THE EFFECT OF BITTER LEAF (*VERNONIA AMYGDALINA*) ON MICROBIAL LOAD AND SENSORY PROPERTIES OF OGI

BY

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BEING A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, INSTITUTE OF APPLIED SCIENCES (IAS), KWARA STATE POLYTECHNIC, ILORIN,

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CERTIFICATION

This is certify that this project is the original work carried out and reported by **WAHAB BASHEEROH AYOMI** with matric number **ND/23/SLT/FT/0080** to the Department of Science Laboratory Technology, Microbiology unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfilment of the requirements of the award of National Diploma (ND) In Science Laboratory Technology

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DEDICATION

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

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All praise, glory, and adoration belong to the Almighty God who made it possible for me to successfully complete this program and this project.

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ABSTRACT

Ogi, a traditional fermented cereal product widely consumed in Nigeria and other parts of West Africa, is known for its nutritional value and cultural significance. However, its high moisture content and lack of effective preservation methods make it highly susceptible to microbial spoilage, leading to reduced shelf life, quality degradation, and potential health risks. This study investigated the effect of bitter leaf (Vernonia amygdalina) extract on the microbial load and sensory attributes of ogi produced from sorghum. Three concentrations of bitter leaf extract (0.7 g, 1.0 g, and 1.25 g per 308 g of ogi) were added to different ogi samples, while two untreated control samples were used for comparison. Microbial analysis was conducted weekly over a two-week period using standard culture media (Nutrient Agar, MRS Agar, SDA, and Yeast Extract Agar). Sensory evaluation was also carried out using a 9-point hedonic scale to assess taste, aroma, texture, and overall acceptability. The results showed that bitter leaf extract significantly reduced microbial growth in treated ogi samples, especially at the 1.0 g concentration, which achieved the best balance between microbial inhibition and sensory acceptability. While the highest concentration (1.25 g) offered stronger antimicrobial activity, it negatively impacted taste and aroma due to increased bitterness. The study concludes that bitter leaf extract can serve as an effective natural preservative in ogi, improving shelf life and microbial safety without compromising sensory quality when used in optimal concentrations. This approach offers a cost-effective, health-conscious, and locally available alternative to synthetic preservatives, particularly suitable for rural and resource-limited settings.
Keywords: *Ogi, Vernonia amygdalina, bitter leaf, microbial load, sensory evaluation, natural preservative, fermented foods.*

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Ogi is a traditional fermented cereal gruel commonly consumed in many parts of West Africa, particularly Nigeria. It is usually made from maize (*Zea mays*), millet (*Pennisetum glaucum*), or sorghum (*Sorghum bicolor*) and is widely used as a weaning food and adult breakfast due to its palatability and ease of digestion (Omoregie & Osagie, 2020). Despite its nutritional value and cultural relevance, Ogi in its wet-milled form is highly perishable. This is largely due to its high moisture content, which fosters microbial proliferation, leading to spoilage, nutrient degradation, and potential foodborne illnesses (Adebayo-Tayo et al., 2019).

Conventional preservation methods such as refrigeration and chemical additives are widely used to prolong shelf life. However, refrigeration is not always practical in many rural or underdeveloped regions due to erratic power supply. Additionally, concerns about the long-term health impacts of synthetic preservatives including carcinogenicity, allergic reactions, and toxicity have spurred interest in safer, natural alternatives (Eze & Onwuka, 2020).

Vernonia amygdalina, commonly known as bitter leaf, is a leafy vegetable indigenous to Africa and used both in culinary and medicinal contexts. It contains a rich profile of phytochemicals, including flavonoids, saponins, alkaloids, tannins, and phenolic compounds, which are known to exhibit antimicrobial and antioxidant properties (Oloyede et al., 2022). These bioactive constituents make bitter leaf a potential candidate for natural food preservation.

In many African countries, especially Nigeria, cereal-based fermented foods occupy a central position in the daily diet due to their affordability, nutritional content, and cultural acceptability. Among such foods, *Ogi* a fermented slurry made traditionally from maize (*Zea mays*), sorghum

(*Sorghum bicolor*), or millet (*Pennisetum glaucum*)—has stood the test of time as a staple consumed by both children and adults (Omoregie & Osagie, 2020). Ogi is usually prepared as a porridge or pap after fermenting and wet-milling grains, and is particularly valued for its soft texture and sour taste, making it ideal as a weaning food for infants.

Despite its nutritional and socio-cultural significance, the production and consumption of Ogi come with notable challenges. Chief among these is its high perishability, particularly in its wet-milled form. The fermentation process, while beneficial for flavor development and microbial acidification, also predisposes Ogi to microbial spoilage if not properly handled or stored (Okafor et al., 2021). Wet Ogi contains high moisture and is stored in non-sterile environments, often at ambient temperatures. These conditions favor the rapid growth of spoilage organisms, including bacteria (*Escherichia coli*, *Staphylococcus aureus*), yeasts, and molds (*Aspergillus niger*, *Penicillium spp.*), which compromise both food safety and sensory quality.

Food spoilage does not only result in economic losses for producers and vendors but also poses health risks for consumers due to the potential production of mycotoxins, biogenic amines, and pathogenic metabolites (Egharevba & Kunle, 2019). To mitigate these challenges, food processors have traditionally relied on preservation techniques such as drying, refrigeration, and chemical additives. While drying extends shelf life, it alters the desirable soft consistency of Ogi. Refrigeration is effective but often impractical in rural or resource-limited settings due to erratic power supply. Meanwhile, synthetic preservatives, though effective, are under increasing scrutiny for their adverse health effects including allergies, gastrointestinal distress, carcinogenicity, and hormonal disruption (Eze & Onwuka, 2020).

In response to these issues, there has been a global shift toward exploring plant-based natural preservatives. Plants used in traditional medicine and cuisine often contain a variety of phytochemicals that exhibit antimicrobial, antioxidant, and anti-inflammatory properties. One such plant that has drawn significant attention in recent years is *Vernonia amygdalina*, commonly known as bitter leaf.

Vernonia amygdalina is a leafy shrub widely found in West and Central Africa and used extensively in both cooking and traditional herbal medicine. Known for its bitter taste, the leaf has been used to treat ailments such as malaria, diabetes, gastrointestinal disturbances, and infections (Adebayo-Tayo et al., 2019). The medicinal efficacy of bitter leaf is largely due to its rich phytochemical composition, which includes flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic compounds (Oloyede et al., 2022). These compounds have been shown in numerous studies to exert bacteriostatic, bactericidal, fungistatic, and fungicidal effects, depending on the target microorganism and the concentration applied.

The mechanism of antimicrobial action of bitter leaf compounds is multifaceted. Flavonoids, for example, are known to inhibit bacterial adhesion, disrupt cytoplasmic membranes, and bind to bacterial enzymes, impeding metabolism (Lambert, 2017). Saponins increase membrane permeability, causing leakage of cellular contents. Tannins form irreversible complexes with microbial proteins, disrupting structural and metabolic functions. Alkaloids interfere with nucleic acid synthesis and enzymatic activity. These biochemical actions can significantly reduce microbial load when such extracts are introduced into perishable food systems.

The antioxidant properties of bitter leaf are equally important. Oxidation is a key factor in the deterioration of fermented foods, leading to rancidity, discoloration, and the loss of sensory appeal. The polyphenols and vitamin C in bitter leaf scavenge free radicals, thereby delaying oxidative spoilage. Moreover, bitter leaf's ability to lower the pH of food matrices makes the environment less hospitable for spoilage microorganisms, mimicking the preservative effect of organic acids used in industrial fermentation (Shahidi & Zhong, 2015).

Incorporating bitter leaf extract into the production of Ogi holds promise not only as a means to reduce microbial contamination but also as a potential enhancer of shelf life and product safety while aligning with the growing demand for clean-label and organic food products. The appeal of using such a locally available and culturally familiar plant lies in its accessibility and cost-effectiveness. Unlike synthetic preservatives, bitter leaf does not require complex processing, refrigeration, or importation. Its integration into Ogi production could empower local processors, reduce postharvest losses, and enhance public health.

However, despite these promising attributes, limited research exists on the application of *Vernonia amygdalina* as a natural preservative specifically in fermented cereal products like Ogi. Most prior studies focus either on its medicinal applications or its antimicrobial effects in laboratory settings rather than in actual food systems. Furthermore, while its antimicrobial potential is well-documented, the impact of bitter leaf extract on the sensory quality of Ogi such as taste, aroma, texture, and appearance has not been extensively evaluated. The bitterness of the extract, if not balanced properly, could reduce consumer acceptability, especially among children and individuals unfamiliar with its flavor profile (Udochukwu et al., 2020).

Finally, the need to preserve Ogi effectively without compromising health or sensory quality underscores the significance of exploring locally available, natural preservatives. Bitter leaf (*Vernonia amygdalina*) represents a viable candidate due to its known antimicrobial and antioxidant properties. This study investigates the incorporation of bitter leaf extract into Ogi to assess its ability to reduce microbial load and extend shelf life without compromising sensory attributes such as taste, aroma, and texture. The findings may contribute to safer and more sustainable food preservation practices, particularly in resource-limited communities.

1.2 Statement of the Problem

Ogi, although nutritionally beneficial and widely consumed, is highly perishable due to its high moisture content and the conditions under which it is traditionally produced and stored. Spoilage of Ogi is a common occurrence, particularly in rural and low-income settings where access to refrigeration is limited. The uncontrolled proliferation of microorganisms in Ogi leads to undesirable changes in taste, smell, color, and texture, and more critically, poses a health risk due to potential contamination by pathogenic microbes and spoilage fungi (Okafor et al., 2021).

While synthetic preservatives are commonly used in food systems to address spoilage issues, they are not without health risks and are often rejected by consumers seeking natural or organic alternatives. Moreover, many of these chemicals are either unavailable or too expensive for small-scale producers in developing countries.

Bitter leaf, a widely accessible medicinal plant in Africa, is known to possess potent antimicrobial and antioxidant properties. However, there is limited empirical research evaluating its application as a preservative in traditional food products like Ogi. Additionally, there is inadequate understanding of how the extract affects the sensory characteristics of Ogi, particularly with regard to taste and overall acceptability.

This study seeks to investigate whether bitter leaf extract can be used to reduce microbial contamination and extend the shelf life of Ogi without compromising its sensory appeal.

1.3 Justification of the Study

With increasing global interest in sustainable and natural food systems, there is a pressing need to develop preservation techniques that are affordable, culturally acceptable, and environmentally friendly. Synthetic preservatives, although effective, are often associated with undesirable side effects and long-term health concerns. Their rejection by informed consumers has created a demand for safer, plant-based alternatives.

Bitter leaf is abundant in most parts of Nigeria and other African countries and is already accepted as both a food and medicinal herb. Using bitter leaf extract as a preservative in Ogi could enhance the safety and stability of the product while addressing the limitations of traditional preservation methods. Additionally, this research provides an opportunity to scientifically validate indigenous knowledge, aligning traditional practices with modern food safety standards.

Moreover, this study could serve as a foundation for further exploration of other indigenous plants with similar bioactive properties, contributing to the development of a broader database of natural food preservatives.

1.4 Aim of the Study

Aim of this study is to evaluate the effect of *Vernonia amygdalina* (bitter leaf) extract on the microbial load and sensory properties of Ogi.

1.5 Objectives of the Study

The specific objectives of this study are to:

1. Determine the microbial load of Ogi samples treated with and without bitter leaf extract over a storage period.
2. To evaluate and compare the sensory properties (taste, aroma, texture, and color) of Ogi treated with varying concentrations of bitter leaf extract and unsupplementtted or untreated Ogi controls.

3. Assess the rate and nature of microbial and sensory changes in Ogi samples during storage.
4. Compare the preservation effectiveness of bitter leaf-treated samples with untreated controls.

1.6 Literature Review

The preservation of fermented foods using natural plant extracts has gained increasing interest in food science and public health. Bitter leaf (*Vernonia amygdalina*) is one of the indigenous African plants recognized for its antimicrobial potential. Its integration into food products such as Ogi could address issues related to food spoilage, microbial safety, and consumer demand for organic, preservative-free products. This literature review provides an overview of Ogi and its spoilage issues, examines the phytochemical properties of bitter leaf, and evaluates previous studies on its use in food systems, especially fermented cereals.

1.6.1 Cereal Grains Used in Ogi Production

Ogi, a traditional fermented cereal porridge widely consumed in West Africa, particularly Nigeria, can be prepared from a variety of cereal grains including maize (*Zea mays*), millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*). Each of these grains has unique nutritional, botanical, and cultural characteristics that influence the quality and sensory attributes of the resulting ogi.

Maize (*Zea mays*)

Maize is one of the most widely cultivated cereal crops globally and a staple in many African diets. Believed to have originated from Central America, maize was introduced to Africa in the 16th century and is now grown across the continent. Maize grains are rich in carbohydrates and contain modest amounts of protein and micronutrients. In ogi preparation, white or yellow maize is typically used (Iken & Amusa, 2019).

Fig. 1.1: Tree and Grain of Maize (*Zea mays*)

Sorghum (*Sorghum bicolor*)

Sorghum is native to the African savannah and is one of the oldest cultivated grains. It is highly drought-resistant and well-suited to semi-arid regions. Sorghum grains range in color from white to red or brown and are rich in dietary fiber, iron, and antioxidants. Sorghum ogi tends to have a deeper flavor and color compared to maize ogi (Obilana, 2020).

Fig. 1.2: Tree and Grain of Sorghum (*Sorghum bicolor*)

Millet (*Pennisetum glaucum*)

Millet, another ancient cereal crop, is cultivated extensively in northern Nigeria and other Sahelian regions. It is highly resilient to poor soil and low rainfall. Millet grains are small, round, and typically pale yellow. They are rich in B vitamins and essential amino acids. Millet-based ogi is less common than maize or sorghum but is favored in specific cultural and regional contexts. These grains form the core raw materials for ogi production, each contributing unique nutritional and sensory properties to the final product (Adekunle, 2017).

Fig. 1.3: Tree and Grain of Millet (*Pennisetum glaucum*)

1.6.2 Traditional Method of Ogi Preparation

Ogi is traditionally produced using simple, indigenous techniques passed down through generations in West African households. The process involves natural fermentation, without the addition of starter cultures or synthetic additives, and is largely conducted under non-sterile, ambient conditions.

Materials Used

- Grains (maize, sorghum, or millet)
- Clean water
- Grinding mill (manual or electric)
- Muslin cloth or sieve
- Fermentation container (plastic or calabash bowl)

Step-by-Step Traditional Method

1. **Selection and Cleaning of Grains**: The chosen cereal grains maize, sorghum, or millet are thoroughly cleaned to remove dirt, stones, chaff, and other impurities.
2. **Soaking:** The cleaned grains are soaked in clean water at room temperature for 48 to 72 hours to soften the kernels and allow spontaneous fermentation by naturally occurring lactic acid bacteria and yeasts (Omoregie & Osagie, 2020).
3. **Wet Milling:** The soaked grains are wet-milled using a stone or electric grinder to produce a smooth, thick slurry.
4. **Sieving:** The milled slurry is filtered through a fine muslin cloth or sieve to separate the starch-rich extract from the fibrous chaff. Water is added intermittently to facilitate the sieving process.
5. **Fermentation:** The filtrate is allowed to stand undisturbed for 24 to 48 hours, during which natural fermentation occurs. This step imparts the characteristic sour flavor and slightly acidic pH, which aids in preservation.
6. **Decanting and Sedimentation:** After fermentation, the supernatant (clear water) is carefully decanted, leaving behind the thick ogi paste settled at the bottom.
7. **Storage or Cooking:** The ogi paste may be stored in a cool environment for short periods or cooked into a smooth, pap-like meal by adding boiling water and stirring until thickened.

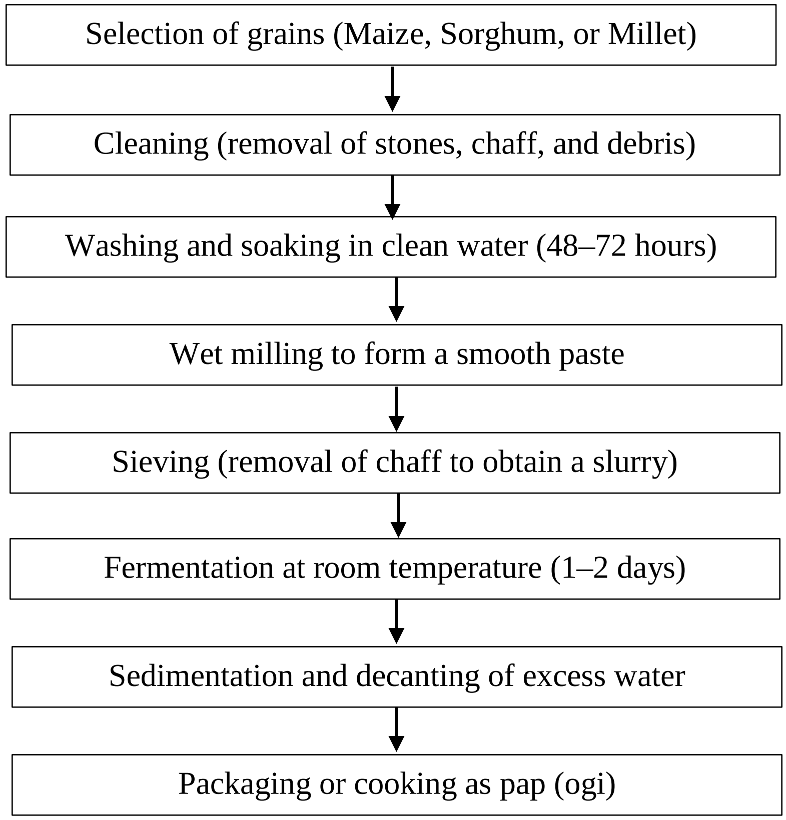

Fig. 1.4: Flowchart of Traditional Method of Ogi Preparation
(Adapted from Omoregie & Osagie, 2020)

1.7 Overview of Ogi

Ogi is a fermented cereal porridge traditionally made by soaking grains (usually maize, millet, or sorghum), wet-milling, and allowing them to ferment naturally for 2–3 days. The fermentation process is spontaneous and typically carried out under non-sterile conditions, allowing naturally occurring lactic acid bacteria and yeasts to drive the biochemical transformation of the grains (Omoregie & Osagie, 2020).

As a result of fermentation, Ogi develops a distinctive sour taste, improved digestibility, and enhanced nutrient bioavailability. However, its high water content and lack of pasteurization render it highly susceptible to spoilage shortly after preparation. Within a few days of ambient storage, Ogi can develop off-odors, discoloration, mold growth, and undesirable changes in texture, making it unfit for consumption. Despite its nutritional benefits, the perishable nature of Ogi presents a serious food safety challenge, particularly in rural areas without access to refrigeration or proper storage facilities (Oboh et al., 2022).

1.8 Microbial Load in Ogi

The microbial ecology of Ogi includes both beneficial and harmful microorganisms. Beneficial microbes, such as *Lactobacillus spp.*, *Pediococcus*, and some strains of *Saccharomyces cerevisiae*, contribute to acid production, improve shelf stability, and enhance nutritional value. However, spoilage and pathogenic organisms—including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Aspergillus niger*, and *Penicillium spp.* can contaminate Ogi during or after fermentation, especially if handled improperly (Okafor et al., 2021).

These undesirable microorganisms pose a threat not only to food quality but also to human health. Spoiled Ogi may contain toxins or exhibit undesirable sensory properties such as putrid smell, slimy texture, and color change. Therefore, reducing the microbial load in Ogi is essential to extending its shelf life and ensuring consumer safety.

1.9 Preservation Techniques for Fermented Foods

Preservation strategies for fermented foods can be broadly categorized into physical, chemical, and biological methods:

- **Refrigeration** is one of the most effective physical methods used to slow microbial metabolism and delay spoilage by maintaining food at low temperatures. However, its application is often limited in many low-income and rural areas due to inconsistent electricity supply and the high cost of refrigeration infrastructure (Okafor et al., 2021).
- **Drying**, another physical technique, involves reducing the moisture content of food to levels that inhibit microbial growth. While this method extends the shelf life of ogi, it

alters its soft, smooth texture, which is a critical sensory property that makes ogi desirable, especially for infants and the elderly (Akinmoladun et al., 2021).

- **Chemical preservatives**, including sodium benzoate, nitrates, and potassium sorbate, are widely used in commercial food processing to inhibit microbial activity. Although these additives are effective, they are increasingly scrutinized for their potential health risks, such as allergic reactions, gastrointestinal irritation, and possible links to carcinogenicity (Eze & Onwuka, 2020).
- **Natural preservatives**, particularly those derived from plants, are gaining widespread acceptance due to their broad-spectrum antimicrobial properties, lower toxicity, and cultural acceptability. Phytochemicals such as flavonoids, alkaloids, and saponins found in many traditional herbs exhibit antimicrobial and antioxidant activities that support food preservation. Plant-based preservation methods have been endorsed by global food safety agencies such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as sustainable and safer alternatives, especially suitable for traditional and rural food systems (Shahidi & Zhong, 2015).

1.10 Sensory Properties of Ogi

Sensory quality is a crucial determinant of consumer acceptance. For Ogi, this includes characteristics such as:

- **Taste** – Should be mildly sour but not unpleasantly bitter or bland.
- **Aroma** – Should retain a clean, slightly fermented smell.
- **Texture** – Should be smooth and lump-free, with no sliminess.
- **Color** – Should remain uniform and free from discoloration.

Spoilage alters these sensory attributes and renders Ogi unpalatable. Natural preservatives like bitter leaf must therefore be assessed not only for microbial inhibition but also for their effect on sensory properties to ensure consumer acceptance (Omoregie & Osagie, 2020).

1.11 Bitter Leaf (*Vernonia amygdalina*) as a Natural Preservative

1.11.1 Botanical Background and Origin

Vernonia amygdalina, commonly known as bitter leaf, is a small shrub that belongs to the Asteraceae (Compositae) family. It is indigenous to tropical Africa, especially West and Central African countries like Nigeria, Cameroon, and Ghana, where it is widely cultivated for both culinary and medicinal purposes. The plant can grow up to 2–5 meters tall and thrives in well-drained soils under full sunlight or partial shade. It is drought-tolerant and requires minimal agronomic inputs, making it suitable for both rural subsistence and urban backyard cultivation (Oboh et al., 2022).

Bitter leaf is highly valued in African traditional medicine and local cuisine. The leaves are typically washed or boiled to reduce their intense bitterness before being incorporated into soups or herbal decoctions. Its widespread use is attributed to its accessibility, affordability, and rich phytochemical profile, which confer antimicrobial, antioxidant, anti-inflammatory, and even antidiabetic properties (Egharevba & Kunle, 2019).

In recent years, attention has shifted toward its potential use as a natural food preservative, especially in traditional fermented foods like ogi, due to the antimicrobial properties of its bioactive compounds.

Bitter leaf, scientifically known as *Vernonia amygdalina*, is a perennial shrub that belongs to the plant family Asteraceae (Compositae), which includes many other medicinal and aromatic plants. It is one of the most widely used indigenous leafy vegetables and medicinal plants in tropical Africa, particularly in West and Central Africa, including countries like Nigeria, Ghana, and Cameroon (Oboh et al., 2022).

Origin and Distribution

Vernonia amygdalina is native to tropical Africa, where it grows abundantly in the wild and is also cultivated for domestic and commercial use. It is commonly found in home gardens, farmlands, and forest edges. The plant has also been introduced to parts of Asia and the Caribbean for its culinary and ethnomedicinal benefits.

Morphological Features

- Bitter leaf is a woody shrub that can grow between 2 to 5 meters tall.
- The leaves are elliptical, green, and bitter, giving the plant its common name.
- It has pale purple flowers and produces small seeds used for propagation.
- The bitter taste is due to its rich content of bioactive phytochemicals such as sesquiterpene lactones, flavonoids, alkaloids, and saponins (Egharevba & Kunle, 2019).

Cultivation

Bitter leaf thrives in well-drained, loamy soils under full sun or partial shade. It is a drought-resistant plant, making it suitable for cultivation in various agroecological zones. Propagation is typically done through stem cuttings, which root easily when planted in moist soil. The leaves can be harvested continuously once the plant is established, making it a low-input, high-yield vegetable crop (Akinmoladun et al., 2021).

Farmers and traditional medicine practitioners value bitter leaf for its culinary versatility, healing properties, and economic potential in both local markets and traditional healthcare systems.

Fig. 1.5: *Vernonia Amygdalina* (Bitter Leaf) (Omoregie & Osagie, 2020).

1.11.2 Nutritional Composition of Bitter Leaf (*Vernonia amygdalina*)

Vernonia amygdalina (bitter leaf) is not only valued for its medicinal and preservative properties but also for its rich nutritional profile, which contributes to its use in traditional diets across Africa. The leaves are consumed either as vegetables in soups or as herbal infusions and have been analyzed for their macronutrient and micronutrient content.

1. Macronutrients

i. **Protein:** Bitter leaf contains a relatively high level of crude protein (approximately 20–35% dry weight), making it a good plant-based protein source in local diets (Akinmoladun et al., 2021).

ii. **Carbohydrates:** The carbohydrate content ranges between 10–15%, contributing to its energy value.

iii. **Fats:** The fat content is low (typically 3–6%), mainly consisting of beneficial unsaturated fatty acids.

iv. **Crude Fiber:** Bitter leaf is rich in dietary fiber (12–18%), which aids digestion and supports gut health (Oboh et al., 2022).

2. Micronutrients

i. **Vitamins:**

- **Vitamin C (ascorbic acid):** Acts as a natural antioxidant, with concentrations ranging from 120–200 mg/100 g fresh leaf.
- **Vitamin A (beta-carotene):** Important for vision and immune function.
- **B-complex vitamins:** Including folate, niacin, and riboflavin, which support metabolism.

ii. **Minerals:**

- **Calcium (Ca):** Supports bone development-ranges from 150–300mg/100g.
- **Iron (Fe):** Important for red blood cell formation-approximately 5–12 mg/100 g.
- **Potassium (K), Magnesium (Mg), and Phosphorus (P)** are also present in appreciable amounts (Adebayo-Tayo et al., 2019).

3. Phytochemicals (Functional Compounds)

Beyond basic nutrition, bitter leaf is rich in bioactive compounds such as:

- Flavonoids
- Saponins
- Alkaloids

- Tannins
- Phenolic compounds

These contribute to its antimicrobial, anti-inflammatory, and antioxidant properties (Egharevba & Kunle, 2019).

Table 1.1: Summary table of Nutritional Content per 100g (Dry Weight Basis)

Nutrient	Approximate Value
Protein	20–35%
Carbohydrates	10–15%
Crude Fiber	12–18%
Fat	3–6%
Vitamin C	120–200 mg
Calcium	150–300 mg
Iron	5–12 mg
Flavonoids, Alkaloids, etc.	Present in high levels

1.11.3 Phytochemical Composition

Bitter leaf is known for its rich composition of bioactive phytochemicals, including:

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).

These compounds not only inhibit microbial growth but also contribute to the plant's medicinal effects. Their presence in bitter leaf supports its use in preserving perishable food items like Ogi.

1.11.4 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).

1.11.5 Mechanism of Action of Bitter Leaf Extract

The efficacy of *Vernonia amygdalina* as a food preservative can be attributed to several mechanisms:

i. **Membrane disruption** – Saponins and alkaloids compromise microbial membrane integrity, causing leakage of cellular contents.

ii. **Enzyme inhibition** – Tannins and phenolics bind with microbial enzymes, disrupting metabolism.

iii. **pH alteration** – Bitter leaf lowers the pH of food, creating an acidic environment that inhibits the growth of most spoilage organisms.

iv. **Antioxidant protection** – Polyphenols neutralize free radicals, preventing oxidation and spoilage of lipids and vitamins (Lambert, 2017; Shahidi & Zhong, 2015).

These actions collectively contribute to extending the shelf life and safety of perishable food products.

1.11.6 Antimicrobial Activity

Numerous studies have confirmed the antimicrobial potency of *Vernonia amygdalina*. Adebayo-Tayo et al. (2019) reported that aqueous extracts of bitter leaf significantly inhibited the growth of *E. coli*, *Salmonella typhi*, and *Aspergillus niger* in laboratory conditions. The extract showed a dose-dependent reduction in microbial counts, indicating that higher concentrations were more effective in suppressing microbial activity.

1.11.7 Sensory Implications

While bitter leaf is effective microbiologically, its bitter taste poses a sensory challenge in food applications. Udochukwu et al. (2020) noted that bitterness was perceptible even at low concentrations in cereal-based foods. However, moderate concentrations were still considered acceptable in sensory evaluations, particularly among adults who were familiar with the taste of bitter leaf. This study aims to determine a concentration that maintains microbial safety without compromising sensory qualities such as taste, aroma, and texture.

1.12 Theoretical Framework

This study is guided by three interrelated theories that explain how natural preservatives like *Vernonia amygdalina* (bitter leaf) can inhibit spoilage and extend the shelf life of traditional fermented foods such as Ogi:

1.12.1 Hurdle Technology Theory

The Hurdle Technology Theory proposes that a combination of multiple mild preservation methods each acting as a "hurdle" can be used synergistically to inhibit microbial growth, ensuring food safety and stability without compromising quality (Leistner, 2000). Instead of relying on one strong preservative method (e.g., high heat or heavy chemical use), several less severe techniques are applied together. In this study, the hurdles include:

- **Natural fermentation** (which reduces pH)
- **Antimicrobial compounds** from bitter leaf extract (e.g., flavonoids, alkaloids, saponins)
- **Possible water activity modulation** (through phytochemical interactions)

These hurdles together form a robust defense against spoilage organisms in Ogi, creating a microbial environment that is difficult to survive or adapt to. This theory supports the use of *Vernonia amygdalina* as a complementary preservation method in conjunction with natural lactic acid fermentation.

1.12.2 Natural Antioxidant Theory 2

The Natural Antioxidant Theory asserts that plant-derived antioxidant compounds can delay or prevent food spoilage by neutralizing free radicals and inhibiting oxidative reactions in food systems (Shahidi & Zhong, 2015). In the case of bitter leaf, it is rich in antioxidants such as:

- **Flavonoids**
- **Tannins**
- **Polyphenols**
- **Vitamin C**

These compounds protect Ogi from oxidative rancidity, discoloration, and nutrient loss during storage. Their activity helps maintain the sensory and nutritional quality of Ogi over time. This theory is especially relevant in explaining how bitter leaf preserves not only microbial safety but also sensory integrity.

1.12.3 Water Activity and Microbial Growth Theory

This theory emphasizes the role of **free water** (measured as water activity, a_w) in microbial growth. According to Scott (1957), different microorganisms require different minimum water activity levels to survive and reproduce. Most spoilage bacteria require a_w > 0.90, while molds and yeasts can grow at lower values.

Although Ogi is inherently high in water activity, bitter leaf's phytochemical composition may indirectly influence water availability by:

- Binding free water through tannins and fibers
- Changing the osmotic environment, making it less favorable for microbes
- Working synergistically with pH and antimicrobials to inhibit growth

Thus, even without reducing moisture content drastically, bitter leaf extract contributes to microbial control by lowering the water activity environment favorable to spoilage organisms.

Table 1.2: Summary of Theoretical Framework

Theory	**Core Principle**	**Application in Study**
Hurdle Technology Theory	Multiple mild barriers together inhibit microbial growth	Fermentation (low pH) + bitter leaf phytochemicals + potential reduction in a_w
Natural Antioxidant Theory	Antioxidants delay spoilage by neutralizing free radicals	Bitter leaf preserves sensory and nutritional quality during storage
Water Activity and Microbial Growth	Microbes need free water (a_w) to survive	Bitter leaf may reduce available water and alter the food matrix

These three theories form the conceptual foundation of the study and support the rationale for using *Vernonia amygdalina* as a multi-functional natural preservative in Ogi.

1.13 Empirical Review/ Related Studies

An empirical review provides an overview of relevant studies that have investigated similar or related research topics, particularly those that include data collection, experimentation, and analysis. In this context, the empirical review highlights previous research on the use of natural plant extracts especially *Vernonia amygdalina* (bitter leaf) in food preservation, with a focus on fermented cereal products like Ogi.

Adebayo-Tayo et al. (2019), in their study on the antimicrobial potential of bitter leaf extract, Adebayo-Tayo and colleagues evaluated aqueous extracts of *Vernonia amygdalina* on the microbial quality of fermented maize paste. The results revealed a significant reduction in total viable counts (TVC), coliforms, and fungi in treated samples compared to controls. The extract was most effective at higher concentrations (up to 3.0%), and its antimicrobial effects were attributed to the presence of flavonoids, tannins, and alkaloids. The study concluded that bitter leaf could serve as a natural preservative, although it did not assess its impact on sensory attributes.

Omoregie & Osagie (2020), this research investigated the functional and sensory properties of Ogi treated with plant-based extracts. The authors compared different indigenous plant extracts, including bitter leaf, scent leaf, and garlic. Bitter leaf-treated Ogi demonstrated significant microbial stability over a 7-day period and maintained acceptable sensory characteristics such as color, aroma, and texture. However, some respondents noted a mild bitter aftertaste. The study emphasized the potential of using bitter leaf as a dual-function preservative—combining safety with sensory viability.

Udochukwu et al. (2020), this comparative study explored the efficacy of different natural plant extracts in preserving maize-based Ogi. Using *Vernonia amygdalina*, *Ocimum gratissimum* (scent leaf), and *Zingiber officinale* (ginger), the researchers monitored microbial load and sensory perception over 10 days. Bitter leaf extract was among the most effective in reducing microbial growth, particularly molds and coliforms. However, its strong bitter flavor affected acceptability at higher concentrations (above 2.5%). The study recommended further work on balancing antimicrobial effectiveness with taste acceptability.

Okafor et al. (2021), this study focused on the microbial and sensory assessment of fermented sorghum Ogi stored under ambient conditions. It reported rapid microbial proliferation in untreated samples, including increases in *E. coli*, *Staphylococcus aureus*, and various fungi. The researchers suggested that incorporating natural antimicrobials such as bitter leaf could extend shelf life, though no direct experimentation with bitter leaf was performed. This highlights a knowledge gap that the current study seeks to address.

Egharevba & Kunle (2019), this work provided a comprehensive phytochemical analysis of *Vernonia amygdalina* and its antibacterial effects on foodborne pathogens. Using methanol and aqueous extractions, the authors tested against *Salmonella*, *Klebsiella*, and *Pseudomonas* species, reporting strong inhibition zones. However, the study was conducted under in vitro conditions and did not apply the extracts to real food systems like Ogi, indicating the need for applied studies such as the current one.

1.13.1 Gaps Identified in Empirical Literature

- **Limited focus on sensory effects**: While many studies have confirmed the antimicrobial potential of bitter leaf, few have systematically evaluated its impact on sensory properties in actual food products.

- **Lack of concentration optimization**: There is limited research on determining the ideal concentration of bitter leaf extract that balances microbial inhibition with consumer acceptability.
- **Scarcity of long-term storage studies**: Most existing studies are short-term (≤10 days), with minimal data on microbial and sensory changes over extended storage (e.g., 28 days).
- **Few studies on fermented sorghum Ogi**: Most empirical research has focused on maize-based Ogi, leaving sorghum variants underexplored.

1.13.2 Contribution of the Current Study

This study contributes to filling the identified gaps by:

- Evaluating both microbial and sensory properties of Ogi treated with bitter leaf extract.
- Using multiple concentrations to determine the most effective and acceptable dosage.
- Monitoring changes over four weeks, providing extended storage data.
- Applying bitter leaf to sorghum-based Ogi, expanding the empirical literature beyond maize-based variants.

1.14 Summary of Literature Review

The literature reviewed in this chapter highlights the significance of Ogi as a widely consumed fermented cereal product in West Africa, particularly in Nigeria. Despite its nutritional value, Ogi is highly perishable due to its high moisture content, spontaneous fermentation process, and lack of post-processing preservation. These factors make it highly susceptible to microbial contamination during storage, leading to spoilage, reduced shelf life, and food safety concerns.

Conventional methods of preservation, such as refrigeration and the use of synthetic chemical additives, are either inaccessible in many rural and semi-urban communities or are associated with adverse health implications. This has led to growing interest in natural and plant-based preservation strategies, especially those that align with indigenous knowledge systems and local food habits.

Among the natural preservation options, *Vernonia amygdalina* (bitter leaf) has emerged as a promising candidate due to its well-documented antimicrobial and antioxidant properties. Studies have shown that bitter leaf contains bioactive compounds including flavonoids, tannins, alkaloids, saponins, and phenolics that are effective in inhibiting a wide range of spoilage and pathogenic microorganisms. These compounds also possess antioxidant properties that help delay oxidative deterioration in food systems, thereby extending shelf life and preserving sensory qualities such as taste, aroma, and texture.

The literature also acknowledges the theoretical foundations supporting the use of bitter leaf in food preservation. The Hurdle Technology Theory explains how multiple preservation methods such as acidification, antimicrobial agents, and low water activity work synergistically to inhibit microbial growth. The Natural Antioxidant Theory supports the idea that plant-based antioxidants delay spoilage and nutrient loss. Finally, the Water Activity and Microbial Growth Theory reinforces the importance of reducing free water to limit microbial proliferation.

However, despite these promising findings, there remains a noticeable gap in empirical research that specifically investigates the use of bitter leaf in fermented cereal products like Ogi. Most existing studies focus either on the phytochemical and medicinal properties of *Vernonia amygdalina* or on its laboratory-tested antimicrobial effects. Few have explored its application in traditional food systems under real storage conditions, and even fewer have evaluated its impact on the sensory acceptability of such products.

This study, therefore, seeks to fill this knowledge gap by evaluating the dual role of bitter leaf extract in preserving Ogi both in terms of microbial inhibition and sensory quality retention. It aims to identify the optimal concentration that maximizes preservation while maintaining or enhancing consumer acceptability. By doing so, it contributes to the growing body of knowledge on natural food preservation and offers practical insights for food safety in low-resource settings.

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)

2.2.3 Equipment

Petri-dishes, inoculating loops, refrigerator, incubators, hot air oven, test tube, beakers, comical flask, grinder, gas cooker, pot, cups, spoons and 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:

- $SBC1^-$: 308.5 g of sorghum + 0.7 g of bitter leaf
- $SBC2^-$: 308.0 g of sorghum + 1.0 g of bitter leaf
- $SBC3^-$: 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.

2.3.1 Control Setup

Two additional control samples were prepared:

- Control 1: 310 g of sorghum soaked in potable/clean water
- Control 2: 310 g of sorghum soaked in distilled water

2.3.2 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:

- Sample 1: +0.7 g bitter leaf

- Sample 2: +1.0 g bitter leaf
- Sample 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.

2.4 Sterilization of Equipment

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 plastic containers were washed with soap 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 seven days interval for first two weeks and 14 days interval of the first 2 weeks. 50ml of water was decanted from samples every two days, and 30 ml of distilled water was replaced, including the control samples.

2.7.2 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 two weeks.

2.7.3 Enumeration of Bacteria and Fungi

Bacterial and fungal colonies that developed on culture plates were counted and recorded.

Enumeration was conducted every seven days for two weeks and 14 days after the two weeks interval.

2.7.4 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.

All microbiological analysis and sensory properties evaluation were done every 7 days interval for first two weeks and 14 days interval after the first two weeks.

CHAPTER THREE

RESULT

3.1 ENUMERATION OF BACTERIAL & FUNGAL CULTURE

TABLE 3.1: Week 1&2 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$^-$	$1.2 x 10^4$		
		S + BLC1$^-$	2.86×10^4		
	MA	S + BLC2$^-$	2.12×10^4	2.46×10^4	$1.92 x 10^4$
		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$^-$	$2.32 x 10^4$		
		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	$2.36 x 10^4$	$1.8 x 10^4$
		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 SENSORY EVALUATION RESULTS

TABLE 3.2: Week 1&2 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

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion of Findings

This study evaluated the effect of *Vernonia amygdalina* (bitter leaf) on the microbial load and sensory properties of Ogi made from sorghum. The results demonstrate that bitter leaf extract significantly reduced microbial proliferation over a 28-day storage period and helped maintain acceptable sensory quality, particularly at moderate concentrations.

The microbial analysis showed that Ogi samples treated with bitter leaf extract (S + BLC1$^-$, S + BLC2$^-$, S + BLC3$^-$) exhibited lower colony-forming units (CFU/ml) across all media (NA, MA, MRS, SDA, and Yeast Extract) compared to the untreated controls (C. NW and C. DW). This supports the antimicrobial potential of *Vernonia amygdalina* due to its phytochemicals, including flavonoids, tannins, alkaloids, and saponins as described by (Adebayo-Tayo et al., 2019; Egharevba & Kunle, 2019).

S + BLC2$^-$ (1.0 g bitter leaf extract) was not consistently the most effective in suppressing microbial growth across all storage days. While *S + BLC2*$^-$ showed relatively low microbial counts in Week 1, by Week 2, some media such as MRS and SDA reported results like "too numerous to count" (TNTC), indicating a spike in microbial activity. In contrast, other treatments, notably *S + BLC3*$^-$ (1.25 g), demonstrated more stable suppression of microbial counts in later weeks. Additionally, *S + BLC1*$^-$ (0.7 g) showed lower microbial activity in some assays (e.g., Yeast Extract Agar) at Week 2 and Week 4, suggesting dose-dependent variability in performance.

These findings indicate that while *S + BLC2*$^-$ initially performed well, it did not maintain superior microbial control across the entire 28-day storage period. This finding contradicts some earlier assumptions and emphasizes the importance of dosage optimization when using natural plant extracts as preservatives (Adebayo-Tayo et al., 2019; Udochukwu et al., 2020). It also reinforces the Hurdle Technology Theory (Leistner, 2000), which advocates for combining multiple preservation factors including pH, temperature, and antimicrobials to inhibit spoilage organisms effectively. Furthermore, the observations align with the Water Activity and Microbial Growth Theory (Scott, 1957), as the bitter leaf extract may have altered the water activity or osmotic conditions of ogi, thereby influencing microbial behavior indirectly.

Sensory evaluation across the three storage periods (Day 7, 14, and 28) revealed that Ogi samples treated with bitter leaf extract especially S + BLC2$^-$ had the highest acceptability scores. Parameters such as taste, odor, appearance, and overall acceptability were better maintained compared to the control samples. S + BLC2$^-$ received the highest taste and general acceptability scores in Day 7.

By Day 14 and Day 28, all samples experienced natural deterioration, but treated samples retained more desirable sensory characteristics than the controls.

S + BLC3$^-$ (1.25g), though effective microbiologically, had reduced sensory scores due to bitterness, reflecting the need for concentration optimization (Udochukwu et al., 2020).

These findings validate the Natural Antioxidant Theory (Shahidi & Zhong, 2015), which explains how plant-based antioxidants delay oxidative spoilage and help maintain food quality during storage.

The integration of bitter leaf extract in Ogi production effectively delayed spoilage, preserved sensory integrity, and outperformed conventional methods under ambient storage. These findings not only support existing empirical studies but also address previously identified research gaps by demonstrating practical application in fermented sorghum Ogi over an extended period.

4.2 Conclusion

This study concludes that *Vernonia amygdalina* (bitter leaf) extract is an effective natural preservative for Ogi, capable of significantly reducing microbial growth and preserving sensory qualities such as taste, aroma, and appearance over a 28-day storage period. Among the tested concentrations, 1.0g per 308g of Ogi (S + $BLC2^{-}$) was most effective in balancing microbial inhibition with consumer acceptability. The preservative effect is attributed to the plant's bioactive compounds flavonoids, tannins, alkaloids, saponins, and phenolics which align with Hurdle Technology, Natural Antioxidant, and Water Activity theories. Bitter leaf presents a sustainable and culturally appropriate alternative to synthetic preservatives, especially in low-resource settings.

4.3 Recommendations

Based on the findings and conclusions of this study, the following recommendations are proposed to promote the effective use of *Vernonia amygdalina* (bitter leaf) as a natural preservative in Ogi and potentially other traditional food products:

1. Small- and medium-scale Ogi producers, especially those operating in rural and semi-urban areas, are encouraged to adopt bitter leaf extract (at 1.0 g per 308 g of Ogi) as a natural preservative. This will help extend shelf life, reduce spoilage losses, and improve food safety without the use of synthetic additives (Eze & Onwuka, 2020; Adebayo-Tayo et al., 2019).

2. Further research should focus on refining processing methods that reduce the bitterness of *Vernonia amygdalina* without compromising its antimicrobial properties. Techniques such as partial blanching, co-fermentation, or extract blending with flavor-modifying herbs could help improve sensory acceptability while maintaining preservative efficacy (Udochukwu et al., 2020).

3. To support broader application, more longitudinal studies should be carried out to monitor microbial and sensory changes beyond 14 days of storage. Additionally, toxicolOgical assessments are necessary to determine the safety of continuous or high-level consumption of bitter leaf extract in food (Egharevba & Kunle, 2019).

4. Government agencies, agricultural extension services, and food safety bodies should organize training programs and awareness campaigns for local food producers, vendors, and consumers on the benefits and safe use of natural preservatives, particularly bitter leaf (Omoregie & Osagie, 2020).

5. Policymakers and food regulatory authorities (e.g., NAFDAC, SON) should consider developing guidelines and standards for the use of natural plant extracts like *Vernonia amygdalina* in traditional food processing. This could lead to its formal recognition as a safe and approved preservative, encouraging widespread use (Shahidi & Zhong, 2015).

6. Researchers and Food Technologists should explore the use of bitter leaf in preserving other perishable food products, such as fermented legumes, porridges, beverages, and meat alternatives. This would help assess its versatility and broaden its commercial potential (Akinmoladun et al., 2021).

By implementing these recommendations, the use of *Vernonia amygdalina* as a natural preservative can be optimized and scaled up, offering a sustainable solution to food preservation challenges in Nigeria and other developing countries.

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