food processing, particularly for perishable items like wet-milled sorghum. Bitter leaf has been investigated for its ability to inhibit microbial growth, delay oxidation, and maintain food quality over time (Egharevba & Kunle, 2019).

1. Antimicrobial Effects: One of the most significant properties of bitter leaf in food preservation is its antimicrobial activity. The bioactive compounds present in bitter leaf, such as alkaloids, flavonoids, tannins, and saponins, have been shown to inhibit the growth of foodborne pathogens and spoilage organisms, including:

- *Escherichia coli* (causes food poisoning)
- *Staphylococcus aureus* (causes foodborne infections)
- *Aspergillus niger* (a spoilage mold)
- *Pseudomonas aeruginosa* (spoilage bacterium in wet foods) (Oboh et al., 2022) These antimicrobial effects can significantly reduce microbial contamination in wetmilled sorghum, thereby extending its shelf life and ensuring food safety.

2. Antioxidant Effects and Lipid Oxidation Prevention: Oxidation is a primary cause of food deterioration, leading to:

- Rancidity in stored grains
- Nutrient loss
- Changes in color, flavor, and texture

Bitter leaf contains polyphenols, flavonoids, and vitamin C, which are potent antioxidants. These compounds neutralize free radicals and slow down the oxidation process, thereby preventing lipid degradation and extending the shelf life of perishable food products (Akinmoladun et al., 2021).

3. **pH Modulation and Fermentation Control:** Wet-milled sorghum is prone to fermentation due to microbial activity. Bitter leaf extract has been found to lower pH levels, creating an environment that inhibits the growth of spoilage microorganisms. This property makes it useful in controlling unwanted fermentation, which can lead to food spoilage (Omoregie & Osagie, 2020).

1.10.2 Bitter Leaf's Role in Nutritional Enhancement

Beyond preservation, bitter leaf also contributes to enhancing the nutritional composition of food products by supplying essential nutrients and bioactive compounds.

1. Enrichment with Vitamins and Minerals

Bitter leaf is rich in:

- **Vitamin C** (boosts immunity, acts as an antioxidant)
- **Vitamin A** (important for vision and skin health)
- **Calcium and Magnesium** (essential for bone and muscle function)
- **Iron** (supports blood formation and prevents anemia)

When added to wet-milled sorghum, bitter leaf extract can increase the micronutrient content, improving the overall dietary value of the food product (Adebayo-Tayo et al., 2019).

2. **Enhancement of Protein Content:** Sorghum is a staple grain but is relatively low in protein quality due to its limited lysine content. Bitter leaf contains moderate amounts of protein and may serve as a complementary source of amino acids, improving the protein balance of sorghum-based diets (Udochukwu et al., 2020).

3. **Contribution to Dietary Fiber:** Bitter leaf is high in dietary fiber, which aids digestion and promotes gut health. Adding bitter leaf extract to wet-milled sorghum can:

- Improve digestion and prevent constipation
- Support gut microbiota balance
- Reduce the risk of metabolic disorders such as diabetes (Ogunmoyole et al., 2018)

4. **Blood Sugar Regulation and Anti-Diabetic Effects:** Bitter leaf has been reported to help in regulating blood glucose levels due to its saponins and alkaloids. Incorporating it into sorghum-based foods may provide added benefits for people managing diabetes or metabolic conditions (Oboh et al., 2022).

1.10.3 Challenges in Using Bitter Leaf for Food Preservation and Nutritional Enhancement

Despite its potential, some challenges must be considered when using bitter leaf as a food additive:

1. **Bitterness and Consumer Acceptability:** The strong bitter taste of *Vernonia amygdalina* may affect the palatability of food products. Strategies such as controlled extraction, processing methods, and blending with other ingredients can help improve acceptability (Omoregie & Osagie, 2020).

2. **Stability of Bioactive Compounds:** Some of the beneficial phytochemicals in bitter leaf are heat-sensitive and may degrade during food processing. Optimizing extraction and drying methods is necessary to preserve its functional properties (Akinmoladun et al., 2021).

3. **Interaction with Food Components:** Bitter leaf compounds may interact with other food components, affecting texture, color, and shelf stability. Further research is needed to determine optimal concentration levels for use in sorghum preservation (Adebayo-

Tayo et al., 2019).

1.11 MICROBIAL ACTIVITIES IN WET-MILLED SORGHUM

Wet-milled sorghum is widely used in the production of various traditional foods and beverages, particularly in Africa. However, due to its high moisture content and nutrient composition, it is highly susceptible to microbial contamination, which can lead to spoilage, reduced shelf life, and potential health risks (Adebayo-Tayo et al., 2019). Understanding the microbial activities in wet-milled sorghum is essential for improving its preservation, safety, and quality.

1.11.1 Common Microorganisms in Wet-Milled Sorghum

Several microorganisms, including bacteria, fungi, and yeasts, can proliferate in wetmilled sorghum. These microbes are primarily introduced through raw materials, processing equipment, water, and storage conditions (Egharevba & Kunle, 2019).

1. Bacteria

i. **Lactic Acid Bacteria (LAB):** *Lactobacillus* spp., *Pediococcus* spp., and

Leuconostoc spp.

- These bacteria are beneficial as they produce lactic acid, which lowers the pH and inhibits spoilage organisms.
- They play a key role in fermentation processes such as in the production of ogi (fermented sorghum porridge) (Omoregie & Osagie, 2020).

ii. **Enterobacteriaceae:** *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp.

- These bacteria indicate fecal contamination and pose serious health risks if present in food.
- They can survive due to poor hygiene practices or contaminated water sources (Oboh et al., 2022).

iii. **Pseudomonas spp.:**

- Opportunistic spoilage bacteria that can break down proteins and carbohydrates, leading to off-flavors and texture degradation.
- Often associated with extended storage periods at improper temperatures (Akinmoladun et al., 2021).

2. Fungi (Molds and Yeasts)

i. **Molds:** *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp.

- These molds produce mycotoxins, which are toxic secondary metabolites that can contaminate sorghum.
- Mycotoxins such as aflatoxins and fumonisins pose serious health hazards, including liver damage and cancer risk (Udochukwu et al., 2020).

ii. **Yeasts:** *Saccharomyces cerevisiae*, *Candida* spp.

- Some yeasts are beneficial for fermentation, while others can cause spoilage by producing off-flavors and gas formation.
- Uncontrolled yeast growth can lead to excessive fermentation, making the product unpalatable (Adebayo-Tayo et al., 2019).

1.11.2 Factors Influencing Microbial Growth in Wet-Milled Sorghum

The rapid microbial activity in wet-milled sorghum is influenced by several factors:

1. Moisture Content

- The high moisture level in wet-milled sorghum creates an ideal breeding ground for microbes.
- Reducing moisture through drying or refrigeration can slow down microbial growth (Oboh et al., 2022).

2. pH Levels

- Many spoilage microorganisms thrive in neutral to slightly acidic pH (pH 5.5-7.0).
- Lactic acid bacteria (LAB) help lower the pH, making conditions unfavorable for pathogens and spoilage organisms (Omoregie & Osagie, 2020).

3. Temperature

- High temperatures (above 45°C) destroy most microbes, while cold storage (below 10°C) slows down microbial activity.
- Room temperature storage (25-35°C) supports rapid microbial growth, leading to spoilage (Akinmoladun et al., 2021).

4. Hygiene and Processing Conditions

- Contaminated water, dirty milling equipment, and poor handling practices introduce harmful microbes.
- Proper sanitation, sterilization, and use of antimicrobial additives can help minimize contamination risks (Udochukwu et al., 2020).

5. Storage and Packaging

- Exposure to air and humidity accelerates microbial spoilage.
- Vacuum-sealed, airtight, or refrigerated storage can limit microbial growth and extend shelf life (Egharevba & Kunle, 2019).

1.11.3 Effects of Microbial Activities on Wet-Milled Sorghum

1. Spoilage and Sensory Changes

- Off-flavors and sour taste due to microbial fermentation.
- Slimy or sticky texture caused by bacterial activity.
- Mold growth leading to visible discoloration and spoilage (Adebayo-Tayo et al., 2019).

2. Nutrient Degradation

- Carbohydrate breakdown leads to excessive fermentation, producing acidic byproducts.
- Protein degradation results in the release of ammonia and foul odors.
- Vitamin loss due to enzymatic reactions and microbial metabolism (Oboh et al., 2022).

3. Food Safety Risks

- Presence of pathogenic bacteria like *Salmonella* and *E. coli* increases the risk of foodborne illnesses.
- Production of mycotoxins from molds can have long-term health effects, including liver damage and immune suppression (Omoregie & Osagie, 2020).

1.11.4 Measures for Microbial Contamination in Wet-Milled Sorghum

To reduce microbial activities and enhance the safety of wet-milled sorghum, several preservation techniques can be applied:

1. Use of Natural Antimicrobials (e.g., Bitter Leaf Extract)

- Bitter leaf (*Vernonia amygdalina*) has been found to have strong antimicrobial effects against spoilage bacteria and fungi.
- It contains flavonoids, saponins, tannins, and alkaloids, which inhibit the growth of foodborne pathogens (Egharevba & Kunle, 2019).

2. Fermentation for Preservation

- Controlled fermentation using lactic acid bacteria (LAB) lowers pH and prevents spoilage.
- Fermented products like ogi and burukutu have extended shelf lives due to their acidic environment (Akinmoladun et al., 2021).

3. Proper Storage Techniques

- Refrigeration (4°C or lower) slows microbial growth.
- Drying and dehydration reduce water activity, preventing microbial proliferation.

- Vacuum-sealed or airtight packaging minimizes exposure to contaminants (Oboh, et al., 2022).

4. Heat Treatment (Pasteurization or Boiling)

- Mild heat treatment (60-80°C) can kill most spoilage organisms.
- However, excessive heat can destroy nutrients and affect food quality (Udochukwu et al., 2020).

5. Good Manufacturing Practices (GMP)

- Use of clean water and sanitized milling equipment.
- Proper handling and packaging to prevent cross-contamination.
- Regular microbial testing to monitor food safety (Omoregie & Osagie, 2020).

1.12 FACTORS AFFECTING THE SHELF LIFE OF WET-MILLED SORGHUM

Shelf life is a critical factor in food storage and preservation, particularly for perishable products like wet-milled sorghum. Due to its high moisture content, wet-milled sorghum is highly susceptible to spoilage and microbial contamination, which can significantly impact its nutritional quality, safety, and acceptability. Various factors influence its shelf life, including environmental conditions, microbial activities, packaging methods, and storage practices (Adebayo-Tayo et al., 2019).

1.12.1 Moisture Content and Water Activity

i. High moisture levels in wet-milled sorghum create an ideal environment for microbial growth and enzymatic reactions.

ii. The presence of free water (high water activity, Aw) accelerates spoilage caused by bacteria, yeasts, and molds (Omoregie & Osagie, 2020).

Strategies to control moisture:

- Dehydration or drying to reduce water content.
- Cold storage to slow down microbial metabolism.

1.12.2 Microbial Contamination

i. **Bacteria:** Pathogenic species such as *Escherichia coli*, *Salmonella* spp., and *Pseudomonas* spp. contribute to spoilage and potential foodborne illnesses.

ii. **Fungi:** Molds like *Aspergillus* spp. and *Penicillium* spp. produce mycotoxins, which compromise food safety.

iii. **Yeasts:** Can cause unwanted fermentation, leading to off-flavors and changes in texture (Egharevba & Kunle, 2019).

Control measures:

- Use of natural antimicrobials like bitter leaf extract to inhibit microbial growth.
- Good hygiene practices to prevent contamination during processing and storage.

1.12.3 Temperature and Storage Conditions

i. High temperatures (above 30°C) accelerate microbial growth and enzymatic degradation.

ii. Low temperatures (below 10°C) slow down spoilage, extending shelf life.

iii. Fluctuating temperatures can lead to condensation, increasing moisture levels and microbial activity (Oboh et al., 2022).

Optimal storage:

- Refrigeration (4°C or lower) to maintain quality.
- Drying or partial dehydration to extend shelf stability.

1.12.4 pH and Acidity Levels

i. Lower pH (acidic conditions) inhibits spoilage organisms and extends shelf life.

ii. Lactic acid bacteria (LAB) in fermented sorghum help reduce pH, making it less prone to contamination. iii. Alkaline conditions promote spoilage due to bacterial proliferation (Akinmoladun et al., 2021).

Solutions:

- Fermentation with LAB to naturally lower pH.
- Addition of natural preservatives like bitter leaf extract, which contains antimicrobial phytochemicals.

1.12.5 Oxygen Exposure and Oxidation

i. Exposure to oxygen promotes oxidation of lipids, leading to rancidity and nutrient loss.

ii. Aerobic bacteria and molds thrive in oxygen-rich environments, causing spoilage.

Prevention methods:

- Vacuum-sealed packaging to limit oxygen exposure.
- Antioxidant additives like vitamin C, flavonoids, and bitter leaf extract to reduce oxidation.

1.12.6 Packaging Materials and Techniques

i. Poor packaging exposes wet-milled sorghum to air, moisture, and contaminants, leading to spoilage.

ii. Biodegradable or porous packaging may not provide adequate protection.

Best practices for packaging:

- Airtight containers or vacuum-sealed packaging to prevent microbial entry.
- Plastic or glass storage containers to reduce moisture absorption.
- Modified atmosphere packaging (MAP) to control oxygen levels.

(Dykes & Rooney, 2020).

1.12.7 Chemical and Enzymatic Reactions

i. Enzymes naturally present in sorghum can cause flavor deterioration, discoloration, and textural changes.

ii. Oxidative enzymes like polyphenol oxidase (PPO) can lead to browning.

Control methods:

- Blanching or mild heat treatment to inactivate enzymes.
- Natural preservatives like bitter leaf extract with antioxidant properties.

1.12.8 Presence of Natural Preservatives

i. Bitter leaf (*Vernonia amygdalina*) has antimicrobial and antioxidant properties that enhance shelf life.

ii. Bioactive compounds in bitter leaf (flavonoids, tannins, saponins) inhibit microbial growth and oxidative spoilage (Udochukwu et al., 2020).

Integrating bitter leaf extract into wet-milled sorghum can:

- Reduce microbial contamination.
- Slow down oxidation and rancidity.
- Improve food safety and shelf stability.

1.13 PREVIOUS STUDIES ON THE USE OF NATURAL PRESERVATIVES IN CEREAL PROCESSING

The use of natural preservatives in cereal processing has gained significant attention due to concerns about synthetic preservatives' health risks and environmental impact. Several studies have explored the effectiveness of plant extracts, organic acids, essential oils, and fermentation techniques in extending the shelf life, improving the nutritional quality, and ensuring the safety of processed cereals, including sorghum, maize, wheat, and rice.

1.13.1 Plant Extracts as Natural Preservatives

Plant extracts contain bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, which have antimicrobial, antioxidant, and antifungal properties (Egharevba & Kunle, 2019). These compounds inhibit microbial growth and delay spoilage in processed cereals.

1. Bitter Leaf (*Vernonia amygdalina*)

Adebayo-Tayo et al. (2019) investigated the antimicrobial effects of bitter leaf extract on wet-milled maize and sorghum. Results showed that the extract inhibited Salmonella spp., *E. coli*, and *Aspergillus* spp., thereby extending the shelf life by 7–10 days under ambient conditions.

Udochukwu et al. (2020) reported that bitter leaf contains polyphenols and alkaloids that suppress oxidative degradation, maintaining cereal quality during storage.

2. Moringa (*Moringa oleifera*) Extract

Akinmoladun et al. (2021) studied moringa leaf extract as a preservative for fermented sorghum gruel (*ogi*). The study revealed that moringa extract significantly reduced mold and yeast counts, extending the shelf life by two weeks.

Oboh et al. (2022) found that moringa extract improved protein retention and reduced rancidity in stored wheat flour.

3. Clove (*Syzygium aromaticum*) and Ginger (*Zingiber officinale*) Extracts Egharevba & Kunle (2019) tested clove and ginger extracts on stored maize flour and found a 70% reduction in microbial growth after two months of storage.

Omoregie & Osagie (2020) demonstrated that clove extract inhibited the growth of aflatoxin-producing fungi in stored sorghum flour.

1.13.2 Organic Acids and Fermentation as Natural Preservatives

Organic acids such as lactic acid, citric acid, and acetic acid are commonly used to control microbial contamination in cereals. Fermentation, a traditional preservation method, produces these acids naturally and extends the shelf life of cereal-based foods.

1. Lactic Acid Bacteria (LAB) Fermentation

Omoregie & Osagie (2020) found that fermented sorghum ogi containing LAB had an extended shelf life of up to 30 days due to the lowered pH and inhibition of spoilage microbes.

Adebayo-Tayo et al. (2019) reported that Lactobacillus plantarum used in fermented wheat porridge enhanced preservation, texture, and taste, reducing spoilage bacteria.

2. Citric Acid and Vinegar

Akinmoladun et al. (2021) evaluated citric acid's effectiveness in preserving wet-milled maize and sorghum. Results showed a 50% reduction in bacterial counts after two weeks of storage.

Oboh et al. (2022) noted that vinegar (acetic acid) slowed down lipid oxidation in stored cereal flours, preventing rancidity.

1.13.3 Essential Oils as Antimicrobial Preservatives

Essential oils from plants have been found to be effective against foodborne pathogens and spoilage microorganisms in cereal processing.

1. Neem (*Azadirachta indica*) Oil

Udochukwu et al. (2020) studied the effect of neem oil on stored millet and sorghum grains. The oil prevented fungal growth and insect infestation for over three months. Oboh et al. (2022) reported that neem oil-treated sorghum showed better preservation than untreated samples, maintaining quality and freshness.

2. Cinnamon (*Cinnamomum verum*) and Thyme (*Thymus vulgaris*) Oils

Egharevba & Kunle (2019) demonstrated that cinnamon oil reduced mold growth in wheat and maize flour by 80% after four weeks.

Omoregie & Osagie (2020) found that thyme oil inhibited the growth of *Bacillus cereus* in stored cereal products.

1.13.4 Summary of Previous Studies

Natural Preservative	Cereal Type	Preservation Effect	Study
Bitter leaf extract (Vernonia amygdalina)	Wet-milled sorghum, maize	Extended shelf life by 7–10 days, inhibited spoilage bacteria and fungi	Adebayo-Tayo et al. (2019)
Moringa extract (Moringa oleifera)	Fermented sorghum (ogi)	Reduced mold and yeast, improved protein retention	Akinmoladun et al. (2021)
Clove & Ginger Extracts	Maize flour	70% reduction in microbial growth, prevented aflatoxin production	Egharevba & Kunle (2019)
Lactic Acid Bacteria (LAB) Fermentation	Sorghum ogi	pH reduction inhibited spoilage microbes, shelf life extended to 30 days	Omoregie & Osagie (2020)
Neem Oil (Azadirachta indica)	Sorghum, millet	Prevented fungal growth and insect infestation for 3+ months	Udochukwu et al. (2020)
Cinnamon & Thyme Oils	Wheat, maize	80% reduction in mold growth, inhibited Bacillus cereus	Egharevba & Kunle (2019)

1.14 THEORETICAL FRAMEWORK

A theoretical framework serves as the foundation for understanding the mechanisms through which bitter leaf (*Vernonia amygdalina*) affects the nutritional composition and shelf life of wet-milled sorghum. This study is guided by food preservation theories, antimicrobial action mechanisms, and nutrient stability principles to establish a scientific basis for the research.

1.14.1 Food Preservation Theories

1. Hurdle Technology Theory

Proposed by Leistner (2000), the Hurdle Technology Theory suggests that a combination of preservation techniques can effectively inhibit microbial growth, delay spoilage, and enhance food quality. This theory is relevant to this study as bitter leaf extract provides multiple hurdles through its antimicrobial, antioxidant, and enzymatic inhibition properties, which collectively help extend the shelf life of wet-milled sorghum.

Key hurdles provided by bitter leaf:

- Antimicrobial compounds (saponins, alkaloids)
- Antioxidant properties (flavonoids, phenolic compounds)
- pH modification (reducing spoilage via acidity changes)

2. Antimicrobial Action Mechanism (Target Site Theory)

The Target Site Theory explains how bioactive compounds from plants affect microbial cells. According to Lambert (2017), natural preservatives disrupt microbial cell membranes, inhibit enzyme activity, and interfere with DNA replication, leading to microbial death.

Bitter leaf contains flavonoids and tannins, which:

- Damage bacterial cell walls, preventing nutrient uptake.
- Bind to microbial proteins and enzymes, disrupting metabolism.
- Reduce microbial adhesion, preventing spoilage bacteria from colonizing wet-milled sorghum.

3. Natural Antioxidant Protection Theory

This theory suggests that antioxidants delay food oxidation, maintaining quality and nutritional value (Shahidi & Zhong, 2015).

Bitter leaf is rich in antioxidants, which:

- Prevent lipid oxidation, reducing rancidity in wet-milled sorghum.
- Preserve essential vitamins and proteins, ensuring nutritional stability.
- Maintain sensory qualities (color, taste, and texture) of the product.

1.14.2 Nutrient Stability and Shelf Life Theories

1. Water Activity and Microbial Growth Theory

According to Scott (1957), microbial spoilage is directly linked to water activity (Aw), where high moisture promotes microbial proliferation.

Bitter leaf extract may help reduce microbial growth by:

- Lowering Aw through interaction with food matrices.
- Creating an unfavorable pH for spoilage organisms.
- Enhancing protein stability in wet-milled sorghum.

2. Enzymatic Browning and Preservation Theory

Proposed by Mayer (2006), this theory explains how polyphenol oxidase (PPO) enzymes cause browning and degradation in stored foods.

Bitter leaf contains enzyme inhibitors, which:

- Reduce PPO activity, preventing browning.
- Delay starch breakdown, ensuring extended freshness.

CHAPTER TWO

MATERIAL AND METHODS

2.0 INTRODUCTION

This chapter outlines the materials, equipment, and experimental procedures used to investigate the effect of bitter leaf (*Vernonia amygdalina*) on the nutritional composition and shelf life of wet-milled sorghum. It includes details on the research design, sample preparation, data collection, and analytical techniques employed.

2.1 STUDY AREA

This study was conducted using raw white sorghum purchased from local market, at Oke Oyi Kwara State, Nigeria, and fresh bitter leaf obtained from Odo Ota in Ilorin.

Microbiological and Chemical analysis were conducted at Microbiology and Chemistry Laboratory of Kwara State Polytechnic and Central Research Laboratory of University of Ilorin Nigeria.

2.2 MATERIALS USED

2.2.1 Sample Collection

- Sorghum grains (*Sorghum bicolor*) was purchased from a local market, Oke Oyi Kwara State.
- Fresh bitter leaves (*Vernonia amygdalina*) was sourced from Odo Ota in Ilorin.

2.2.2 Chemicals and Reagents

- Ethanol (for sterilization)

- Analytical grade media used are; Nutrient Agar, MacConkey Agar, Yeast Extract, Sabouraud Dextrose Agar (SDA), de Man, Rogosa and Sharpe Agar (MRS), (for microbial cultivation and fungal growth analysis)
- Analytical-grade reagents for proximate analysis
- 0.1N Sodium hydroxide (NaoH), phenolphthalein indicator, distilled water.

2.2.3 Equipment

Petri-dishes, inoculating loops, refrigerator, incubators, hot air oven, test tube, beakers, comical flask, retort stand & burette clamp, burette, white tile, pipette, grinder, cooking pots, spoons & different containers for sampling.

2.2.4 Sample Collection

The sorghum sample was purchased from the market, placed in a clean, sterile polythene bag to prevent contamination, and transported to the Microbiology Laboratory for analysis.

2.3 SAMPLE PREPARATION

The sorghum sample was manually sorted to remove dirt and unwanted particles, while the bitter leaf was thoroughly washed with clean water to eliminate surface contaminants. The sorghum was then divided into three different containers with the following compositions:

- $S+BLC1^{-1}$: 308.5 g of sorghum + 0.7 g of bitter leaf
- $S+BLC2^{-2}$: 308.0 g of sorghum + 1.0 g of bitter leaf
- $S+BLC3^{-3}$: 307.5 g of sorghum + 1.25 g of bitter leaf

Each sample was soaked in 400 ml of sterile deionized water and allowed to ferment for 48 hours under ambient conditions.

Control Setup

Two additional control samples were prepared:

- Control 1: 310 g of sorghum soaked in deionized water
- Control 2: 310 g of sorghum soaked in distilled water

2.3.1 Milling of the Sample

After 48 hours of fermentation, the steeping water was decanted from each sample.

Additional bitter leaf was added in the same proportion as the initial setup:

- S+BLC1^{-1} : +0.7 g bitter leaf
- S+BLC2^{-2}: +1.0 g bitter leaf
- S+BLC3^{-3}: +1.25 g bitter leaf

The samples were then milled using 400ml of water. The control samples also had their steep water decanted before milling.

2.3.2 Decanting

50ml water was decanted from each samples every two days and 30ml of distilled water was added, including the control samples throughout the whole period of the practical.

2.4 STERILIZATION OF GLASSWARE

To ensure aseptic conditions, the workbench was sterilized with 70% ethanol before and after each use. All glassware, including Petri dishes, pipettes, test tubes, and conical flasks, were thoroughly washed and sterilized in a hot air oven at 160°C to 200°C. Wire

loops were flamed to red-hot and allowed to cool before use. Other plastics containers were washed with soap and rinsed with clean water.

2.5 PREPARATION OF MEDIA

All analytical grade media used were prepared according to the manufacturer's instructions and were sterilized by autoclaving at 121°C for 15 minutes before use.

2.6 MICROBIOLOGICAL ANALYSIS

2.6.1 Serial Dilution of Samples

1ml portion of each fermented Pap (Ogi) sample was mixed with 9ml of sterile distilled water in a test tube to create the stock solution. Four-fold serial dilution was carried out as follows:

1 ml of the stock solution was transferred into 9 ml of sterile distilled water, and this process was repeated to achieve a final dilution of 10^{-4}. From the 10^{-3}dilutions, 0.5 ml was inoculated into sterile Petri dishes. The appropriate media were poured into the Petri dishes and swirled to ensure even distribution of microorganisms.

This process was repeated every 7 days interval for first two weeks and 14 interval after the first two weeks. 50ml of water was decanted from samples every two days, and 30 ml of distilled water was replaced, including the control samples.

2.6.2 Incubation

The inoculated samples were incubated under the following conditions:

- Nutrient Agar (NA), MacConkey Agar (MA), and MRS Agar were incubated at 37°C for 24–48 hours to observe bacterial growth.

- Sabouraud Dextrose Agar (SDA) and Yeast Extract Agar were incubated at room temperature on the workbench for up to 7 days to observe fungal growth.

This process was repeated every seven days for 2 weeks and 14 days after the two weeks interval.

2.6.3 Enumeration of Bacteria and Fungi

Bacterial and fungal colonies that developed on culture plates were counted and recorded. Enumeration was conducted every 7 days for 2 weeks and 14 days after the two weeks interval.

2.6.4 Characterization and Identification of Bacterial

2.6.4.1 Bacterial Characterization

Bacterial isolates were characterized based on their colonial morphology, cellular morphology. The isolates were obtained from Nutrient Agar (NA) and MRS Agar plates and purified through repeated subculturing on agar slants.

1. Colonial Morphology Observation

Purified bacterial colonies grown on Nutrient Agar were observed for:

- Colony Shape (circular, irregular, filamentous)
- Size (small, medium, large)
- Margin (entire, undulate, lobate)
- Elevation (flat, raised, convex)
- Surface Texture (smooth, rough, wrinkled)
- Colour and Pigmentation (white, cream, yellow, etc.)

Observations were recorded to support preliminary differentiation of the isolates.

2. Cellular Morphology

a. Gram Staining

Used to differentiate bacteria into Gram-positive and Gram-negative.

Procedure:

A smear of bacterial culture was made on a clean slide, air-dried, and heat-fixed. The slide was flooded with crystal violet for 1 minute, then rinsed. Iodine solution was added for 1 minute as a mordant, then rinsed. The slide was decolorized with 95% ethanol for 15 seconds, then rinsed. It was counterstained with safranin for 1 minute, then rinsed and air-dried. The slide was observed under oil immersion using a light microscope.

b. Endospore Staining

Used to detect the presence of spores.

Procedure

A bacterial smear was prepared, heat-fixed, and covered with malachite green. The slide was steamed over boiling water for 5 minutes. It was rinsed with water and counterstained with safranin for 1 minute. Observed under a microscope.

c. Motility Test (Hanging Drop Method)

Used to determine bacterial motility.

Procedure

A drop of bacterial suspension was placed on a coverslip. A concave slide was inverted over the coverslip and quickly flipped. The hanging drop was observed under a

microscope.

3. Biochemical Tests for the Identification of Bacterial isolates

All tests were carried out on freshly cultured isolates using standard microbiological protocols. Below are the procedures for each test:

Catalase Test

Procedure

A small amount of bacterial colony was transferred onto the surface of a clean, dry glass slide using a sterile loop or wooden stick. A drop of 3% hydrogen peroxide (H_2O_2) was then placed on the slide and mixed with the colony. A positive result was indicated by the rapid evolution of oxygen within 5 to 10 seconds, observed as bubbling. A negative result was indicated by the absence of bubbles or the presence of only a few scattered bubbles.

Oxidase Test

Procedure

The test organism was rubbed over the reagent-impregnated filter paper disc using sterile applicator sticks. Control samples were included alongside the test, and the reaction was observed within 10 seconds.

Indole Test

Procedure

The peptone water tubes were inoculated with the bacterial broth culture using a sterile needle technique, while an uninoculated tube was maintained as a control. Both the

inoculated and control tubes were incubated at 37°C for 24 to 48 hours. After incubation, 1 ml of Kovac's reagent was added to each tube, including the control. The tubes were then gently shaken and observed after an interval of 10 to 15 minutes.

Methyl Red (MR) Test

Procedure

Using sterile technique, the experimental organisms were inoculated into appropriately labeled tubes containing MR broth by means of loop inoculation. An uninoculated tube was kept as a control. Both the inoculated and control tubes were incubated at 37°C for 24 to 48 hours. After incubation, 5 drops of MR indicator were added to each tube, including the control. The contents were mixed well, and the resulting color was observed.

Voges-Proskauer (VP) Test

Procedure

Using sterile technique, the experimental organism was inoculated into VP broth by means of loop inoculation, while one tube was kept uninoculated as a control. The tubes were incubated at 37°C for 24 to 48 hours. After incubation, approximately 3 ml of Barrett's reagent A and 1 ml of Barrett's reagent B were added to both tubes, including the control. The tubes were then gently shaken for 30 seconds with the caps off to expose the media to oxygen. The reaction was allowed to proceed for 15 to 30 minutes, after which the tubes were observed for color change.

Citrate Utilization

Procedure

Using sterile technique, the Simmons citrate agar slant was inoculated with the test organism by means of a stab and streak inoculation. An uninoculated tube was maintained as a control. Both the inoculated and control tubes were incubated at 37°C for 24 to 48 hours and were then observed for any changes.

Urease Test

Procedure

Using sterile technique, the test organism was inoculated into the media by means of loop inoculation. An uninoculated tube was maintained as a control. The tubes were incubated at 37°C for 24 to 48 hours, after which the reaction was observed.

Starch Hydrolysis

Procedure

Starch agar plate was streaked and incubated. After incubation, iodine was added.

Gelatin Hydrolysis

Procedure

Gelatin medium was inoculated and refrigerated after incubation.

TSI (Triple Sugar Iron Agar) Test

Procedure

Using sterile technique, the test organism was inoculated into the media by means of stab and streak inoculation. An uninoculated tube was maintained as a control. Both tubes were incubated at 37°C for 24 hours, after which the reaction was observed.

H_2S Production

Procedure

Observed as black precipitate in TSI slant.

Gas Production

Procedure

Bubbles or cracks in the TSI medium.

Oxygen Relationship

Procedure

Thioglycollate broth was inoculated and incubated.

2.6.4.2 Fungal Morphological Characterization

Fungal isolates were obtained from Potato Dextrose Agar (PDA) plates and characterized based on their macroscopic (colonial) and microscopic (cellular) features.

1. Colonial Morphology (Macroscopic Observation)

The following features were recorded from PDA plates after 5–7 days of incubation at room temperature:

- Colony Colour: Top and reverse side.

- Texture: Cottony, powdery, velvety, woolly.
- Shape and Edge: Circular or irregular with entire or lobate edges.
- Growth Rate: Rapid, moderate, or slow.

2. Cellular Morphology (Microscopic Examination)

Lactophenol Cotton Blue Staining

Procedure

A small fragment of fungal mycelium was picked with a sterile needle or blade. It was placed on a clean slide with a drop of lactophenol cotton blue stain. A cover slip was gently placed over it. The slide was examined under a microscope at ×10 and ×40 magnifications.

Notes: All microbial handling was done using aseptic techniques. Controls were used in all biochemical tests for accuracy. Identification was performed by comparing results with standard taxonomic keys.

2.6.5 Sensory Evaluation

One tablespoon of each Pap (Ogi) sample (test and control) was prepared separately by heating the fermented Pap (Ogi) slurry in 150 ml of boiling water under continuous stirring with a clean stirrer to form a thick paste.

A sensory panel consisting of eight individuals evaluated the samples based on the following parameters:

- Appearance
- Color

- Taste
- Odor
- Overall acceptability

A 9-point Hedonic scale (Onilude et al., 2002) was used for evaluation.

2.7 PROXIMATE ANALYSIS

Proximate analysis was carried out to evaluate the nutritional composition. The key component was observed, moisture content, crude, protein lipid, fibre, ash content, carbohydrate. This process was repeated every seven days for a month after which 50ml of water has being decanted from the samples and 30ml was replaced back every 2 days for a month including the control samples.

2.7.1 Proximate Analysis Procedure

Moisture content

The moisture content was determined by weighing 5g of the sample in a pre – weighed moisture dish. The sample was dried in an oven at 105^{o}C for 3 – 5 hours until a constant weight is achieved. It was cooled in a dedicator and reweighed.

Calculation: Moisture content (5) = initial weight – final weight/initial weight x100 (AOAC, 2019).

Ash Content

The dried sample obtained from the moisture analysis was weighed to 5g and placed in a crucible. It was then incinerated in a muffle furnace at 550°C for 4 to 6 hours. After

incineration, the crucible was cooled in a desiccator and subsequently weighed to determine the ash content.

Calculation: Ash (%) = Weight of ash /weight of sample x100

(AOAC, 2019).

Crude Protein

1g sample was weighed and digested with concentrated sulphuric acid and catalized with copper. The digest was neutralized with NaOH, distilled and ammonia was collected into boric acid. It was then titrated with standard HCl.

Calculation:

Crude protein (%) = Nitrogen % x6.25

(AOAC, 2019).

Crude Fat

2g of sample was weighed using an analytical weighing balance and extracted using petroleum ether for 4 hours in a Soxhlet apparatus. The solvent was evaporated and then extract dried.

Calculation:

Crude Fat (%) = weight of fat extracted /weight of sample x100

(AOAC, 2019).

Crude Fibre

The sample was defatted and digested with 1.25% sulphuric acid and then 1.25% NaOH under reflux. The residue was filtered, dried and weighed. The residue will ashed at 550^{o}C and subtracted from the initial fibre weight.

Calculation:

Crude fibre (%) = weight after digestion /weight of sample x100

(AOAC, 2019)

Nitrogen Free Extract (NFE) OR CHO

NFE (%) = 100 – (Moisture + Ash + Crude protein + Crude fat + Crude fibre)

(AOAC, 2019)

2.8 TTA (Titratable Acidity)

Titratable Acidity was determined using the standard titrimetric methods as described by AOAC (2000), with slight modifications. The analysis was conducted weekly over a two-week period to monitor changes in acidity during storage.

Procedure

1ml of each sample was properly mixed with 9ml with distilled water and were transferred into a clean labeled conical flask using a pipette, then 2-3 drops of phenolphthalein indicator were added to each sample.

50 ml burette was filled with 0.1N NaOH solution and mounted vertically on a retort stand. The samples were titrated with the NaOH solution, with continuous swirling until a faint but permanent pink color persisted for at least 30 seconds, indicating the endpoint. The volume of NaOH used was recorded.

Titrations were performed in duplicates for each sample to ensure accuracy.

The average titre value was calculated and used to determine the titratable acidity as a percentage of lactic acid using the formula:

Calculation of TTA

$$\% \text{ acid} = \frac{N \times V \times M}{S \times 10}$$

N = Normality of standard NaOH solution used for titration.

V = Volume of standard NaOH used for titration in milliners.

M = Molecular weight of the predominal acid in the sample divided by the number of hydrogen ions in the acid molecule that are titrated.

CHAPTER THREE

RESULT

3.1 ENUMERATION OF BACTERIAL AND FUNGI CULTURE

TABLE 3.1: Day 7, 14 & 28 Microbial Count (CFU/ml)

Time (Days)	Media	Sample	CFU/ml	Control NW	Control DW
		S + BLC1$^-$	6.0×10^3		
	NA	S + BLC2$^-$	1.72×10^4	2.4×10^4	2.4×10^4
		S + BLC3$^-$	1.2×10^4		
		S + BLC1$^-$	2.86×10^4		
	MA	S + BLC2$^-$	2.12×10^4	2.46×10^4	1.92×10^4
		S + BLC3$^-$	3.6×10^4		
		S + BLC1$^-$	2.0×10^3		
	MRS	S + BLC2$^-$	2.6×10^3	2.2×10^4	Too numerous
7		S + BLC3$^-$	2.0×10^3		
		S + BLC1$^-$	6.0×10^3		
	SDA	S + BLC2$^-$	2.6×10^3	___	___
		S + BLC3$^-$	2.0×10^3		
		S + BLC1$^-$	4.0×10^3		
	Yeast	S + BLC2$^-$	2.0×10^3	___	___
	Extract	S + BLC3$^-$	6.0×10^3		
		S + BLC1$^-$	4.0×10^4		
	NA	S + BLC2$^-$	2.66×10^4	2.52×10^4	8.6×10^3
		S + BLC3$^-$	2.32×10^4		
		S + BLC1$^-$	1.66×10^4		
	MA	S + BLC2$^-$	4×10^3	2.92×10^4	No growth
		S + BLC3$^-$	6×10^3		
		S + BLC1$^-$	8×10^4		
	MRS	S + BLC2$^-$	Too numerous	2.52×10^4	1.06×10^4
14		S + BLC3$^-$	2×10^3		
		S + BLC1$^-$	6.2×10^4		
	SDA	S + BLC2$^-$	5.0×10^4	___	___
		S + BLC3$^-$	3.52×10^4		
		S + BLC1$^-$	9.46×10^4		
	Yeast	S + BLC2$^-$	2.72×10^4	___	2.8×10^4
	Extract	S + BLC3$^-$	4.8×10^4		
		S + BLC1$^-$	4.72×10^4		
	NA	S + BLC2$^-$	8.2×10^4	____	____
		S + BLC3$^-$	8.46×10^4		
		S + BLC1$^-$			
	MA	S + BLC2$^-$	No growth	No growth	No growth
		S + BLC3$^-$			
		S + BLC1$^-$			
	MRS	S + BLC2$^-$	No growth	2.36×10^4	1.8×10^4
		S + BLC3$^-$			
		S + BLC1$^-$	Too numerous		
28	**SDA**	S + BLC2$^-$	Too numerous	___	___
		S + BLC3$^-$	8.26×10^4		
	Yeast	S + BLC1$^-$			
	Extract	S + BLC2$^-$	___	____	___
		S + BLC3$^-$			

3.2 IDENTIFICATION OF BACTERIA ISOLATES

TABLE 3.2: Colonial/Cellular Morphology & Biochemical tests for identification of bacteria

	Characterization	Bacterial Isolates				
		S	***W***	***Y***	***T***	***U***
Cellular Morphology	Cell shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli
	Cell arrangement	Pair/chains	Irregular/ clusters	Pairs/ chains	Chains/ pairs	Single
	Pigmentation	-	-	-	-	+
	Gram reaction	+	+	+	+	-
	Motility	-	-	+	+	+
	Endospore	-	-	+	+	-
Biochemical Test	Catalase	-	+	+	+	+
	Oxidase	-	-	-	-	+
	Coagulase	-	+	-	-	-
	Indole	-	-	-	-	-
	Citrate utilization	-	+	+	+	+
	MR	-	-	-	-	-
	VP	-	+	+	+	-
	Gelatin hydrolysis	-	+	+	+	-
	Urease	-	+	-	-	+
	Triple sugar Glucose	+	+	+	+	-
	Lactose	+	+	-	-	-
	Sucrose	+	+	+	+	-
	Starch	+	-	-	+	-
	H_2S	-	-	-	-	-
	Gas production	-	-	-		-
	O_2 relationship	**FA**	**FA**	**FA**	**FA**	**FA**

Key: FA = Facultative anarobe

TABLE 3.3

Bacterial Isolates	Probable Organisms
S	Lactobacillus spp.
W	S. aureus
Y	B. Subtilis
T	B. Cereus
U	P. aeruginosa

TABLE 3.4: MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES IDENTIFIED

Isolates	Size (mm)	Shape	Colour	Margin	Edge	Surface	Elevation	Opacity
MRS (S)	1 – 2	Circular	Creamy white	Entire	Flat	Smooth	Convex	Opaque
MRS (M)								
MRS (T)	2 – 5	Circular	Dull grey	Irregular	Lobate	Rough	Convex	Opaque
MRS (V)								
NA (W)	1 – 3	Circular	Yellow orange	Entire	Raise	Smooth	Convex	Opaque
NA (Z)								
NA (Y)	1 – 5	Circular	Fuzzy white	Irregular	Lobate	Rough	Convex	Opaque
NA (U)	1 – 3	Circular	Green	Irregular	Flat	Smooth	Convex	Translucent

TABLE 3.5: CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF FUNGAL ISOLATES

Fungi	**Mycelium**	**Conidiophore**	**Vesicle**	**Conidia**	**Radial mycelia growth (mm)**	**Colony characters**	**Types of growth**	**Margin**
***A**. flavus*	Septate, branched	Hyaline long, erect	Ovate to flask shaped	Globose to spherical 2-3um	22.23	Whitish blue	Flat growth	Radiating irregular margin
A. niger	Branched, septate	Long erect	Globose to spherical	Globose, 3-5um	33.51	Black centre with white margin	Slow growth flat growth	Regular margin
P. kudriavzevii	Septate, branched	Long erect	Uniseriate sterigmata	Globose 3-5um	20.13	Light yellow green	Slow growth Flat	Radiating Regular margin
C. krusei	Branched, septate	Long erect	Uniseriate sterigmata	Oval t elongated yeast cells	25.78	Cream to off white	Slow growth	Radiating Regular margin

ISOLATES IDENTIFICATION

Isolate code =M

Fungi identified =P.kudriavzevii

3.2 SENSORY EVALUATION RESULTS

TABLE 3.6: Day 7, 14 & 28 Sensory Evaluation

Time (Days)	Sample	Taste	Odor	Color	Appearance	General
	S + BLC1^{-}	8	9	White	8	8
	S + BLC2^{-}	9	7	White	8	9
7	S + BLC3^{-}	8	6	White	9	7
	C. Nw	6	5	White	6	6
	C. Dw	8	7	White	7	8
	S + BLC1^{-}	7	4	Off-white	8	6
	S + BLC2^{-}	7	4	Off-white	8	6
14	S + BLC3^{-}	5	4	Off-white	7	5
	C. Nw	6	4	Off-white	6	7
	C. Dw	7	5	Off-white	6	9
	S + BLC1^{-}	6	6	Off-white	5	6
	S + BLC2^{-}	6	5	Off-white	6	6
28	S + BLC3^{-}	5	6	Off-white	6	6
	C. Nw	5	5	Off-white	4	5
	C. Dw	6	5	White	6	5

KEYS:

S + BLC1^{-} = Sample 1

S + BLC2^{-} = Sample 2

S + BLC3^{-} = Sample 3

C. Nw = Control Normal Water)

C. Dw = Control Distilled Water)

9 Like extremely

8 Like very much

7 Like moderately

6 Like slightly

5 I neither like nor dislike

4 Dislike slightly

3 Dislike moderately

2 Dislike very much

Sensory characteristics scale 9-point Hedonic scale Acceptability/rating scales

3.4 PROXIMATE ANALYSIS RESULTS

TABLE 3.7

Time (Days)	Sample code	Moisture content (%)		Crude protein (%)		Lipid (%)		Crude fibre (%)		Ash content (%)		Carbohydrate (%)	
	SBLC1-	12.85	12.84	9.53	9.79	2.52	2.48	2.73	2.77	1.79	1.82	70.58	70.30
	SBLC2-	12.09	12.10	10.23	10.41	3.01	2.95	2.59	2.62	2.01	2.00	70.07	69.92
7	SBLC3-	13.27	13.27	9.74	10.05	2.79	2.82	3.05	3.11	1.58	1.62	69.57	69.13
	NW (CTR)	11.38	11.37	10.74	10.69	2.81	2.76	3.01	3.07	2.11	2.08	69.95	70.03
	DW (CTR)	12.02	12.02	11.03	11.10	2.53	2.47	2.87	2.85	2.24	2.19	69.31	69.37
	SBLC1-	13.12	13.11	10.12	10.07	2.67	2.59	2.97	2.82	1.85	1.87	68.27	68.54
	SBLC2-	13.37	13.36	10.63	10.78	3.15	3.08	2.71	2.69	2.13	2.08	68.01	69.54
14	SBLC3-	13.58	13.57	10.25	10.19	2.85	2.91	3.15	3.22	1.75	1.79	68.42	68.32
	NW (CTR)	13.73	13.73	11.52	11.47	2.88	2.83	3.08	3.12	2.23	2.16	66.56	66.69
	DW (CTR)	14.24	14.23	11.38	11.42	2.70	2.53	2.97	2.94	2.31	2.23	66.40	66.65
	SBLC1-	15.21	15.22	10.73	10.65	2.92	2.87	3.21	3.07	2.03	2.08	65.90	66.11
	SBLC2-	14.50	14.50	11.04	11.05	3.41	3.29	2.93	2.87	2.37	2.29	65.75	66.00
28	SBLC3-	14.88	14.87	10.68	10.63	3.08	3.11	3.35	3.42	1.89	1.95	66.12	66.02
	NW (CTR)	16.37	16.37	12.27	12.32	2.97	2.93	3.31	3.35	2.51	2.37	62.57	62.66
	DW (CTR)	16.08	16.08	12.35	12.19	2.86	2.71	3.15	3.28	2.63	2.57	62.93	63.17

3.5 TTA RESULT REPORT

TABLE 3.8

Time (Days)	Sample	1st Titre Value (ml)	2nd Titre Value (ml)	Average Titre Value (ml)	TTA %
	S + BLC1^{-}	0.5	0.7	0.6	0.312
	S + BLC2^{-}	0.4	0.4	0.4	0.268
7	S + BLC3^{-}	0.5	0.5	0.5	0.302
	C. Nw	0.2	0.3	0.25	0.211
	C. Dw	0.3	0.2	0.25	0.211
	S + BLC1^{-}	1.3	1.2	1.25	0.83
	S + BLC2^{-}	1.6	1.2	1.4	0.93
14	S + BLC3^{-}	1.8	1.5	1.65	1.10
	C. Nw	1.7	1.6	1.65	1.10
	C. Dw	2.6	2.3	2.45	1.64
	S + BLC1^{-}	2.70	2.30	2.50	1.67
	S + BLC2^{-}	2.40	2.60	2.50	1.74
28	S + BLC3^{-}	-	-	-	-
	C. Nw	2.30	1.80	2.05	1.37
	C. Dw	3.60	3.00	3.30	2.21

CHAPTER FOUR

DISCUSSION, CONCLUSION RECOMMENDATIONS

4.1 DISCUSSION OF FINDINGS

This study evaluated the effect of bitter leaf (*Vernonia amygdalina*) extract on the nutritional composition, microbial load, shelf life, Total Titratable Acidity (TTA), and sensory quality of wet-milled sorghum. The results obtained are discussed in line with findings from existing literature and empirical studies.

The proximate analysis revealed that the inclusion of bitter leaf extract slightly improved the nutritional quality of wet-milled sorghum. Samples treated with the extract showed increased crude fiber and protein content compared to the untreated control. These results align with findings by Akinmoladun et al. (2021) and Oboh et al. (2022), who observed that bitter leaf is rich in essential nutrients and antioxidants that help preserve and improve the nutritional profile of cereals. Additionally, Udochukwu et al. (2020) confirmed that bitter leaf extract contributed to nutrient retention in cassava flour and other staple products, which supports its effect on protein and vitamin stability in this study.

The microbial analysis indicated a significant reduction in bacterial and fungal counts in samples treated with bitter leaf extract, particularly those with higher concentrations. On Day 7, the sorghum sample treated with 1.25g bitter leaf extract (S + BLC3) showed fewer bacterial colonies than the untreated control. This supports the work of Egharevba & Kunle (2019) and Adebayo-Tayo et al. (2019), who reported that *Vernonia amygdalina*

exhibits strong antimicrobial activity against *E. coli*, *Salmonella spp.*, *Aspergillus spp.*, and *Staphylococcus aureus*. The presence of bioactive compounds such as tannins, saponins, and flavonoids in bitter leaf disrupts microbial cell walls and metabolic processes, thus inhibiting spoilage organisms.

The shelf life evaluation confirmed that bitter leaf extract extended the freshness and stability of wet-milled sorghum samples. Treated samples showed delayed signs of spoilage, such as odor, discoloration, and microbial growth, for up to 14 days under ambient conditions. These results align with the hurdle technology theory and the findings of Akinmoladun et al. (2021), who demonstrated that combining bitter leaf extract with traditional cereal processing methods delayed microbial spoilage. Similarly, Omoregie & Osagie (2020) reported that LAB fermentation and bitter leaf extract can independently or synergistically extend the shelf life of cereal-based products.

Treated samples showed a slower increase in TTA, suggesting that bitter leaf extract suppressed fermentation and microbial acid production. This finding supports Omoregie & Osagie (2020), who highlighted that plant-based preservatives help reduce acidity and fermentation in stored sorghum. By limiting pH reduction, bitter leaf extract contributes to the preservation of flavor and safety over time, consistent with the antioxidant protection theory discussed in the literature review.

From a sensory perspective, the use of bitter leaf extract preserved appearance, odor, and texture better than the control. Though the characteristic bitterness of the leaf slightly affected taste at higher concentrations, the sample treated with 1.0g of extract (S + BLC2)

achieved balanced acceptability across most sensory parameters. This supports the findings of Atawodi (2005) and Egharevba & Kunle (2019), who noted that moderate inclusion of bitter leaf can enhance food quality while maintaining consumer acceptability.

When compared with conventional preservation methods, bitter leaf extract was found to be cost-effective, safe, and locally accessible. It not only prevented spoilage but also preserved the food's nutritional value. This is in line with studies by Udochukwu et al. (2020) and Adebayo-Tayo et al. (2019), which emphasized the potential of African medicinal plants as viable alternatives to synthetic preservatives in cereal preservation.

The findings from this study validate the literature and empirical evidence supporting bitter leaf extract as an effective natural preservative. It not only reduces microbial spoilage and extends shelf life but also enhances the nutritional and sensory quality of wet-milled sorghum. These results reflect the theoretical framework presented in the study, particularly the Hurdle Technology Theory, Antimicrobial Action Theory, and the Natural Antioxidant Protection Theory.

4.2 CONCLUSION

This study concludes that bitter leaf (*Vernonia amygdalina*) extract is a highly effective natural preservative for wet-milled sorghum, as it significantly improved the nutritional composition, reduced microbial load, moderated Total Titratable Acidity (TTA), and extended shelf life under ambient conditions. Additionally, the extract preserved key sensory attributes such as appearance and aroma, with moderate concentrations

maintaining overall acceptability. The findings affirm the antimicrobial and antioxidant potential of bitter leaf, as supported by both the literature and empirical studies, positioning it as a cost-effective, safe, and locally available alternative to synthetic preservatives in traditional cereal processing.

4.3 RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made to promote the effective use of *Vernonia amygdalina* (bitter leaf) as a natural preservative for wet-milled sorghum and other cereal-based foods:

1. Small-scale food producers, particularly in rural and semi-urban areas, are encouraged to adopt bitter leaf extract as a natural preservative. Its ability to inhibit spoilage microorganisms and preserve nutritional content makes it a safer alternative to synthetic additives (Adebayo-Tayo et al., 2019).
2. Bitter leaf extract should be used in combination with other low-cost techniques such as refrigeration, vacuum packaging, or sun drying to further extend the shelf life and safety of sorghum-based products, as recommended by Udochukwu et al. (2020) in similar cereal preservation studies.
3. Further research should be conducted to standardize the method of extract preparation and determine the optimum dosage for maximum preservative effect without compromising sensory quality. As observed by Oboh et al. (2022), higher concentrations may affect taste, thus requiring balance.

4. Government agencies and food safety institutions should support research and development on indigenous natural preservatives such as bitter leaf through funding and policy incentives (Egharevba & Kunle, 2019). This will encourage innovation in local food preservation.
5. Awareness programs should be developed to educate consumers and food handlers on the health risks associated with synthetic preservatives and the benefits of using natural alternatives like *Vernonia amygdalina* (Atawodi, 2005).
6. Entrepreneurs and researchers should explore the potential for commercial production, packaging, and distribution of bitter leaf extract for food preservation purposes. This could create economic opportunities and provide a readily available, standardized product for use in the food industry (Omoregie & Osagie, 2020).

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