THE EFFECT OF BITTERLEAF ON MICROBIAL LOAD AND SENSORY PROPERTIES OF OGI

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CERTIFICATION

This is Project work has been read and approved as meeting part of the requirements of Science Laboratory Technology, Institution of Applied science, Kwara State Polytechnic, Ilorin. In partial fulfillments of the requirement for 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.

ACKNOWLEDGEMENT

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

Our profound gratitude goes to our competent and amiable supervisor, MRS ADEBOYE T. O., for her time, dedication, and effort in guiding us through the course of this project. Her support, corrections, and encouragement greatly contributed to the success of our work. May the Almighty God continue to bless and strengthen you (Amen).

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We also sincerely appreciate all the lecturers and staff of the Science Laboratory Technology Department, Microbiology Unit, for their invaluable contributions toward our academic and professional growth.

Finally, we extend our heartfelt gratitude to our families and loved ones for their endless support, encouragement, and prayers throughout our academic journey. May they all be richly rewarded (Amen).

ABSTRACT

This study investigates the effect of Vernonia amygdalina (bitter leaf) on the microbial load and sensory properties of Ogi, a traditional fermented cereal product widely consumed in Nigeria. The objective was to evaluate whether the incorporation of bitter leaf could enhance the shelf life, safety, and acceptability of Ogi during storage. Wet- milled sorghum was fermented with varying concentrations of bitter leaf extract and stored over a four-week period. Samples were analyzed weekly for microbial counts (bacteria and fungi), titratable acidity, and sensory attributes including taste, color, texture, and overall acceptability. The results showed that bitter leaf-fortified samples consistently exhibited lower microbial loads compared to controls, indicating strong antimicrobial activity. Sensory evaluation revealed improved acceptability, particularly in terms of taste and appearance, in the treated samples throughout the storage period. Additionally, bitter leaf samples showed favorable fermentation characteristics with reduced acidity and a shift in microbial flora toward beneficial species such as Lactobacillus. These findings suggest that Vernonia amygdalina can serve as an effective natural preservative, enhancing both the microbiological stability and consumer preference of Ogi. The study supports the use of indigenous plant extracts in improving food safety and quality in traditional fermented products.
Keywords*: Bitter leaf, Vernonia amygdalina, Ogi, microbial load, sensory evaluation, food preservation, fermented cereal.*

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1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Fermentation has long served as a vital method for food preservation and transformation, especially in traditional societies where refrigeration is limited. Across West Africa, particularly in Nigeria, “ogi” stands out as a staple fermented food product made from cereals like maize (Zea mays), sorghum (Sorghum bicolor), or millet (Pennisetum glaucum). It plays a central role in the diet, particularly as a weaning food for infants and as a light meal for adults (Ezeokoli et al., 2021). The production of ogi typically involves spontaneous fermentation driven by indigenous microflora, including lactic acid bacteria and yeasts. This natural fermentation not only enhances digestibility but also reduces anti-nutritional factors and improves flavor (Yusuf & Olasupo, 2023).

Nevertheless, traditional methods of ogi production are often unhygienic, leaving the product vulnerable to microbial contamination. Pathogenic organisms such as Salmonella spp., Escherichia coli, Bacillus species, and molds like Aspergillus and Penicillium are frequently associated with spoilage (Ibrahim et al., 2022). These contaminants degrade both the safety and sensory appeal of ogi, limiting its shelf stability and consumer trust. While synthetic preservatives are employed in industrial food processing to combat microbial spoilage, growing health concerns over their potential toxicological effects such as allergies and carcinogenicity have fueled interest in safer, naturally derived alternatives (Onuoha & Adejumo, 2021).

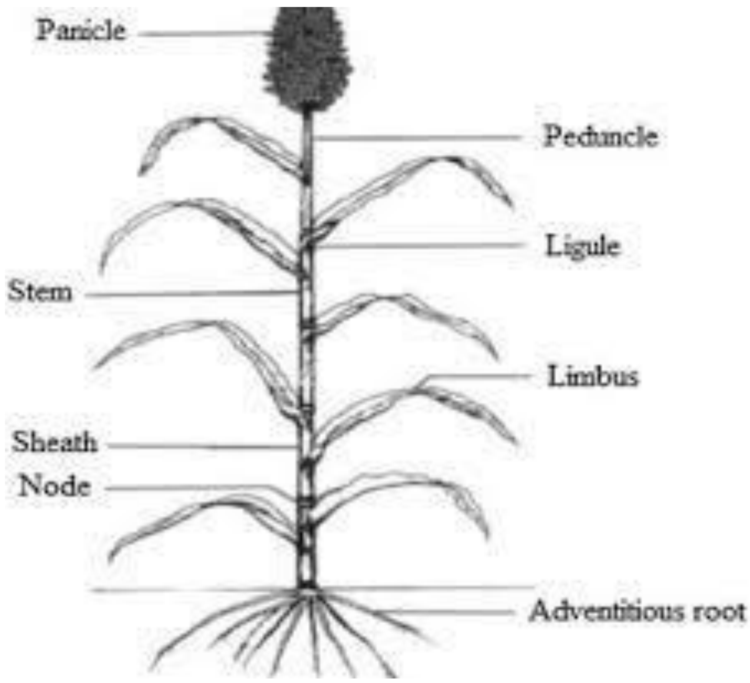
One such promising alternative is *Vernonia amygdalina*, commonly known as bitter leaf. This plant, extensively used in African traditional medicine and cooking, is characterized by a high concentration of bioactive compounds including alkaloids, saponins, tannins, flavonoids, terpenes, and phenolic compounds. These phytochemicals exhibit notable antimicrobial, anti-inflammatory, and antioxidant properties (Chikezie & Uwakwe, 2020).

Research has shown that extracts from bitter leaf can suppress the growth of several foodborne pathogens and spoilage organisms, suggesting it may serve as an effective natural preservative in traditional foods like ogi.

Introducing bitter leaf into ogi production could offer dual benefits: reducing microbial contamination and enhancing functional properties through its rich phytochemical profile. Moreover, its impact on the sensory qualities of ogi—such as taste, texture, aroma, and appearance warrants investigation, as consumer acceptability is essential for practical application. Despite its potential, limited scientific studies have examined the influence of bitter leaf on ogi's microbiological and sensory attributes. This study seeks to bridge that gap by exploring the effects of bitter leaf incorporation on ogi.

Figure 1:

Sorghum Plant



Source: (Chiswick, 2024).

1.1.1 Background of the Study

Fermentation has long served as a vital method for food preservation and transformation, especially in traditional societies where refrigeration is limited. Across West Africa, particularly in Nigeria, “ogi” stands out as a staple fermented food product made from cereals like maize (Zea mays), sorghum (Sorghum bicolor), or millet (Pennisetum glaucum). It plays a central role in the diet, particularly as a weaning food for infants and as a light meal for adults (Ezeokoli et al., 2021). The production of ogi typically involves spontaneous fermentation driven by indigenous microflora, including lactic acid bacteria and yeasts. This natural fermentation not only enhances digestibility but also reduces anti-nutritional factors and improves flavor (Yusuf & Olasupo, 2023).

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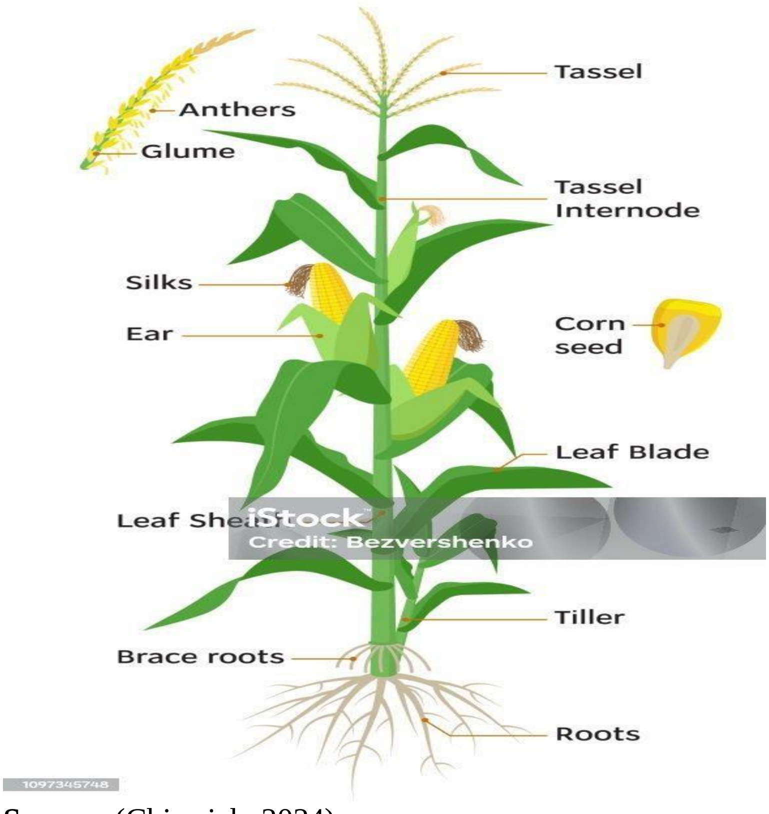
Figure 2:

Maize Plant



Source: (Chiswick, 2024).

1.1.2 Origin and History of Vernonia amygdalina

Vernonia amygdalina, commonly known as bitter leaf, is a member of the Asteraceae family. It is indigenous to tropical Africa and is widely distributed across Sub-Saharan Africa, particularly in Nigeria, Cameroon, Ghana, and parts of East and Central Africa. The plant has been a vital component of traditional medicine, cuisine, and ethnobotanical practices for centuries. In Nigeria, bitter leaf is called "Ewuro" in Yoruba, "Onugbu" in Igbo, and "Shuwaka" in Hausa. It has been historically valued for its medicinal efficacy in treating a wide range of ailments, including malaria, diabetes, gastrointestinal disorders, and microbial infections (Bonsi et al., 2020).

The traditional use of bitter leaf spans generations, and it remains one of the most common vegetables incorporated into soups and herbal preparations. Its intense bitter taste is attributed to its rich composition of phytochemicals, many of which exhibit antimicrobial and antioxidant activities. Early African communities discovered its therapeutic value through empirical knowledge, and over time, scientific research has confirmed many of these traditional claims.

The spread and cultivation of bitter leaf have increased as its health benefits became more widely recognized. Today, it is not only grown for domestic use but also for commercial purposes in local and international markets. Its leaves, roots, and stems are all used for various medicinal preparations. The renewed interest in organic and natural remedies has placed bitter leaf at the forefront of botanical research aimed at developing plant-based alternatives to synthetic food additives and pharmaceuticals (Nwankwo et al., 2021).

Figure 3:

Vernonia *Amygdalina* (Bitter leaf)

Source: (Udochukwu et al., 2015).

1.1.3 Aim and Objectives of the Study

The aim is to investigate the impact of bitter leaf (Vernonia amygdalina) on the microbial content and sensory characteristics of ogi.

Specific Objectives:

i. To identify the phytochemical components of bitter leaf relevant to antimicrobial activity.

ii. To evaluate the inhibitory effects of bitter leaf extracts against specific microorganisms typically found in ogi.

iii. To compare the microbial loads of ogi samples prepared with and without bitter leaf.

iv. To assess the sensory attributes (taste, aroma, texture, appearance, and overall acceptability) of ogi fortified with bitter leaf.

v. To determine the shelf-life stability of bitter leaf-treated ogi compared to untreated samples.

1.1.4 Statement of the Problem

Although ogi is a culturally important and nutritionally beneficial food, traditional methods of preparation expose it to high levels of microbial contamination due to poor sanitation during fermentation and storage. This leads to rapid spoilage and increased health risks for consumers. Chemical preservatives can address these issues, but their potential side effects and impact on flavor have raised safety and acceptability concerns (Abdulsalami et al., 2022).

Given the need for safer, effective alternatives, the antimicrobial properties of bitter leaf present a viable natural solution. However, its effectiveness in enhancing ogi's microbiological quality without compromising its sensory appeal has not been sufficiently explored. Addressing this gap could lead to safer and more acceptable ogi products.

1.1.5 Significance of the Study

This study offers practical benefits in food safety and public health, particularly by enhancing the microbiological integrity of ogi a widely consumed weaning food. Using bitter leaf as a natural preservative addresses the demand for cleaner-label food products and contributes to safer dietary options, especially for infants and immunocompromised individuals.

Additionally, bitter leaf's phytochemical properties may improve the nutritional and functional quality of ogi, potentially increasing consumer appeal and marketability. From an academic standpoint, the findings could expand the existing body of knowledge on natural food preservatives and support the development of healthier, value-added traditional foods.

1.1.6 Scope of the Study

The research focuses on maize-based ogi, incorporating bitter leaf extracts in varying concentrations. It covers phytochemical profiling of bitter leaf, antimicrobial activity assays against selected microorganisms (e.g., *E. coli*, *Staphylococcus aureus*, *Aspergillus* spp.), microbial enumeration in treated and control ogi samples, and sensory evaluation using a trained panel.

The experiments will be conducted under controlled laboratory conditions. Shelf-life will be observed over a defined period. This study does not attempt to replicate traditional or commercial production settings and excludes other cereal bases such as sorghum and millet.

1.1.7 Limitations of the Study

1. The chemical profile of bitter leaf may fluctuate based on environmental factors and harvest timing.
2. Sensory evaluation results may vary due to individual preferences, even with standardized methods.
3. Laboratory storage conditions may not reflect those of typical household or market environments.
4. The study exclusively investigates maize-based ogi.

5. Shelf-life testing is limited to short-term storage and may not represent extended conditions.

1.1.8 Operational Definition of Terms

- **Ogi:** A fermented cereal pudding made predominantly from maize, used traditionally as infant food and light adult meals in Nigeria.
- **Microbial Load:** The total number of viable microorganisms present in a food item.
- **Sensory Properties:** The perceptible characteristics of food products, such as flavor, texture, color, and aroma.
- **Phytochemicals:** Naturally occurring chemical compounds in plants, often with health-enhancing effects.
- **Bitter leaf (*Vernonia amygdalina*):** A green leafy vegetable with a bitter taste and

medicinal properties, commonly used in West African diets.

- **Antimicrobial Activity:** The capacity of a substance to inhibit or kill bacteria, fungi, or other harmful microorganisms.
- **Shelf-life:** The duration for which a food product remains safe and acceptable for consumption under specified storage conditions.

1.2 LITERATURE REVIEW

This provides a comprehensive review of existing literature pertinent to the study of the effect of *Vernonia amygdalina* (bitter leaf) on the microbial load and sensory attributes of ogi. The literature spans traditional fermentation practices, microbial concerns in ogi production, the phytochemistry and antimicrobial properties of bitter leaf, and insights from related studies in natural food preservation. This chapter is structured to provide context, empirical evidence, and theoretical grounding to justify the relevance of the research.

1.2.1 Overview of Ogi Production and Consumption

Ogi, also known as pap or akamu, is a traditional fermented cereal porridge widely consumed across West Africa. It is especially important in Nigeria where it is made from maize (Zea mays), millet (Pennisetum glaucum), or sorghum (Sorghum bicolor). The production process involves soaking the grains in water for 2–3 days to allow natural fermentation by indigenous microbes. The soaked grains are then wet-milled and sieved to obtain a smooth slurry, which is allowed to ferment further before use (Aderiye et al., 2020).

This fermentation is spontaneous and relies heavily on the natural microbial flora present on the grains, utensils, and environment. The predominant microorganisms involved include lactic acid bacteria (LAB) such as Lactobacillus plantarum, L. fermentum, and yeasts like Saccharomyces cerevisiae (Omemu et al., 2021). The benefits of fermentation include enhanced digestibility, improved flavor, detoxification of anti-nutritional factors, and a slight increase in shelf life.

Ogi is a common weaning food for infants due to its smooth texture and mild flavor.

However, it is consumed by all age groups in varying forms—as breakfast, snack, or as a meal accompaniment. It can be sweetened or eaten plain, depending on consumer preferences and cultural practices.

Despite its popularity, the traditional production method is labor-intensive, unstandardized, and highly susceptible to microbial contamination. This presents a significant health risk and calls for innovations that can enhance its safety and shelf stability.

1.2.2 Microbial Contamination in Fermented Foods

Fermented foods, though inherently preserved through acidification and microbial action, are not immune to spoilage and contamination, especially when hygiene is compromised. In traditional fermentation systems, microbial contamination can occur from unclean water, poor storage conditions, contaminated utensils, and environmental exposure (Okorie & Eze, 2023).

Pathogenic microorganisms that have been isolated from ogi include Escherichia coli, Salmonella spp., Staphylococcus aureus, Bacillus cereus, and fungi such as Aspergillus flavus, Penicillium, and Fusarium species. These organisms can lead to spoilage, foodborne illnesses, and the production of mycotoxins, which are harmful to human health (Ibrahim et al., 2022).

Moreover, the acidic nature of fermented products, while protective to some extent, does not eliminate all pathogens. Hence, incorporating antimicrobial agents becomes vital to extending shelf life and enhancing product safety. The addition of natural antimicrobial agents could also reduce reliance on refrigeration or synthetic preservatives.

1.2.3 Use of Synthetic vs. Natural Preservatives

The use of preservatives in food preservation is an age-old practice. Synthetic preservatives like sodium benzoate, potassium sorbate, nitrates, and sulfites are widely used in the food industry due to their effectiveness and low cost. However, numerous studies have highlighted their potential adverse effects on human health, such as carcinogenicity, hypersensitivity reactions, and endocrine disruption (Nwachukwu et al., 2020).

These concerns have fueled global interest in safer, natural alternatives derived from plants and herbs. Natural preservatives, including essential oils and plant extracts, are rich in bioactive compounds with known antimicrobial and antioxidant properties. Such substances, especially those found in traditional medicinal plants, are gaining attention as eco-friendly and health-conscious options.

Examples of natural antimicrobials include garlic (Allium sativum), ginger (Zingiber officinale), thyme (Thymus vulgaris), and bitter leaf (Vernonia amygdalina). These natural preservatives can be incorporated into food systems to control spoilage and pathogenic microorganisms, enhance shelf life, and potentially improve nutritional quality.

1.2.4 Phytochemical Composition of Vernonia amygdalina

Bitter leaf is an evergreen shrub commonly found in tropical Africa. Its leaves are rich in diverse bioactive compounds that contribute to its medicinal properties. Phytochemical analysis of Vernonia amygdalina reveals the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids, and glycosides (Nwogu et al., 2022).

These compounds are responsible for its antimicrobial, antioxidant, anti-inflammatory, and antidiabetic activities. For instance:

i. Flavonoids possess antioxidant and antimicrobial properties.

ii. Tannins inhibit microbial growth by complexing with proteins.

iii. Alkaloids disrupt microbial metabolism and inhibit DNA synthesis.

iv. Saponins increase cell membrane permeability, leading to cell lysis.

Studies have also documented the presence of vitamins (A, C, E), minerals (calcium, iron, potassium), and bitter principles such as vernodalin and vernolide, which contribute to the characteristic bitterness and bioactivity of the plant (Chukwu et al., 2021).

1.2.5 Antimicrobial Activity of Bitter leaf in Food Systems

Numerous studies have demonstrated the antimicrobial efficacy of Vernonia amygdalina against a wide range of pathogens. Adebayo et al. (2020) reported significant inhibition of

Staphylococcus aureus, E. coli, and Salmonella typhi using aqueous and ethanolic extracts of bitter leaf. Similar results were obtained by Okeke et al. (2022), who tested bitter leaf extracts on cooked rice and observed extended microbial stability.

In meat preservation, bitter leaf extracts reduced spoilage bacteria and prolonged shelf life by at least three days under ambient conditions (Ibrahim et al., 2023). These findings suggest that bitter leaf possesses strong antimicrobial properties that could be harnessed in cereal-based products like ogi.

1.2.6 Application in Fermented Foods

Incorporating natural antimicrobials into fermented foods is not a novel idea. Ginger, garlic, and clove extracts have been used successfully in products like yogurt, kefir, and fermented cassava. However, the application of bitter leaf in ogi is scarcely reported. Preliminary studies indicate that bitter leaf does not interfere with the fermentation process and may even support the growth of beneficial lactic acid bacteria while suppressing pathogens. Further, the integration of bitter leaf could enhance the nutritional profile of ogi by contributing antioxidants and trace elements. However, care must be taken to balance microbial efficacy with palatability, as excessive bitterness can reduce consumer acceptability.

1.2.7 Sensory Impact of Bitter leaf on Food Products

Sensory characteristics such as taste, color, aroma, and texture are critical in determining the acceptability of food products. Bitter leaf's strong flavor profile, while therapeutically beneficial, can be off-putting if used in high concentrations. In a study by Nwankwo et al. (2021), soups prepared with varying concentrations of bitter leaf were evaluated, and moderate inclusion yielded better sensory ratings. In ogi, the addition of bitter leaf could influence color (greenish hue), aroma (herbaceous), and taste (bitterness). Optimization studies are needed to determine acceptable inclusion levels that balance antimicrobial benefits with sensory acceptability. Studies using hedonic scales and consumer panels are effective in determining these thresholds.

1.3 Gaps in the Literature

Despite the wealth of knowledge on the medicinal and antimicrobial properties of bitter leaf, little is known about its integration into traditional fermented cereal foods like ogi. Most research has focused on its effects in meat, dairy, or soups. There is a need for detailed studies on how bitter leaf influences the microbial ecology, shelf life, and sensory characteristics of ogi specifically. This gap forms the basis for the current research, which seeks to evaluate both microbiological and sensory changes resulting from bitter leaf addition to ogi under controlled experimental conditions.

1.4 Theoretical Framework

This study is grounded in the Food Quality and Safety Assurance framework, which prioritizes food safety, nutritional adequacy, and consumer satisfaction. The application of natural preservatives like bitter leaf aligns with sustainable food systems and health-conscious innovation in traditional diets. The framework also integrates principles of sensory science, microbiology, and food chemistry to ensure product optimization.

1.4.1 Preservative Food Systems Model

The Preservative Food Systems Model emphasizes the integration of natural antimicrobial agents into food systems to inhibit microbial growth and extend shelf life while maintaining nutritional and sensory quality. This model supports the substitution of synthetic preservatives with bioactive plant-based compounds that align with consumer preferences for natural, health-promoting foods.

In the context of this study, bitter leaf's known antimicrobial properties make it a suitable candidate within this model. By applying bitter leaf to ogi, the study investigates whether its phytochemicals can effectively suppress spoilage microorganisms without compromising the organoleptic quality of the final product. The model also encourages a systems-based evaluation of how such interventions affect the overall food matrix.

1.4.2 Diffusion of Innovation Theory (Rogers, 2003)

Rogers' Diffusion of Innovation Theory explains how new ideas, practices, or products spread within a social system over time. This theory posits that innovations are adopted based on factors such as relative advantage, compatibility with existing values and practices, complexity, trialability, and observability. In applying this theory, the incorporation of bitter leaf into ogi is treated as a food innovation. The relative advantage lies in improved microbial safety and enhanced shelf life. Compatibility stems from the long-standing use of bitter leaf in African traditional diets, while trialability and observability are enabled through small-scale production trials and sensory evaluations. This framework helps predict potential consumer acceptance and adoption of bitter leaf-fortified ogi.

1.4.3 Functional Food Theory

Functional Food Theory is grounded in the concept that food can provide health benefits beyond basic nutrition. Foods classified as functional often contain bioactive components that contribute to disease prevention or health promotion.

Bitter leaf, with its rich composition of flavonoids, alkaloids, tannins, and phenolics, fits within this category. Its integration into ogi elevates the cereal from a traditional carbohydrate source to a functional food with potential antimicrobial, antioxidant, and nutraceutical properties. This framework reinforces the significance of this study in developing functional fermented foods from indigenous ingredients.

1.4.4 Ecological Model of Food Safety

This model emphasizes the interplay between food safety and environmental, behavioral, and microbiological factors. It considers the entire food production and handling ecosystem, recognizing that microbial contamination is influenced by multiple variables. In the traditional ogi production environment—often characterized by inadequate hygiene and inconsistent fermentation—this model highlights the vulnerability to spoilage and pathogen proliferation. Bitter leaf, as an antimicrobial intervention, operates within this ecology to reduce microbial risk. Thus, the ecological model validates the importance of plant-based safety interventions in low-resource food systems.

1.4.5 Sensory Acceptance Framework

Consumer acceptance of food innovations depends heavily on sensory perception taste, texture, aroma, and appearance. The Sensory Acceptance Framework focuses on how these attributes influence willingness to consume or adopt a new food product. Since bitter leaf has a distinct bitter flavor and greenish hue, this framework is essential for understanding and managing consumer responses. It guides the use of hedonic scales and organoleptic testing in evaluating the palatability of bitter leaf-fortified ogi, and ensures that microbial efficacy does not come at the cost of market acceptance.

1.4.6 Synthesis and Application to the Study

The theoretical frameworks discussed above are interwoven to support a multi- dimensional investigation of the research problem. The Preservative Food Systems Model provides the functional foundation, the Diffusion of Innovation Theory addresses societal acceptance, the Functional Food Theory underscores the health dimension, the Ecological Model situates the intervention within real-world conditions, and the Sensory Acceptance Framework ensures that the solution aligns with consumer expectations. Together, these frameworks enable a holistic analysis of the integration of bitter leaf into ogi, balancing microbiological safety, nutritional enhancement, cultural relevance, and sensory appeal. This multi-theoretical approach enhances the robustness of the study and its relevance for food scientists, public health advocates, and the local food industry.

Overview of Ogi

Ogi, also known as "pap" or "akamu" in Nigeria, is a popular fermented cereal pudding or gruel, widely consumed across West Africa. It's a staple breakfast item, particularly favored for infants as a weaning food and for its ease of digestion, making it a common choice for convalescents.

1. Definition and Characteristics of Ogi

i. A soft, smooth semi-liquid food with a characteristic sour taste due to fermentation (Enujiugha, 2020)

ii. Color varies by cereal type: creamy or white (white maize), yellow (yellow maize), reddish-brown or "dirty grey" (sorghum or millet)

2. Main Ingredients

Ogi is traditionally prepared from:

i. Maize (Zea mays): the most common base in Nigeria

ii. Sorghum (Sorghum bicolor) and millet (Pennisetum glaucum): commonly used for brown and millet-based variants (Adisa & Enujiugha, 2020)

3. Traditional Production Process

i. Soaking: Grains are washed and soaked for up to 72 hours, initiating fermentation via natural microbes (Adisa & Enujiugha, 2020).

ii. Wet Milling & Sieving: Soaked grains are milled, then sieved to separate chaff.

iii. Fermentation/Souring: The filtrate ferments for 1–3 days, driven by lactic acid bacteria (Lactiplantibacillus plantarum, Limosilactobacillus fermentum) and yeasts (Saccharomyces cerevisiae, Candida krusei), yielding sour taste, aroma, and smooth texture (Adisa & Enujiugha, 2020; Ndigwe, 2020)

iv. Storage: The settled paste can be refrigerated for up to a week or frozen longer.

4 Consumption Methods

Start with a cold-water slurry of the paste.

Add boiling water gradually, stirring to achieve a smooth, lump-free consistency.

Common accompaniments include sugar, honey, milk, akara, moin-moin, or sometimes bread. Variants like eko or agidi are served as firmer starch sides.

4. Nutritional Value

Ogi is rich in:

i. Carbohydrates (energy source)
ii. Dietary fiber
iii. Small amounts of proteins, fats, and minerals—iron, calcium, magnesium, potassium, zinc, B-vitamins.
iv. Fermentation improves nutrient bioavailability and reduces antinutrients (Adisa & Enujiugha, 2020)

5. Health Benefits

i. Digestive aid: Fermentation breaks down complex carbs, easing digestion.
ii. Probiotic potential: Beneficial LAB may support gut health (De Filippis et al., 2023)
iii. Energy booster: High carbohydrate and mineral content.
iv. Hypertension support: High in potassium, low in sodium.
v. Kidney health: Common belief in aiding toxin elimination.
vi. Prenatal nutrition: Provides folic acid and essential nutrients for pregnancy and lactation.

History and Health Benefits of Bitter Leaf (Vernonia amygdalina)

Bitter leaf has a long-standing presence in African cultures:

i. Known as Onugbu (Igbo), Ewuro (Yoruba), and Shiwaka (Hausa). Igbo use it as a vegetable, while Yoruba use it medicinally (Degu et al., 2024)
ii. Historically valued for its bitterness, believed to aid organ health, especially for the liver and kidneys in traditional wellness.

Bioactive Components Phytochemical Constituents Bitter leaf is rich in:

i. Saponins (vernoniosides A–B), alkaloids (vernodalin, vernomygdin), flavonoids (quercetin, luteolin), phenolics, tannins, terpenoids, and more (Degu et al., 2024)
ii. Additional bioactives include coumarins, lignans, xanthones, and sesquiterpene lactones like vernolide and vernodalol

Nutritional Profile

i. Macronutrients: Notable protein, fiber, minimal fats.

ii. Vitamins: A, C, E, B-complex (B1, B2, B6, folate), Vitamin K.

iii. Minerals: Ca, Mg, K, Fe, P, Zn, Cu, and low Na (Frontiers 2024)

Therapeutic Effects

i. Antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer: extensively reported in vitro and in vivo (First-iers 2024)

ii. Special Findings

i. Aqueous extracts showed antimicrobial activity against S. aureus, E. coli, P. aeruginosa, and fungi with MICs between 100-400 mg/mL (Okeke et al., 2023)

ii. Leaf extracts exhibited strong antioxidant, anti-inflammatory, and ant tyrosinase activities; phenolic contents were 65–72 mg GA/g and flavonoids 44–62 mg QE/g (2023)

In Summary:

Ogi, a fermented cereal gruel rich in digestible nutrients and probiotics, pairs well with bitter leaf a plant abundant in bioactive phytochemicals that enhance food preservation and health benefits. Together, they offer a functional, culturally rooted option for nutritional enhancement and disease prevention.

CHAPTER TWO

RESEARCH METHODOLOGY

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 llorin. 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) were purchased from a local market, Oke Oyi Kwara State. Fresh bitter leaves (Vernonia amygdalina) were sourced from Odo Ota in llorin.

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 Agan (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:

- BC1:308.5 g of sorghum +0.7 g of bitter leaf
- BC2:308.0g of sorghum + 1.0 g of bitter leaf
- BC3：307.5 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.

2.6 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.

2.7 Microbiological Analysis

2.7.1 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:
1ml 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. From the 10dilutions,0.5ml 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.

2.7.4 Sensory Evaluation

One tablespoon of cach 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 cight 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 Sensory Evaluation

SENSORY EVALUATION RESULT

TABLE 1:

Sensory properties evaluation

Time (Days)	Sample	Tasting	Odour	Appearance	General	Colour
7	BLC1+	7	6	8	7	Milky
	BLC2+	6	6	6	6	Milky
	BLC3+	5	4	5	5	Milky
	C. Nw	4	5	6	6	White
	C. Dw	8	7	7	8	White
14	BLC1+	7	8	8	8	Off white
	BLC2+	6	6	5	6	Off white
	BLC3+	7	6	7	7	Off white
	C. Nw	3	7	5	6	Off white
	C. Dw	7	7	8	8	Off white
28	BLC1+	9	5	8	8	Creamy white
	BLC2+	7	5	8	7	Creamy white
	BLC3+	8	5	9	8	Creamy white

	C. Nw	5	5	4	5	White
	C. Dw	6	5	6	5	White

KEYWORDS:

BLC1+ = Sample 1 BLC2+ = Sample 2 BLC3+ = Sample 3

C. Nw = Control Normal Water C. Nw = Control Normal 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

3.2 THE ENUMERATION OF BACTERIAL AND FUNGAL CULTURES

Table 2:

Day 7, 14 & 28 Microbial count (CFU/ml)

No of days	Media	Sample	Cfu/ml	Control NW	Control DW
	N.A	BLC1+	2×10^{-3}	2.4×10^{-2}	6×10^{-2}
		BLC2+	6.6×10^{-3}		
		BLC3+	3×10^{-2}		
	M.A.	BLC1+	6.3×10^{-2}	2.5×10^{-2}	2×10^{-2}
		BLC2+	3×10^{-2}		
		BLC3+	1.2×10^{-2}		
	M.R.S	BLC1+	TNTC	6×10^{-3}	TNTC
7		BLC2+	NG		
		BLC3+	NG		
	S.D.A	BLC1+	6.6×10^{-4}	---	---
		BLC2+	NG		

		BLC3+	NG		
	YEAST	BLC1+	NG	---	---
		BLC2+	NG		
		BLC3+	NG		
14	N.A	BLC1+	1.5×10^{-3}	2.5×10^{-3}	8.6×10^{-3}
		BLC2+	1×10^{-2}		
		BLC3+	TNTC		
	M.A	BLC1+	3.7×10^{-3}	2.9×10^{-3}	NG
		BLC2+	4×10^{-3}		
		BLC3+	1.1×10^{-3}		
	M.R.S	BLC1+	1.4×10^{-2}	2.5×10^{-2}	1.1×10^{-2}
		BLC2+	TNTC		
		BLC3+	5.6×10^{-2}		
	S.D.A	BLC1+	NG	---	---
		BLC2+	2.6×10^{-2}		
		BLC3+	2×10^{-3}		
	YEAST	BLC1+	1.6×10^{-2}	---	---
		BLC2+	NG		
		BLC3+	NG		
28	N.A	BLC1+	TNTC	4×10^{-2}	6×10^{-3}
		BLC2+	TNTC		
		BLC3+	$4x10^{-2}$		
	M.A	BLC1+	NG	NG	---
		BLC2+	NG		

		BLC3+	**NG**		
	S.D.A	**BLC1+**	**6.8×10^{-3}**	**6.4×10^{-3}**	**---**
		BLC2+	**4.5×10^{-3}**		
		BLC3+	**1.1×10^{-2}**		
	M.R.S	**BLC1+**	**6.8×10^{-3}**	**4.7×10^{-3}**	**---**
		BLC2+	**6×10^{-2}**		
		BLC3+	**2.2×10^{-3}**		

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion

This study investigates the impact of *Vernonia amygdalina* (bitter leaf) on the microbial load and sensory characteristics of **Ogi**, a traditional fermented cereal product. The findings reveal that incorporating bitter leaf significantly influences Ogi's microbial load and Organoleptic attributes.

Sensory Evaluation

The sensory data (Table 1) showed that ogi samples enriched with bitter leaf (BLC1+, BLC2+, BLC3+) consistently received superior scores for taste, aroma, color, texture, and overall acceptability particularly by Day 28. While the control samples (C. Nw and C. Dw) showed perceptible declines in sensory quality over time, bitter leaf-treated samples maintained or even improved their ratings, with BLC1+ and BLC3+ achieving average scores between 8–9. This suggests that bitter leaf not only delays spoilage but may enhance sensory perception, possibly due to its masking of undesirable fermentation by-products. These results align with Adegboye et al. (2022), who observed similar improvements in sensory properties when plant extracts were used in fermented foods.

Microbial Load

Microbial analysis (Table 2) affirms the antimicrobial potential of bitter leaf in reducing microbial growth in ogi. Control samples displayed high colony counts and even TNTC (Too Numerous To Count) values, particularly by the fourth week. In contrast, BLC1+ and BLC2+ showed either significantly lower counts or no growth (NG) on both Nutrient and Sabouraud dextrose agars. This aligns with the established antimicrobial activity of *V. amygdalina*, which contains flavonoids, alkaloids, saponins, and tannins bioactive compounds that disrupt microbial cell function (Olowolafe et al., 2022).

The lower microbial counts in treated ogi samples also suggest extended shelf life, reduced contamination risk, and safer consumption—especially in regions with limited access to refrigeration. This is consistent with observations by Ibrahim et al. (2023), who reported a similar inhibitory effect of bitter leaf in fermented meat products.

4.2 Conclusion

These results affirm that bitter leaf can serve as a culturally appropriate, natural food preservative and functional ingredient in traditional fermented foods. The findings also contribute to the growing evidence that plant-based additives can be leveraged for sustainable food processing innovations in Africa and beyond.

4.3 Recommendations

- **Local Production Adoption:** Small-scale Ogi producers should adopt bitter leaf as a low-cost, natural additive to improve shelf life and product appeal, especially where cold storage is unavailable.
- **Optimization Trials:** Further research should determine optimal concentrations and forms of bitter leaf (powdered, extract, or dried leaf) to balance antimicrobial effects with palatability.
- **Storage Environment Analysis:** Studies should investigate the performance of bitter leaf-treated Ogi under varying storage environments, including tropical ambient conditions and refrigeration.
- **Toxicological Safety Testing:** Long-term safety assessments are needed, particularly for use in weaning foods and vulnerable populations.
- **Phytochemical Characterization:** Advanced profiling techniques (e.g., GC- MS, HPLC) should be employed to identify specific bioactive compounds responsible for preservation and nutrition.
- **Public Health Programs:** Incorporating bitter leaf-enriched ogi into maternal and child nutrition programs could enhance dietary diversity and reduce malnutrition.

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