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

BY

ABDULROSHEED WALIYAT YETUNDE
HND/23/SLT/FT/0639

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

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) DEGREE IN SCIENCE LABORATORY TECHNOLOGY, INSTITUTE OF APPLIED SCIENCES (IAS), MICROBIOLOGY UNIT

JULY, 2025

CERTIFICATION

This is certify that this project is the original work carried out and reported by ABDULROSHEED, Waliyat Yetunde with matriculation number HND/23/SLT/FT/0639 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 fulfillment of the requirements of the Award of Higher National Diploma (HND) In Science Laboratory Technology

MRS. ADEBOYE T. O. **(PROJECT SUPERVISOR)**	**DATE**
MRS. AHMED T. **(HOU MIROBIOLOGY)**	**DATE**
DR. USMAN A. **(HEAD OF DEPARTMENT)**	**DATE**
External Examiner	**DATE**

DEDICATION

This project is dedicated to the Almighty God for His guidance and strength throughout our academic journey. We al-so 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).

Special appreciation goes to our indefatigable Head of Department, DR. USMAN A., for his continuous support and leadership, as well as to our Head of Unit, MRS. HAMMED, for her guidance and concern throughout the course of this project.

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).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study investigated the effects of incorporating bitter leaf (Vernonia amygdalina) into ogi on its microbial load, sensory properties, and nutritional composition over a 28-day storage period. Three fortified samples (BLC1-, BLC2-, BLC3-) and two control samples (using normal water and distilled water) were monitored at 7, 14, and 28 days. Results showed that the bitter leaf-fortified ogi exhibited significantly lower microbial counts, with some media showing no growth, indicating the antimicrobial potential of V. amygdalina. Sensory evaluation revealed improved acceptability in terms of taste, odour, appearance, and general preference, particularly in BLC3-. Nutritional analysis indicated higher crude protein and lower moisture content in the fortified samples, suggesting better nutritional quality and shelf stability. Titratable acidity measurements showed that the test samples had a more controlled fermentation profile compared to controls. Beneficial microbes such as Lactobacillus spp. were isolated from test samples, while spoilage organisms like Staphylococcus aureus and Pseudomonas aeruginosa were found in controls. Fungal isolates like Aspergillus niger and Candida krusei were more prevalent in control samples. These findings support the potential of bitter leaf as a natural preservative and nutritional enhancer in traditional fermented foods like ogi.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Taxonomy and Description of Bitter leaf

Bitter leaf is commonly known by its scientific name, *Vernonia amygdalina*, a member of the Asteraceae family. This plant is widely distributed in tropical Africa and is well recognized for its characteristic bitter taste and broad ethnobotanical applications. The genus *Vernonia* comprises over 1,000 species, but *V. amygdalina* is the most extensively studied in terms of medicinal, nutritional, and industrial applications (Omoregie & Folashade, 2020). It is a perennial shrub or small tree that can grow up to 3 meters in height, distinguished by its rough bark and ovate leaves. *V. amygdalina* is a non-aromatic plant and does not have the typical fragrance of culinary herbs, but its chemical profile compensates for its distinct bitterness.

Vernonia amygdalina, widely recognized in African traditional medicine, has undergone taxonomic revisions and is now firmly classified under the Asteraceae family, supported by molecular tools like DNA barcoding (Chukwuma et al., 2022). Accurate scientific naming is essential for distinguishing it from other Vernonia species with differing phytochemical profiles and ensures consistency in food, pharmacological, and microbiological research (Ekpenyong et al., 2021; Ibrahim et al., 2021). The species name 'amygdalina' reflects the bitter taste of its leaves, attributed to compounds such as vernodalin and vernomygdin (Abbah et al., 2020). Its identification supports standardization in experimental protocols, facilitates international research collaboration, and underpins its inclusion in pharmacopeias and global regulatory frameworks (Ezekwesili et al., 2023). In food science, particularly studies involving sorghum processing and preservation, the correct naming allows reproducibility and traceability of results, linking antimicrobial and antioxidant effects to the plant’s validated bioactive profile (Nkongho et al.,

2021; Adedayo et al., 2022). Overall, the taxonomy of V. amygdalina is central to its application in ethnobotany, nutrition, and biotechnology.

Fig.1: Bitter leaf

Source: (Ekpenyong et al., 2021).

Varieties of Bitter Leaf

Vernonia amygdalina, commonly referred to as bitter leaf, is not typically divided into formally recognized botanical varieties like many cultivated crops. However, within various regions of Africa**,** local ecotypes and landraces of *V. amygdalina* exist, often differentiated by leaf size**,** bitterness level, and growth habit (Afolabi et al., 2020). These differences are largely influenced by environmental conditions and traditional cultivation practices rather than distinct genetic markers. In Nigeria, for example, farmers refer to different types such as "short broad-leafed," "long narrow-leafed," and "smooth-edged" types, although these are not officially classified in botanical nomenclature. Despite the lack of formal taxonomic distinction, local naming and preference play a significant role in determining which variety is cultivated for culinary or medicinal use (Ogundele et al., 2021). The absence of formal varietal registration presents a challenge to standardization and commercial-scale production. Nonetheless, morphological differences are well-documented, and efforts are being made to characterize these variations

genetically (Nwafor et al., 2022). In some cases, these forms are even regarded as separate types in local seed systems.

Researchers have proposed classifying *V. amygdalina* based on leaf texture, bitterness intensity, and oil content**,** which can indirectly affect its nutritional and medicinal applications. According to Ayoade et al. (2021), two common field types are often mentioned among farmers: "wild bitter leaf" (more bitter and less commonly consumed directly) and "domesticated bitter leaf" (less bitter and more widely used in cooking).

Fig. 2: Wild bitter leaf and domesticated bitter leaf

Source: (Ayoade et al., 2021)

The wild variant is usually found growing in the wild or forest margins and is noted for its intense bitter compounds such as lactones and steroidal saponins. In contrast, the domesticated version is usually propagated through stem cuttings and is commonly seen in home gardens and farms. These differences reflect not only phenotypic variation but also varying levels of bioactive compounds,

which can influence food formulation, such as when used in sorghum-based products. While both types belong to the same species, they may offer different benefits in terms of shelf-life enhancement and antimicrobial activity, which are vital for sorghum preservation. Still, more studies are needed to establish if these field distinctions correlate with molecular or biochemical differences. This underscores the need for further genetic mapping and conservation efforts.

In Cameroon and parts of East Africa, ethnobotanical surveys have identified region-specific types of *Vernonia amygdalina* used differently based on cultural preferences (Mbanya et al., 2020). In these regions, the plant may be known by names like “ndolé” or “mubirizi,” and local knowledge indicates that certain varieties are more appropriate for medicinal teas, while others are used in sauces or as feed. These distinctions are based on organoleptic properties (taste, smell, texture) rather than official botanical classification. Ethnopharmacological records suggest that farmers recognize varieties based on growth rate, pest resistance, and leaf yield. However, formal agronomic studies validating these variants are still limited. Such variation is vital in food-based studies since some varieties may contain higher concentrations of antioxidants or antimicrobial agents relevant for shelf-life extension. As bitter leaf becomes increasingly commercialized, researchers and agricultural extension officers are calling for the establishment of varietal banks and germplasm collections. These would help protect against biodiversity loss and encourage targeted use in food processing and nutrition.

Efforts are underway to standardize these types using molecular markers and phytochemical profiling. Recent advances in DNA barcoding and fingerprinting have helped researchers begin to distinguish between clones and phenotypes of *V. amygdalina* grown in different ecological zones (Chikezie et al., 2023). By analyzing inter-simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD), researchers have identified distinct genetic lines that may qualify as

cultivars. This has significant implications for food science applications, especially in fortifying sorghum-based products with the most suitable variety of bitter leaf. In this light, different ecotypes may offer different phytochemical benefits, such as higher flavonoid content or stronger antimicrobial efficacy, impacting the nutritional value and shelf life of food. Hence, cataloging and conserving these genetic resources is necessary for future food security efforts. Although still early, the progress in varietal identification signals the need for breeding programs tailored to nutritional enhancement.

Ultimately, even without officially recognized varietal classifications, the diverse local names, physical characteristics, and uses of *V. amygdalina* across Africa reflect its agroecological adaptability and versatility. For food-based research, this informal varietal differentiation provides a valuable framework for selecting appropriate types for nutritional and preservation purposes. For instance, some variants may produce more aqueous extract, making them more effective for wet-milled sorghum enhancement (Uche et al., 2021). Others might be less bitter and better suited for food applications without extensive processing. Researchers are therefore encouraged to document local knowledge while also conducting scientific analyses to link traditional classifications to measurable attributes. This dual approach strengthens both academic validation and community engagement, ensuring that traditional practices inform modern food science. In future work, especially in sorghum fermentation and storage, selecting the right bitter leaf variant may significantly influence the outcome of such functional applications.

Cultivation and origin

Bitter leaf (*Vernonia amygdalina*) is native to tropical Africa, where it has been used for centuries in food, medicine, and cultural rituals. Its origin is traced to the sub-Saharan regions**,** particularly West and Central Africa, with Nigeria, Cameroon, and Ghana being major areas of natural

occurrence and usage (Okoye et al., 2021). It grows wild in forests and is also domesticated for culinary and therapeutic purposes. Over time, its cultivation has expanded to East and Southern Africa due to its adaptability to diverse environments and increasing awareness of its health benefits (Oladipo et al., 2020). Ethnobotanical evidence supports its long-standing use in traditional African medicine, especially for malaria, diabetes, and gastrointestinal issues. Although largely African in origin, the plant is also grown in tropical Asia and parts of the Caribbean for medicinal purposes. Its spread beyond Africa is driven by migration and international trade in herbal medicine. The history of *V. amygdalina* underlines its multi-functional value across many African societies. Today, its economic importance is increasing due to its nutraceutical potential**.**

Bitter leaf thrives in warm, humid tropical climates, making it well-suited for cultivation in lowland regions with rainfall between 1000–2000 mm/year. It prefers loamy, well-drained soils**,** but is highly tolerant to a variety of soil types, including sandy or lateritic soils (Ndukwu & Omaliko, 2022). The plant grows best under full sunlight but can also survive in partially shaded environments. Cultivation requires minimal input; it is a hardy plant resistant to pests and drought once established. Bitter leaf can be propagated through stem cuttings, which root easily when planted in moist soil. Although it can be grown from seeds, vegetative propagation is more common due to higher success rates. The plant typically reaches 2–5 meters in height and can be harvested multiple times throughout the year. Leaves are plucked regularly, promoting bushy growth and increasing yield. Bitter leaf's adaptability makes it an ideal crop for rural and urban agriculture alike.

Modern cultivation of *Vernonia amygdalina* involves spacing plants 60–100 cm apart**,** applying organic manure or compost to enhance growth, and routine pruning to encourage leafy biomass production. Farmers often intercrop bitter leaf with maize, cassava, or okra to maximize land use

efficiency (Ezeokoli et al., 2023). Pest management is usually minimal due to the plant's natural phytochemical defenses, which repel many common insects. However, fungal leaf spots and root rot may occur in overly wet conditions, requiring drainage or fungicide application. Commercial-scale cultivation often integrates irrigation and organic soil enhancement to boost yield and reduce stress during dry seasons. Harvesting starts within 8–12 weeks after planting, with frequent leaf plucking done every 2–3 weeks. The harvested leaves are either used fresh, dried, or processed into extracts. Post-harvest handling involves washing, squeezing, and drying**,** especially when used in food or storage-enhancing applications.

In many African countries, especially Nigeria, bitter leaf cultivation is a source of income and employment for rural households. It is sold fresh in local markets or processed into powders, extracts, and herbal teas. Women play a major role in the cultivation and processing of bitter leaf, especially in subsistence farming systems (Abah et al., 2020). Increasing urban demand has led to more organized farming systems, including the use of greenhouses and hydroponic techniques in peri-urban areas. With rising awareness of its medicinal properties, bitter leaf is also being considered for commercial cultivation and export. Additionally, it contributes to food security, as it is easy to grow, requires minimal resources, and adds nutritional and antimicrobial benefits to traditional dishes like soups and porridges. Governmental and research institutions are beginning to promote bitter leaf cultivation under agro-industrial schemes, particularly those focusing on nutraceuticals and functional foods, including its potential role in enhancing sorghum-based products**.**

Phytochemical Properties

Bitter leaf (Vernonia amygdalina) is widely studied for its rich phytochemical profile, which contributes to its bitter taste and numerous therapeutic benefits. The leaf contains a variety of

bioactive compounds including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds (Ezeokoli et al., 2021). These phytochemicals are known to exhibit antioxidant, anti-inflammatory, antimicrobial, and antimalarial activities. Flavonoids and phenolic acids in particular play a key role in scavenging free radicals, thereby protecting cells from oxidative damage. The bitter taste is primarily attributed to sesquiterpene lactones, which are also responsible for the plant's antimicrobial and antimalarial effects (Adamu et al., 2020). This complex mixture of secondary metabolites makes bitter leaf a valuable plant for both food and medicinal applications.

Studies have demonstrated that bitter leaf extracts contain high levels of polyphenols and flavonoids, which contribute significantly to its strong antioxidant capacity (Nworu et al., 2022). These antioxidants help neutralize reactive oxygen species (ROS), reducing oxidative stress linked to chronic diseases. The leaf also contains alkaloids and saponins that exhibit potent antimicrobial activities against a broad spectrum of bacteria and fungi (Abdullahi et al., 2020). This property is especially relevant for food preservation and enhancing shelf life, as the phytochemicals can inhibit microbial growth in processed foods such as wet-milled sorghum. The antimicrobial properties have been linked to the disruption of microbial cell walls and inhibition of enzyme activities essential for pathogen survival. Thus, the phytochemical composition supports both health and food safety applications.

The phytochemical content in bitter leaf varies depending on factors such as geographical location, soil quality, harvesting time, and extraction method (Ogundipe et al., 2021). For instance, leaves harvested during the rainy season tend to have higher flavonoid and phenolic contents due to increased plant metabolism. Different solvents used in extraction (water, ethanol, methanol) also influence the type and concentration of phytochemicals obtained, affecting their bioactivity

(Umechuruba & Nwosu, 2023). Water and ethanol extracts are most commonly used, with ethanol extracts often showing higher concentrations of phenolic compounds. Understanding these variations is critical when applying bitter leaf in food systems, such as in wet-milled sorghum, to ensure consistent nutritional and preservative effects.

Nutritional composition

Bitter leaf (*Vernonia amygdalina*) is widely recognized not only for its medicinal properties but also for its rich nutritional profile. It contains a significant amount of essential nutrients such as proteins, carbohydrates, dietary fiber, vitamins, and minerals that contribute to its value as a leafy vegetable (Adebayo et al., 2021). The leaves are particularly rich in vitamins A, C, and E, which function as antioxidants and support immune health. Moreover, the presence of B-complex vitamins like riboflavin and niacin aids in energy metabolism. These nutrients are important for human health and are among the reasons why bitter leaf is commonly used in traditional diets across Africa, often incorporated into soups and stews to enhance both flavor and nutritional quality.

In addition to vitamins, bitter leaf is an excellent source of minerals crucial for various physiological functions. Studies have shown that it contains appreciable amounts of calcium, potassium, magnesium, and iron (Ogunlade et al., 2020). Calcium and magnesium are vital for bone health, while potassium plays a key role in maintaining electrolyte balance and cardiovascular function. The iron content is beneficial for preventing anemia, especially in populations prone to iron deficiency. The dietary fiber content in bitter leaf also supports digestive health by promoting regular bowel movements and reducing cholesterol levels. This combination of nutrients makes bitter leaf a functional food with multiple health benefits beyond basic nutrition.

The nutritional composition of bitter leaf varies depending on environmental factors, maturity stage, and processing methods. Fresh leaves tend to have higher vitamin C levels, which can decrease upon drying or cooking due to heat sensitivity (Ijeh & Ejike, 2022). However, processing techniques like blanching and fermentation can enhance the bioavailability of some nutrients while reducing antinutritional factors such as oxalates and phytates. These antinutrients, if consumed in excess, may interfere with mineral absorption. Hence, proper preparation of bitter leaf not only improves taste but also optimizes its nutritional benefits. Overall, the diverse nutrient content and health-promoting compounds in bitter leaf make it a valuable addition to diets aimed at improving nutrition and health outcomes.

Table 1: Nutritional Composition of Bitter Leaf (*Vernonia amygdalina*)

Nutrient	Approximate Content per 100g (Dry Weight)	Function/Benefit
Protein	18–22%	Supports tissue repair, immune function
Carbohydrates	40–45%	Provides energy
Dietary Fiber	15–18%	Aids digestion, lowers cholesterol
Vitamin A (β-carotene)	5,000–8,000 IU	Promotes vision, skin, and immune health
Vitamin C	50–200 mg	Antioxidant, enhances iron absorption
Vitamin E	4–6 mg	Antioxidant, supports skin and heart health
Riboflavin (B2)	0.2–0.4 mg	Energy metabolism, red blood cell formation
Niacin (B3)	0.5–0.8 mg	Supports energy metabolism and nervous system
Calcium	200–300 mg	Bone development and nerve function
Potassium	400–600 mg	Regulates blood pressure and fluid balance
Magnesium	100–150 mg	Muscle function, enzyme activity
Iron	5–8 mg	Prevents anemia, supports oxygen transport
Moisture Content	8–12% (dry leaf)	Affects shelf life and storage stability
Ash Content	9–11%	Reflects total mineral content

Source: (Ijeh & Ejike, 2022).

Health and medicinal use of bitter leaf

Bitter leaf is widely used in traditional African medicine for its broad range of therapeutic properties. One of its most prominent uses is in the management of malaria and fever. The leaf extract contains bioactive compounds such as flavonoids, saponins, alkaloids, and sesquiterpene lactones like vernodalin and vernomygdin, which exhibit strong antimalarial and antipyretic properties. Decoctions made from the leaves are commonly consumed to reduce fever and combat symptoms associated with malaria, making bitter leaf a staple in herbal treatment across many communities.

In addition to its antimalarial effect, bitter leaf is known for its role in supporting digestive health and managing diabetes. The high fiber content helps regulate bowel movement, while the plant's bitter principles stimulate digestive enzymes and bile production, which enhances nutrient absorption and appetite. Furthermore, studies have shown that bitter leaf can significantly reduce blood glucose levels, making it beneficial for people with diabetes. Its antioxidant components also help protect pancreatic cells, thus supporting insulin function and reducing oxidative stress in diabetic individuals.

Bitter leaf also contributes to cardiovascular and liver health. Its rich content of vitamins A, C, and E, along with minerals like potassium and magnesium, helps regulate blood pressure and supports heart function. The hepatoprotective properties of bitter leaf have been demonstrated in studies where its extracts reduced liver enzyme levels, indicating its potential in treating liver-related disorders such as hepatitis and fatty liver disease. Additionally, its anti-inflammatory and antimicrobial properties make it effective in treating infections, wounds, and skin conditions, reinforcing its reputation as a versatile medicinal plant.

Advantages especially in food related research

Bitter leaf (*Vernonia amygdalina*) holds significant advantages in food-related research due to its rich nutritional and bioactive compound profile. Its leaves are known to contain essential vitamins, minerals, and phytochemicals such as flavonoids, alkaloids, tannins, and saponins, which contribute to its antioxidant and antimicrobial properties (Adeyemo et al., 2021). These compounds help inhibit microbial growth in food products, thereby potentially extending shelf life and improving food safety. Researchers have explored bitter leaf as a natural preservative alternative to synthetic chemicals, which are increasingly scrutinized for their health risks. Its inclusion in food formulations can thus enhance nutritional quality while promoting consumer health through functional food development.

In addition to its preservative qualities, bitter leaf exhibits strong antioxidant activity that helps reduce oxidative spoilage in food matrices. Lipid oxidation is a primary cause of rancidity in many food products, leading to off-flavors and reduced nutritional value. Extracts from bitter leaf have been shown to scavenge free radicals effectively, which can mitigate oxidative degradation during food storage (Okoye & Chukwu, 2022). This property is particularly beneficial in processing cereals like sorghum, where oxidative stress can compromise both shelf life and nutrient retention. Incorporating bitter leaf extracts in wet-milled sorghum or other cereal-based foods could improve stability without compromising taste, a crucial factor in consumer acceptance.

Another important advantage of bitter leaf in food research is its potential to improve the nutritional composition of processed foods. The leaves contain appreciable levels of proteins, fibers, vitamins A, C, and E, and minerals such as calcium, potassium, and iron (Ezeokoli et al., 2023). When added to staple foods like sorghum, bitter leaf can enrich the micronutrient content, addressing common dietary deficiencies in populations relying heavily on cereal grains. This is

especially critical in regions where malnutrition is prevalent, and access to diverse diets is limited. Thus, bitter leaf offers a practical means of biofortifying foods naturally and affordably, complementing other fortification strategies.

Bitter leaf also demonstrates antimicrobial effects against several foodborne pathogens and spoilage microorganisms, which can help in reducing foodborne illnesses and spoilage rates. Studies have reported inhibitory action of bitter leaf extracts against bacteria such as *Escherichia coli*, *Staphylococcus aureus,* and *Salmonella* spp. (Ndukwu & Okeke, 2020). This antimicrobial property makes it a promising candidate for natural food preservatives, especially in developing countries where refrigeration and modern preservation technologies may be limited. Integrating bitter leaf in food products or packaging materials could reduce dependency on synthetic preservatives while ensuring microbial safety.

Bitter leaf's bitter taste and aroma can influence sensory properties of food products, which can be advantageous or challenging depending on the application. In some traditional African cuisines, the bitterness is appreciated and associated with health benefits, making bitter leaf a culturally accepted ingredient (Abah et al., 2021). In food product development, bitterness can be modulated through processing techniques such as blanching or drying to optimize consumer acceptability. This flexibility allows researchers and food manufacturers to tailor the sensory profile while leveraging bitter leaf's functional benefits. Overall, its multipurpose advantages in nutrition enhancement, preservation, and food safety position bitter leaf as an important focus in food science research.

Potential Application in Sorghum Processing and Storage

The incorporation of bitter leaf (*Vernonia amygdalina*) in sorghum processing presents promising opportunities for enhancing both the nutritional quality and shelf life of sorghum-based products.

Sorghum is a staple cereal crop in many parts of Africa but is often limited by its relatively low nutrient content and susceptibility to rapid spoilage, especially in wet-milled forms (Adeyemi et al., 2021). Adding bitter leaf, which is rich in antioxidants, vitamins, and bioactive compounds, can help enrich the nutritional profile of sorghum flour or batter. This integration supports food security by improving the nutrient density of sorghum foods, making them more beneficial for vulnerable populations that rely heavily on cereals for daily calories.

From a preservation perspective, bitter leaf contains natural phytochemicals such as flavonoids, phenolic acids, and sesquiterpene lactones, which exhibit strong antimicrobial and antioxidant properties (Chukwuemeka et al., 2022). These compounds can inhibit microbial growth and oxidative degradation that typically cause spoilage in wet-milled sorghum. Incorporating bitter leaf extracts or powders into sorghum batter may delay fermentation, reduce mold growth, and extend shelf life without the need for synthetic preservatives. This is particularly valuable in regions where refrigeration is limited or unavailable, providing a cost-effective and natural solution to post-harvest losses.

In sorghum processing, bitter leaf can also improve sensory qualities such as taste and aroma. While the inherent bitterness of the leaf may be a challenge, careful processing and dosage optimization can balance flavor profiles to enhance consumer acceptance (Ibrahim et al., 2020). Additionally, bitter leaf's fibrous content can contribute to better texture and water retention in wet-milled sorghum products like porridges and beverages. Research indicates that integrating bitter leaf with sorghum can result in novel functional foods that combine traditional flavors with added health benefits, appealing to health-conscious consumers and opening new markets for sorghum-based foods.

Furthermore, bitter leaf's potential extends beyond processing into storage innovations. Extracts from the leaf could be developed into natural food coatings or packaging additives that protect sorghum products from oxidation and microbial contamination during storage and transportation (Nkwocha et al., 2023). Such applications align with the increasing global demand for clean-label, environmentally friendly food preservation methods. By harnessing bitter leaf's bioactive compounds, sorghum processors and farmers could reduce spoilage rates, enhance product safety, and increase the shelf life of sorghum foods, ultimately contributing to sustainable food systems and improved livelihoods.

Taxonomy and Description of Sorghum

The scientific name of sorghum is *Sorghum bicolor* (L.) Moench. It belongs to the family *Poaceae* (also known as Gramineae), which includes many important cereal crops. The species name "bicolor" reflects the variation in grain color found among different cultivars. The genus *Sorghum* contains around 25 recognized species, but *S. bicolor* is the most widely cultivated for food and industrial use (Mace et al., 2021). The name was first described by Carl Ludwig Willdenow and later modified by Conrad Moench. The inclusion of "L." in the name indicates that Linnaeus first classified the plant. Its naming and taxonomy have provided a foundation for global research and classification efforts. This scientific identification is essential for breeding, conservation, and trade purposes. It also helps researchers track genetic modifications and biodiversity. Accurate scientific naming helps distinguish between cultivated and wild sorghum types in studies.

Sorghum has a diploid genome with 2n = 20 chromosomes and has been fully sequenced, enabling genetic improvement and adaptation research. As a C4 plant, *Sorghum bicolor* is known for its efficient carbon fixation mechanism, making it highly productive under high temperatures and low water availability (Zhao et al., 2020). The crop is native to Africa but is now grown in tropical and

subtropical regions worldwide. Due to its robustness, it is one of the top five cereal crops globally, after wheat, rice, maize, and barley. Its scientific name is used in agronomy, food science, and molecular biology to identify studies related to sorghum-based innovations. The taxonomic clarity of *S. bicolor* is vital for international collaborations in sorghum research. It also allows for the correct identification in herbariums, gene banks, and botanical documentation. Studies involving wild relatives such as *Sorghum halepense* and *Sorghum propinquum* often compare them to *S. bicolor* to understand stress resistance traits. These comparisons help breeders develop improved cultivars.

The correct use of the scientific name *Sorghum bicolor* supports global standardization in trade and agriculture. Researchers and policymakers rely on this name when developing international food policies and regulations. It also ensures consistency in scientific publications and databases such as GenBank and GRIN. The species is known for its significance in bioenergy production, where it is utilized for ethanol and biomass fuel due to its high sugar content and drought tolerance (Farré et al., 2022). Proper identification prevents the misapplication of findings to unrelated grass species. It ensures that cultivars developed for dry zones, for example, are not confused with species suited for wet environments. The continued recognition and use of *Sorghum bicolor* in global scientific discourse strengthen efforts in food security and climate-smart agriculture. Hence, its scientific classification remains foundational in botany, food science, and crop improvement programs.

Varieties of Sorghum

Sorghum exhibits significant genetic diversity and is broadly classified into five major races based on spikelet and panicle characteristics: bicolor, guinea, caudatum, kafir, and durra. Each race has unique traits and regional adaptations. For instance, the guinea race is common in West Africa and

is known for its open panicles, while the kafir type is prominent in Southern Africa due to its drought resistance (Mace et al., 2021). Besides these botanical races, sorghum varieties are grouped into types based on end-use: grain sorghum, sweet sorghum, forage sorghum, and broomcorn. Grain sorghums are cultivated mainly for human consumption. Sweet sorghums are used for syrup and ethanol production. Forage sorghums provide livestock feed, and broomcorn is used to make brooms. This classification helps guide cultivation and research based on specific industrial or nutritional goals.

Color is an important distinguishing feature among sorghum varieties. The grains can be white, red, brown, yellow, or black fig. 3, each influencing its nutritional profile and application. White sorghum, such as the variety ‘SAMSORG 17’, is preferred for human consumption due to its mild flavor and low tannin content (Farré et al., 2022). Red and brown sorghums, such as ‘KSV8’ and ‘CSH 14’, contain higher levels of phenolic compounds and tannins, making them more suitable for brewing and animal feed. Black sorghum is rare but valued for its high antioxidant content due to anthocyanins. Yellow sorghum is rich in carotenoids and is beneficial in addressing vitamin A deficiencies. These color differences are not just aesthetic—they reflect biochemical differences that impact taste, digestibility, and antioxidant activity. Thus, sorghum color is crucial in food product development and health-related research. It also affects consumer preference and market value across regions.

Fig.3 Varieties of Sorghum (white, red, brown)

Source: (Farré et al., 2022).

Modern breeding has led to the development of hybrid sorghum varieties that combine favorable traits from different types. For example, dual-purpose varieties like ‘Macia’ are suitable for both food and forage use in dry areas (Zhao et al., 2020). These improved varieties are often more resistant to diseases, pests, and environmental stresses such as drought and heat. In Africa and Asia, breeding programs have introduced early-maturing and high-yielding varieties like ‘SAMSORG 40’ and ‘SAMSORG 14’, enhancing food security in vulnerable areas. Additionally, low-tannin varieties have been developed to improve digestibility and reduce anti-nutritional factors. The genetic diversity among sorghum varieties supports their application in functional foods, bioenergy, and sustainable agriculture. By selecting the right variety based on grain color and agronomic traits, farmers can meet specific market and environmental needs. This makes varietal classification vital in sorghum breeding, nutrition, and food science research (Zhao et al., 2020).

Origin and Cultivation of Sorghum

Sorghum (*Sorghum bicolor*) originated in northeastern Africa, particularly in the region encompassing present-day Ethiopia and Sudan, where it was first domesticated over 5,000 years

ago. It later spread to other parts of Africa and Asia through human migration and trade routes. As an indigenous crop of Africa, sorghum played a central role in early agricultural systems due to its adaptability to hot and arid climates (Zhao et al., 2020). The genetic diversity found in African landraces has provided a rich resource for breeding programs aimed at improving drought tolerance and grain quality. Archeological evidence supports the idea that its cultivation in Africa predated that of maize in many areas, highlighting its historical significance. Its spread into India and China led to the development of new cultivars suited for different environments. Sorghum's ability to thrive in marginal soils contributed to its expansion across the Sahel, India, and parts of southern Europe. It eventually reached the Americas during the transatlantic slave trade, where it was adopted for both food and fodder. Today, it is grown in over 100 countries across diverse agro-ecological zones.

Modern cultivation of sorghum involves both traditional and improved agronomic practices, depending on the region. In Africa and Asia, smallholder farmers often use indigenous knowledge and practices passed down through generations. In contrast, in countries like the United States, Australia, and Brazil, commercial production relies on mechanized farming and high-yielding hybrids (Mace et al., 2021). Sorghum is typically planted at the beginning of the rainy season, as it requires moderate moisture for germination but is tolerant to drought later in its growth. The plant matures within 90–120 days, depending on the variety, and is relatively low maintenance compared to other cereals. It is generally grown as a rainfed crop but can also thrive under irrigation. Fertilizer application, spacing, and pest control vary widely depending on farming systems and scale. Recent innovations include the use of conservation agriculture and intercropping to enhance yields. Sorghum is cultivated for various purposes: grain for human

consumption, forage for livestock, and stalks for fuel or fiber. Sweet sorghum varieties are also increasingly used in biofuel production due to their high sugar content.

The global demand for sorghum has grown due to its resilience and versatility. It is particularly valuable in regions facing climate change challenges, as it can produce satisfactory yields even under water stress. Its cultivation supports food security and income generation, especially in Sub-Saharan Africa, where it serves as both a staple and a cash crop (Farré et al., 2022). Additionally, international organizations have recognized its potential and are investing in research and development to enhance its productivity. Genetic improvement programs aim to produce climate-resilient, pest-resistant, and nutritionally enhanced varieties. Moreover, sorghum cultivation supports sustainable agriculture by requiring fewer inputs and reducing pressure on natural resources. As awareness grows regarding gluten-free diets and alternative grains, sorghum's cultivation is also increasing in developed countries. Government policies and farmer cooperatives are vital in expanding its cultivation through training, access to seeds, and market linkages. Overall, sorghum’s historical origin and continued cultivation reflect its importance as a global food and economic crop.

Phytochemical Properties

Sorghum (*Sorghum bicolor*) contains a rich profile of phytochemicals, particularly phenolic compounds such as tannins, flavonoids, and phenolic acids. These compounds are primarily concentrated in the outer layers of the grain, especially in pigmented varieties like red and black sorghum (Awika & Rooney, 2021). The most notable class of phytochemicals in sorghum are 3-deoxyanthocyanidins, unique flavonoids not commonly found in other cereals. These compounds exhibit strong antioxidant, anti-inflammatory, and antimicrobial properties. In addition, sorghum contains phytosterols and lignans, which contribute to cardiovascular and hormonal health. The

antioxidant potential of sorghum is comparable to that of fruits and vegetables, making it a promising functional food ingredient. The phytochemical content varies by genotype, growing conditions, and postharvest processing. High-tannin sorghum types, although sometimes considered anti-nutritional, are beneficial in animal feed and in managing postprandial glucose (Li et al., 2020). These bioactive compounds play a significant role in disease prevention, particularly in managing oxidative stress and metabolic disorders.

In food research, sorghum phytochemicals have attracted attention due to their therapeutic benefits. Phenolic compounds in sorghum have shown inhibitory activity against enzymes linked to diabetes, cancer, and cardiovascular diseases (Mukurumbira et al., 2021). Tannin-rich varieties, when processed appropriately, retain beneficial effects without adverse impacts on digestibility. Processing methods such as fermentation, germination, and thermal treatment can reduce undesirable phytochemicals while enhancing bioavailability. Sorghum-based fermented foods, such as porridges and beverages, often contain higher levels of bioactive compounds after processing. This enhances their appeal in developing nutraceuticals and functional food products. Researchers are investigating sorghum phytochemicals for their potential use in pharmaceuticals, especially for antioxidant and anticancer therapies. These compounds also extend shelf life in sorghum-based foods by preventing lipid oxidation. Additionally, they may act as natural preservatives in food systems. Thus, the phytochemical richness of sorghum positions it as a multi-purpose crop in health-centered food innovation.

Sorghum's phytochemical profile also plays a role in plant defense and environmental adaptation. For instance, tannins deter pests and fungal pathogens, giving sorghum an advantage in pest-prone, low-input agricultural systems. This characteristic reduces the need for synthetic pesticides, supporting sustainable agriculture (Farré et al., 2022). Moreover, these compounds contribute to

sorghum's drought tolerance by regulating osmotic stress. The plant's phytochemicals also interact with soil microbiota, influencing nutrient availability and plant health. In food systems, the color and astringency of sorghum products are directly linked to phytochemical concentration. These factors impact consumer acceptance but also signal high antioxidant activity. Selective breeding now focuses on modifying phytochemical profiles to meet both nutritional and sensory needs. With increasing interest in clean-label and naturally preserved foods, sorghum phytochemicals offer unique industrial advantages. Their multifunctional roles reinforce the importance of sorghum in food security and health promotion initiatives.

Nutritional Composition

Sorghum is a nutrient-dense cereal, offering an excellent source of energy, dietary fiber, protein, and essential minerals. The average composition of whole grain sorghum includes approximately 70–75% carbohydrates, 10–12% protein, 3–5% fat, and 1–3% ash (Olagunju et al., 2020). It is also rich in micronutrients such as iron, phosphorus, magnesium, potassium, and zinc. Unlike wheat and barley, sorghum is gluten-free, making it suitable for individuals with celiac disease or gluten intolerance. Its protein is mainly composed of kafirins, which are prolamins specific to sorghum. Though kafirins are less digestible than other cereal proteins, processing techniques like germination and fermentation can improve their bioavailability. Sorghum also contains B vitamins, including niacin, thiamine, and riboflavin. Its nutritional profile supports sustained energy release and digestive health due to its high fiber and complex carbohydrate content. Sorghum's low glycemic index contributes to blood sugar regulation, making it suitable for diabetic-friendly diets. The energy contribution of sorghum is significant in many developing regions, where it serves as a dietary staple. Its caloric value is similar to that of maize and rice, providing around 330–350 kcal per 100 grams. The dietary fiber in sorghum aids bowel health, cholesterol reduction, and

satiety, which is beneficial for weight control. Whole grain sorghum retains its bran and germ, preserving most of its nutrients during milling. Fortified sorghum products are also being developed to address micronutrient deficiencies, particularly in low-income populations. Several studies have shown that sorghum consumption can improve iron and zinc status in children when appropriately prepared. Additionally, polyunsaturated fatty acids present in sorghum, particularly linoleic acid, contribute to cardiovascular health. Sorghum is increasingly used in infant and complementary foods due to its digestibility and nutrient density when processed correctly. Hence, its composition supports its integration into global nutrition strategies. It offers not only sustenance but also health-enhancing benefits.

The nutritional quality of sorghum varies with genotype, soil condition, and processing methods. Brown and red sorghum varieties often contain more antioxidants and fiber than white varieties, which are commonly used in porridges and beverages (de Morais Cardoso et al., 2021). Processing methods such as decortication, cooking, fermentation, and malting influence the retention and availability of nutrients. While decortication removes fiber and phytochemicals, fermentation improves mineral bioavailability by reducing phytates. Sorghum's adaptability allows it to retain nutritional integrity even under suboptimal agricultural conditions. This resilience, combined with its nutritional richness, makes sorghum a candidate for addressing global malnutrition. Innovations in food technology are further enabling its use in snacks, pasta, flour blends, and ready-to-eat meals. Sorghum's role in dietary diversification and chronic disease prevention is gaining recognition worldwide. Thus, it represents a valuable crop for enhancing both food security and public health outcomes.

Table 3: Nutritional Composition of Sorghum (Per 100g, Whole Grain)

Nutrient	Approximate Content	Nutritional Benefit
Carbohydrates	70–75%	Primary source of energy; includes complex carbs for sustained release
Protein	10–12%	Contains kafirins; supports growth and repair
Fat	3–5%	Includes polyunsaturated fats like linoleic acid for heart health
Ash (Minerals)	1–3%	Indicates total mineral content
Dietary Fiber	6–10%	Promotes digestive health and satiety
Energy	330–350 kcal	Provides significant caloric value
Iron	3–5 mg	Supports oxygen transport and prevents anemia
Phosphorus	250–300 mg	Essential for bone and cell function
Magnesium	150–170 mg	Important for enzyme function and muscle contraction
Potassium	300–350 mg	Regulates blood pressure and fluid balance
Zinc	1.5–2.5 mg	Immune support and cell division
Niacin (B3)	2.5–4.0 mg	Aids energy metabolism and skin health
Thiamine (B1)	0.2–0.4 mg	Supports nervous system function
Riboflavin (B2)	0.1–0.3 mg	Supports energy release from food
Gluten	0%	Gluten-free; suitable for celiac or gluten-sensitive individuals

Source: (Olagunju et al., 2020).

Potential Application

Potential applications of sorghum in processing and storage are extensive due to its unique nutritional and phytochemical properties. Sorghum's high antioxidant content helps in extending the shelf life of food products by reducing lipid oxidation and microbial spoilage, making it an excellent natural preservative in processed foods (Awika & Rooney, 2021). Its gluten-free nature allows it to be used in a variety of specialty diets and gluten-free products, including baked goods, porridges, and snacks, broadening consumer options. Sorghum flour can be used to improve the texture, nutritional profile, and moisture retention of food products, enhancing both quality and consumer acceptability. Additionally, sorghum's resistance to drought and pests translates to a

stable raw material supply, supporting sustainable food production. Processing methods like fermentation and malting not only improve digestibility and nutrient availability but also introduce beneficial flavors and textures, thereby increasing sorghum's versatility in food industries (Olagunju et al., 2020). Sorghum's potential as a functional ingredient in composite flours and as a carrier of bioactive compounds also supports its role in developing nutraceuticals and health-oriented foods. These attributes make sorghum a promising crop for innovation in food technology and preservation strategies.

Wet milled Soghurm(Ogi)

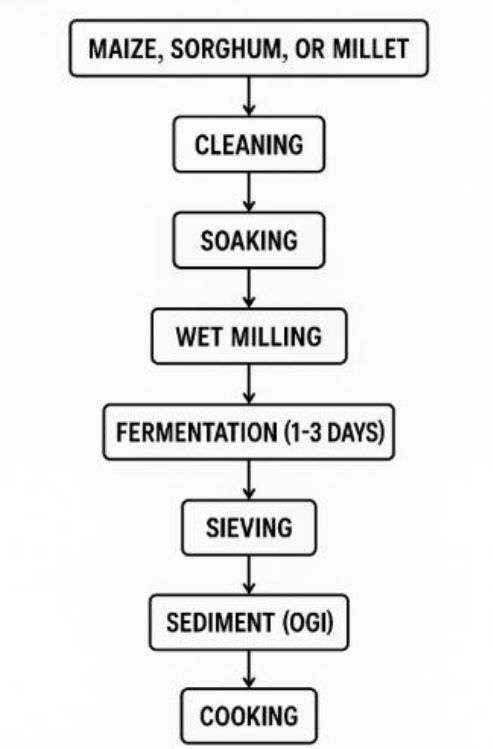
The production process begins with sorting and cleaning of maize grains to remove impurities such as dirt, stones, and damaged kernels, ensuring the quality of the final product. After cleaning, the maize undergoes dehulling fig. 4, where the outer husk or bran is removed to reduce fiber content and enhance fermentation. The dehulled maize is then subjected to wet milling, where it is ground into a fine slurry called ogi slurry. This slurry serves as the substrate for microbial fermentation. Traditionally, this preparation follows a sequence of steps including washing, steeping in water for up to 72 hours, wet milling, sieving to remove fibrous residues, and sedimentation to separate the starch-rich portion from the water. The sedimented slurry is then allowed to ferment naturally. During fermentation, naturally occurring microorganisms, primarily lactic acid bacteria (LAB) and some yeasts, proliferate in the ogi slurry (Olagunju et al., 2020). The slurry is typically incubated for about 2 to 4 days at ambient temperature, allowing these beneficial microbes to ferment the carbohydrates into organic acids, mainly lactic acid. This fermentation process lowers the pH, improves the nutritional quality, enhances flavor, and acts as a natural preservative. In some controlled production processes, inoculation with a known starter culture at a concentration of about 10^9 colony-forming units per gram (cfu/g) is performed to ensure consistent and rapid

fermentation, improve safety, and reduce spoilage by unwanted microbes. In addition, parboiling may be applied to the ogi slurry after fermentation to gelatinize starches, improve digestibility, and extend shelf life. The microbiological dynamics during fermentation depend on the duration, typically ranging from 48 to 96 hours, and environmental conditions such as temperature and oxygen availability. Proper microbial balance and control during these stages are crucial for producing high-quality ogi with desirable taste, texture, and shelf stability (Farré et al., 2022).

Fig. 4: Preparation of ogi in a controlled environment

Source: (Olagunju et al., 2020)

Fig.5: Traditional preparation of Ogi



Source: (Farré et al., 2022).

Grains that can be used

The most commonly used grains for ogi preparation are maize (corn), sorghum, and millet. Maize ogi is widely preferred due to its mild taste, smooth texture, and relatively shorter fermentation time. It yields a creamy, slightly sour porridge that is a staple breakfast food for many households. The maize used can be white or yellow, with each offering subtle differences in color and flavor of the final ogi product (Onabanjo et al., 2020). Maize is nutritionally rich in carbohydrates and provides a good energy source, but it is relatively low in protein content, which has led to the inclusion of other grains in ogi production.

Sorghum and millet are also frequently used as alternative grains for ogi preparation, especially in regions where maize is less accessible or during maize shortages. Sorghum ogi tends to have a stronger flavor and darker color due to the presence of tannins and phenolic compounds in the

grain. This variety of ogi is prized for its higher antioxidant content and improved nutritional profile compared to maize ogi (Ogunremi et al., 2021). Millet ogi is similarly rich in essential minerals and offers a slightly different taste profile, often described as having a more natural, grainy flavor with a mildly roasted or nut-like taste. Both sorghum and millet ogi require a longer fermentation time, which enhances their probiotic benefits and improves digestibility by reducing anti-nutritional factors such as phytates.

In addition to maize, sorghum, and millet, other grains such as guinea corn and even rice have been experimented with in ogi production. Guinea corn, a variety of sorghum, shares similar characteristics with sorghum and is used in certain localities to produce ogi with a distinct taste and nutritional benefits. Rice ogi, though less traditional, has gained popularity as a gluten-free alternative, particularly for individuals with celiac disease or gluten intolerance (Akinola et al., 2020). Each grain imparts unique sensory, nutritional, and functional properties to ogi, allowing for diversity in consumption and catering to different dietary needs. Overall, the choice of grain influences the texture, taste, nutrient content, and health benefits of the final fermented product.

Procedure for preservation of ogi

Traditionally, ogi is highly perishable due to its high moisture content and active microbial population, which promote spoilage by unwanted bacteria, yeasts, and molds. One common preservation method involves refrigeration. Freshly prepared ogi is stored at low temperatures (around 4°C) to slow down microbial activity and enzymatic reactions that cause spoilage. Refrigeration can extend ogi's shelf life from a day or two at room temperature to up to a week, making it more convenient for daily consumption (Ibrahim et al., 2021).

Another effective preservation technique is drying or dehydration, which reduces the moisture content significantly, thereby limiting microbial growth. Dried ogi can be prepared by spreading

the fermented paste thinly and sun-drying or using controlled hot air drying. The dried product can then be milled into a powder, which can be reconstituted with water when needed. This method not only extends shelf life but also reduces storage space and transport costs. Studies have shown that drying ogi preserves most of its nutritional and probiotic qualities, though some loss of heat-sensitive vitamins may occur (Adeyemi et al., 2020).

In modern food processing, packaging innovations also play a key role in preserving ogi. Vacuum packaging or using oxygen-impermeable films can protect ogi from oxidation and contamination by spoilage organisms. Combined with refrigeration or drying, these packaging methods further enhance shelf stability. Additionally, natural preservatives such as extracts from bitter leaf or other plants rich in antimicrobial compounds have been researched for their potential to inhibit spoilage microbes in ogi. These methods help in maintaining the sensory properties and improving safety, thereby making ogi more acceptable for wider commercial distribution (Ojo et al., 2022). Overall, integrating traditional and modern preservation techniques is essential to maintain ogi quality and increase its shelf life.

Problem associated with Conventional & traditional method of preservation

Conventional and traditional methods of food preservation, such as fermentation, drying, smoking, salting, and refrigeration, have been widely used for centuries to extend the shelf life of various foods. However, these methods present several challenges. One major problem is the inconsistency in preservation outcomes due to variations in environmental conditions such as temperature, humidity, and hygiene during processing and storage. For example, traditional sun drying depends heavily on weather conditions, making it unreliable during rainy or humid seasons and increasing the risk of microbial contamination and spoilage (Adeyemi & Oluwole, 2020). Additionally,

traditional fermentation processes may lack standardized control, leading to variable acidity, flavor, and safety profiles, which can affect product quality and consumer acceptability.

Another issue with conventional preservation methods is the relatively short shelf life they provide compared to modern techniques. Fermented products like ogi, if not properly stored, can spoil quickly due to the growth of undesirable microorganisms such as molds and spoilage bacteria (Ibrahim et al., 2021). The absence of adequate temperature control in many traditional settings accelerates spoilage and limits the distribution range of such foods. Moreover, methods like salting and smoking can introduce excessive salt or harmful compounds such as polycyclic aromatic hydrocarbons, posing potential health risks if consumed frequently (Onyango et al., 2021). These health concerns limit the acceptability of traditional preservation among health-conscious consumers.

Traditional preservation methods also often require labor-intensive and time-consuming processes that are less efficient for large-scale production. For instance, manual sorting, washing, soaking, and fermentation steps in traditional ogi preparation may vary widely between households, affecting uniformity and scalability (Akinola et al., 2020). Furthermore, limited access to modern storage facilities in rural areas often results in significant post-harvest losses due to pest infestations and environmental degradation. Finally, traditional methods typically do not provide adequate microbial safety assurance, which is critical for preventing foodborne illnesses, especially in the absence of formal quality control measures. These problems highlight the need for improved or complementary preservation technologies that maintain traditional food characteristics while enhancing safety and shelf life.

Effect of bitter leaf on milled sorghum

The incorporation of bitter leaf (Vernonia amygdalina) into wet-milled sorghum has been shown to positively influence its nutritional composition. Bitter leaf is rich in essential nutrients such as vitamins (A, C, and E), minerals (calcium, iron, and potassium), and bioactive phytochemicals like flavonoids, saponins, and phenolic compounds (Okafor et al., 2021). When added to sorghum during wet milling, these compounds can enhance the overall nutrient profile of the resulting flour or paste, improving its antioxidant capacity and providing additional health benefits. This enrichment is particularly valuable given that sorghum, while rich in carbohydrates and fiber, has relatively low levels of certain micronutrients.

Beyond nutrition, bitter leaf also affects the shelf life of wet-milled sorghum. The phytochemicals in bitter leaf exhibit natural antimicrobial and antioxidant properties, which can inhibit the growth of spoilage microorganisms such as bacteria and fungi that typically reduce the shelf life of sorghum products (Ezeonu et al., 2020). These compounds slow down oxidative rancidity and microbial deterioration, thereby prolonging the freshness and safety of the wet-milled sorghum during storage. This natural preservation effect is especially important in regions with limited access to refrigeration, where extending shelf life through chemical preservatives is less feasible.

However, the addition of bitter leaf may also influence sensory properties, such as taste and color, of the wet-milled sorghum. The characteristic bitter taste of Vernonia amygdalina can alter the flavor profile, potentially affecting consumer acceptability if not balanced properly (Ugbogu & Obiakor, 2022). Despite this, processing techniques such as blanching or controlled extraction of bitter compounds can help mitigate excessive bitterness while retaining the nutritional and preservative benefits. Overall, the effect of bitter leaf on wet-milled sorghum is promising for

improving both its nutritional value and shelf stability, which could enhance the utilization of sorghum as a staple food.

1.1 Literature review on related research

Adebayo et al. (2021) investigated the effect of clove (Syzygium aromaticum) powder addition on fermented maize pap. Their research demonstrated that incorporating clove not only extended the shelf life by inhibiting microbial spoilage but also enriched the antioxidant capacity of the pap. Cloves contain eugenol, a potent phenolic compound with strong antimicrobial and antioxidant properties, which effectively slowed down spoilage microorganisms such as yeasts and molds, thereby improving the product's safety and longevity during storage under ambient conditions.

Olatunde and Akinola (2022) evaluated the incorporation of bitter leaf (Vernonia amygdalina) extract into millet-based ogi. Their findings revealed that supplementation with bitter leaf significantly improved the nutritional profile by increasing the content of essential vitamins and minerals such as vitamin C, calcium, and iron. The antimicrobial effect of bitter leaf extract was evident as the treated ogi showed reduced total viable bacterial counts over a 7-day refrigerated storage period. The researchers attributed this to the high concentration of flavonoids, alkaloids, and other phytochemicals present in bitter leaf, which possess broad-spectrum antimicrobial activity. This enhancement of both shelf life and nutrition positions bitter leaf as a valuable functional additive in cereal-based fermented foods.

Similarly, research by Nwokocha et al. (2020) focused on the synergistic effects of combining clove and ginger extracts in maize pap. Their work highlighted how these spices not only contributed to sensory improvements like aroma and taste but also acted as natural preservatives by suppressing the growth of foodborne pathogens such as Staphylococcus aureus and Escherichia coli. The combination of spices resulted in a significant decrease in pH and an increase in total

phenolic content, creating an unfavorable environment for spoilage microbes. This dual action of preservation and nutritional enhancement underscores the potential of spice supplementation in improving traditional fermented foods.

A study by Eze et al. (2023) explored the effect of bitter leaf powder on the physicochemical and microbial stability of wet milled sorghum slurry. Their findings showed that bitter leaf supplementation delayed lipid oxidation and reduced microbial load, resulting in extended shelf life during storage at both room temperature and refrigeration. The antioxidant properties of bitter leaf helped prevent rancidity, while its antimicrobial compounds curtailed the proliferation of spoilage bacteria. This research emphasized the practical application of bitter leaf as a natural preservative in wet-milled sorghum products, which is directly relevant to enhancing the quality of pap.

Adekunle and Oladipo (2021) reviewed various traditional spices including cloves, bitter leaf, and black pepper for their roles in fortifying ogi and similar fermented cereal products. Their meta-analysis pointed to consistent improvements in protein content, antioxidant capacity, and microbial safety across studies where spices were used as supplements. They also highlighted the consumer preference for naturally preserved products without chemical additives, noting the increased demand for “clean-label” foods. Their work supports integrating spices like bitter leaf and cloves in food formulations not only for preservation but also for boosting the functional and nutritional value of staple fermented cereals.

1.2 Statement of Problem

Ogi, a traditional fermented cereal commonly made from sorghum, maize, or millet, serves as a major weaning and staple food in many parts of Africa, especially Nigeria. However, its short shelf life and limited nutritional composition present significant challenges to food security and

nutritional adequacy, particularly among low-income populations. The rapid microbial spoilage of wet-milled ogi during storage reduces its safety and acceptability, while its low protein and micronutrient content raises concerns about its ability to meet dietary needs. Recent studies have explored natural additives to improve food quality and preservation, with bitter leaf (*Vernonia amygdalina*) showing promise due to its antimicrobial and nutritional properties (Ezekwesili et al., 2020). Despite this, there is limited research on its incorporation into ogi to enhance both shelf life and nutritional value. This study, therefore, seeks to investigate the effect of bitter leaf on the nutritional composition and microbial stability of wet-milled sorghum (ogi).

1.3 Aim

To evaluate the effect of bitter leaf (*Vernonia amygdalina*) supplementation on the nutritional composition and shelf life of wet-milled sorghum (ogi), with the goal of enhancing its nutritional value and microbial stability during storage at ambient condition.

1.4 Objectives

- To determine the effect of different concentrations of bitter leaf on the nutritional composition of wet-milled sorghum (ogi).
- To assess the impact of bitter leaf addition on the microbial stability and shelf life of wet-milled sorghum during storage under ambient conditions.
- To evaluate the sensory properties and consumer acceptability of ogi fortified with varying levels of bitter leaf.
- To determine the total titratable acidity of wet-milled sorghum over the storage period.
- To assess the changes in the nutritional composition of wet-milled sorghum over a 4-week storage period

1.5 Justification of Study

This study is justified by the need to enhance the nutritional quality and extend the shelf life of sorghum-based foods, addressing malnutrition and food spoilage challenges. Incorporating bitter leaf, known for its rich bioactive compounds and antimicrobial properties, offers a natural and sustainable solution. This integration supports food security and promotes the use of indigenous resources in vulnerable communities.

CHAPTER TWO

2.0 Material and Method

2.1 Study Area

This study was conducted using raw white sorghum purchased from local market at oke oyi Kwara state while fresh bitter leaf obtained was 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 grain

- Sorghum grain(*Sorghum bicolor*) was purchased from a local market at Oke oyi kwara state

Bitter leaf

- Fresh bitter leaves (*Vernonia amygdalina*) was sourced from Odo ota in Ilorin

2.2.2 Chemical Reagents

The chemical reagents used in this study included deionized water and 70% ethanol. Deionized water was used for all washing, soaking, and preparation processes to ensure sterility and prevent contamination. Ethanol (70%) was employed to disinfect laboratory surfaces and instruments before and after each procedure.

2.2.3 Equipment

The equipment and materials employed during the research comprised an electrical blender, conical flasks, beakers, Petri dishes, sterile test tubes, sterile syringes, foil paper, a steerer, weighing balance, Bunsen burner, autoclave, heat lamp, and sample containers. These tools were

used for processing, mixing, culturing, and analyzing the ogi samples under hygienic and controlled laboratory conditions.

2.2.4 Media

Five types of microbiological media were used for the cultivation and enumeration of microbial organisms during the study. These included MRS agar for lactic acid bacteria, MacConkey agar for coliforms, Sabouraud Dextrose Agar (SDA) for fungi, Yeast extract for yeast growth and Nutrient agar for general bacterial growth. All media were used according to the manufacturer's specifications and prepared under sterile conditions.

2.3.0 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 the compositions:

- BLC1-: 308.5 g of sorghum + 0.7g ofbitter leaf
- BLC2-: 308.0 g of sorghum + 1.0g of bitter leaf
- BLC3-: 307.5 g of sorghum + 1.25 gof bitter leaf

Control setup

Two additional control samples were prepared

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

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

2.3.1 MILLING OF SAMPLES

After 48hours of fermentation, the steeping water was decanted from each sample. Additional bitter leaf was added in the same proportion as the initial setup:

- BLC1-: 1.5g of bitter leaf
- BLC2-: 2.0g of bitter leaf
- BLC3-: 2.5g of bitter leaf

2.4 STERILIZATION OF GLASSWARES

All glassware were washed with soap and rinsed with distilled water and sterilized using hot air oven at 45^0C for 15minutes. Inoculating loop was heated to redness with spirit lamp and workbench was wiped with 70% ethanol.

2.5 PREPARATION OF MEDIA

Five different media were prepared for microbial analysis: MRS agar (33.6g/L), MacConkey agar (23.5g/L), SDA (32.5g/L) Yeast Extract (11.5g/L), and Nutrient agar (14g/L). Each agar type was accurately weighed into separate conical flasks and reconstituted with 500ml of distilled water. The solutions were stirred thoroughly and gently heated to ensure full dissolution. The media were sterilized using an autoclave set at 120ºC for 15 minutes at 15 psi. After sterilization, the media were cooled slightly for use in microbiological analysis.

2.6 MICROBIOLOGICAL ANALYSIS

2.6.1 Serial Dilution and Inoculation

Serial dilution of each sample was carried out weekly to assess microbial load in each sample during storage. Initially, 9ml of sterilized distilled water was introduced into each of the 16 test tubes placed in racks and autoclaved. 1ml of blended sample was added to the first test tube, mixed thoroughly, and 1ml was transferred to the next tube. This process continued serially until a

dilution gradient was achieved. From each dilution, 0.5ml was aseptically inoculated into sterile Petri dishes containing prepared agar (MRS, MacConkey, SDA, Yeast Extract and Nutrient agar) using the pour plate technique. The media were swirled gently to evenly distribute the inoculum, covered, and incubated for 24 hours at room temperature. This process was repeated weekly for the first 2 weeks and 2 weeks after

2.6.1.1 Decanting

50ml was decanted from each sample every 2 days and 30ml of distilled water was added including the control sample throughout whole period of the practical

2.6.2 Enumeration of Bacterial Isolates

After incubation of the inoculated plates, microbial growth was carefully observed and documented based on visible colony characteristics such as shape, size, color, texture, and elevation. Distinct colonies exhibiting unique morphological features were selected and subcultured to obtain pure isolates. The bacterial and fungal isolates, grown on their respective media, were then subjected to microscopic examination and a series of biochemical tests. These analyses facilitated the accurate identification and classification of the microbial species present in the wet mixed sorghum samples.

2.6.3 Characterization and Identification of Bacterial

2.6.3.1 Bacterial Characterization

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

1. Colonial Morphology Observation

Purified bacterial colonies grown on Nutrient Agar were observed for:

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

Observations were recorded to support preliminary differentiation of the isolates

2. Cellular Morphology

a. Gram Staining

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

Procedure:

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

b. Endospore Staining

Used to detect the presence of spores.

Procedure

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

c. *Motility Test* (*Hanging drop method*)

Used to determine bacterial motility

Procedure

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

3. Biochemical Tests for the identification of bacterial isolates

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

Catalase Test

Procedure

A small amount of bacterial colony was trans ferred onto the surface of a clean, dry glass slide using a sterile loop or wooden stick. A drop of 3% hydrogen peroxide (H_20_2) was then placed on the slide and mixed with the colony. A positive result was indicated by the rapid evolution of oxygen within 5 to 10 seconds, observed as bubbling. A negative result was indicated by the absence of bubbles or the presence of only a few scattered bubbles.

Oxidase Test

Procedure

The test organism was rubbed over the reagent-impregnated filter paper disc using sterile applicator sticks. Control samples were included alongside the test, and the reaction was observed within l0 seconds

Indole Test

Procedure

The peptone water tubes were inoculated with the bacterial broth culture using a sterile needle technique, while an uninoculated tube was maintained as a control. Both the inoculated and control tubes were incubated at 37C for 24 to 48 hours. After incubation, 1 ml of Kovac's reagent was added to each tube, including the control. The tubes were then gently shaken and observed after an interval of 10 to 15 minutes

Methyl Red (MR) Test

Procedure

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

Voges-Proskauer (VP) Test

Procedure

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

Citrate Utilization

Procedure

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

Urease Test

Procedure

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

Starch Hydrolysis

Procedure

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

Gelatin Hydrolysis

Procedure

Gelatin medium was inoculated and refrigerated after incubation

TSI (Triple Sugar Iron Agar) Test

Procedure

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

H_2S Production

Procedure

Observed as black precipitate in TSI slant.

Gas Production

Procedure

Bubbles or cracks in the TSI medium

Oxygen Relationship

Procedure

Thioglycollate broth was inoculated and incubated

2.6.3.2 Fungal Morphological Characterization and Identification

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

1. Colonial Morphology (Macroscopic Observation)

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

- Colony Colour: Top and reverse side
- Texture: Cottony, powdery, velvety, woolly
- Shape and Edge: Circular or irregular with entire or lobate edges
- Growth Rate; Rapid, moderate, or slow

2. Cellular Morphology (Microscopic Examination)

Lactophenol Cotton Blue Staining

Procedure

A small fragment of fungal mycelium was picked with a sterile needle or blade. It was placed on a clean slide with a drop of lactophenol cotton blue stain. A cover slip was gently placed over it. The slide was examined under a microscope at x10 and x40 magnifications

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

2.6.4 Sensory Evaluation

To evaluate the palatability and consumer acceptability of the ogi samples, 150ml of distilled water was boiled on a gas cooker, and a tablespoon of each fermented paste (BLC1-, BLC2-, BLC3-, and control) was added. The mixture was stirred continuously until it formed a consistent porridge. Cooked samples were evaluated based on sensory characteristics such as taste, color, appearance, odour, and Overall acceptability. This was done by a panel of tasters under hygienic conditions.

2.7 Storage of bacterial cultures

The isolated organism were purified using repeated subculturing techniques and stored on agar slant, through slant culture. For further analysis for the first 7 days, 14 days and 28 days.

2.8 Monitoring and Fermentation Maintenance

Weekly, the fermented sorghum paste from each sample was decanted removing the top layer of water and replaced with fresh autoclaved deionized water. This weekly replacement helped simulate household fermentation practice and provided a consistent environment for microbial

activity. The process also helped in monitoring the shelf life of each treated ogi sample by tracking microbial growth over time. The water removed from the paste was collected and analyzed for Total Titratable Acidity (TTA) to monitor changes in acidity as fermentation progressed, which serves as an indicator of spoilage or preservation.

2.9 Proximate Analysis

The solid paste obtained after decantation was used for proximate analysis to determine the nutritional composition of the treated and untreated ogi samples. This included the assessment of moisture content, ash content, crude protein, crude fat, crude fiber, and carbohydrate content. These parameters were evaluated using standard methods such as oven drying, Soxhlet extraction, and Kjeldahl digestion, as appropriate. The aim was to determine whether the inclusion of bitter leaf at various concentrations had any effect on the nutritional profile of the fermented ogi.

2.9.1 Proximate Analysis Procedure

2.9.1.1 Moisture content

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

Calculation: Moisture content (5) = initial weight – final weight/initial weight x100

(AOAC 2019)

2.9.1.2 Ash Content

Dried sample from the moisture analysis will be taken 5 g will be weighed. It will be placed in a crucible and incinerated at 550^{o}C for 4 – 6 hours. It will then be cooled in a desicator and weighed.

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

(AOAC 2019)

2.9.1.3 Crude Protein

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

Calculation:

Crude protein (%) = Nitrogen % x6.25

(AOAC 2019)

2.9.1.4 Crude Fat

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

Calculation:

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

(AOAC 2019)

2.9.1.5 Crude Fibre

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

Calculation:

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

(AOAC 2019)

Nitrogen Free Extract (NFE) OR CHO

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

(AOAC 2019)

2.10 Identification of bacteria Isolate

2.10.1 Cell Shape

A clean glass slide was prepared with a smear of the bacterial isolate. The smear was air-dried, heat-fixed, stained with crystal violet, and viewed under a microscope. Cell shape (coccus, rod, spiral) was observed under oil immersion at 100x objective.

2.10.2 Cell Arrangement

Prepared smear was stained and observed microscopically after heat fixing. The arrangement pattern (single, pair, chains, clusters) was recorded. This helps differentiate species based on how cells group post-division.

2.10.3 Pigmentation

Isolates were cultured on nutrient agar and incubated for 24–48 hours. The colony color (e.g., white, yellow, red, etc.) was visually observed. Pigmentation assists in preliminary identification of bacterial species.

2.10.4 Gram Reaction

Smear was prepared and sequentially stained with crystal violet, iodine, alcohol, and safranin. Slide was observed under oil immersion; Gram-positive appeared purple, Gram-negative pink. This differential stain helps classify bacteria based on cell wall properties.

2.10.5 Motility

A drop of bacterial suspension was placed on a clean slide and covered with a coverslip. Using a light microscope, movement was observed in a hanging drop preparation. Active movement indicated motility; stationary cells indicated non-motile bacteria.

2.10.6 Endospore

Smear of bacterial culture was stained using the Schaeffer-Fulton method (malachite green + safranin). Slide was steamed over heat to allow dye penetration, then rinsed and counterstained.

Endospores appeared green within red/pink vegetative cells under the microscope.

2.11 TITRATABLE ACIDITY

The discussion in the chapter on pH and acidity emphasizes that pH and titratable acidity are not the same. pH is a measure of the amount of free hydrogen ions in a solation. Titratable acidity is a measure of both bound and free hydrogen ions in a solution. It is measured by titration of acid in the food with standardized NaOH solution. There is no fixed relationship between pH and titratable acidity in a food. However, experience has shown that titratable acidity can be relied upon as an indicator that pH is no higher than some maximum value for a particular product formulation. This relationship must be established by experience for the particular ingredients and the way in which they are used. For Ahis reason, the regulation allows use of titratable acidity measuremēnts toeentrel a-process and for documentation of a final maximum pH if the equilibrium pH of a produet is below 4.0. Even if pH measurements with a pH meter are routinely done, titratable acidity is an important analytical method to assure that ingredients contain the expected, amount of acid. Final products may have too much, as well as too little, acid present. Both situations may indicate a problem with product quality. Measurement of titratable acidity can be a very useful way to detect a problem in either direction

Titration Procedure

An accurately measured amount of the sample was put into a beaker. If the sample was a liquid, it was pipetted into the beaker using a properly calibrated pipette, and the sample volume was recorded. If the sample was a slurry that could not be accurately pipetted, it was added directly to

the beaker, and its exact weight was recorded. It was noted that the addition of distilled water to the sample after measurement did not interfere with the titration process. Water could be added to make a slurry easier to handle during titration or to dilute a colored sample, making it easier to observe the pink color change when phenolphthalein was used as the endpoint indicator. If samples were so highly colored that observing the endpoint was difficult, a pH meter was used to determine when the pH reached 8.2.

While the sample was being stirred with a magnetic stirring bar, standard NaOH solution was added from a burette calibrated in milliliters. When the endpoint of the titration was reached, the volume of NaOH solution used was recorded.

2.11.1 Calculation of Titratable Acidity

The percent (%) acid in a sample is calculated predominant acid per 100 grams or 100 milliliters. The equation to calculate titratable acidity is as follow

% Acid**=(NxVxM)/Sx10**

Where:

N = Normality of standard NaOH solution used for titration

V = Volume of standard NaOH used for titration in milliliters

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

S = Sample size in milliliters or grams

CHAPTER THREE

3.0 RESULT

3.1 ENUMERATION OF BACTERIAL CULTURES

Table 1: 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}**	**$2.5x\ 10^{-2}$**	**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}**		

3.2 SENSORY EVALUATION
Table 2

SENSORY EVALUATION RESULT

TABLE 2: Day 7, 14 & 28 Sensory evaluation

Time (Days)	Sample	Tasting	Odour	Appearance	General	Colour
7	BLC1-	6	4	5	7	Off white
	BLC2-	7	4	5	8	Off white
	BLC3-	8	4	5	9	Off white
	C. Nw	5	3	3	4	White
	C. Dw	6	7	6	7	Off 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-	7	7	8	7	off white
	BLC2-	5	2	8	6	off white
	BLC3-	6	5	9	7	off white
	C. Nw	5	5	4	5	off white
	C. Dw	**6**	**5**	**6**	**6**	**White**

KEYWORDS:

BLC1- = Sample 1

BLC2- = Sample 2

BLC3- = Sample 3

C. Dw = Control Distilled 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

3.3 MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES IDENTIFIED

Table 3. Morphological Characteristics of Bacterial Isolates Identified

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

3.4 IDENTIFICATION OF BACTERIA ISOLATES

Table.4 Colonial/Cellular Morphology & Biochemical tests for identification of bacteria

	Morphology & Biochemical Tests	Bacterial Isolates				
		Lactobacillus sp	*S. aureus*	*B. subtilis*	*B. cereus*	*P. aeruginosa*
Cellular Morphology	Cell shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli
	Cell arrangement	Pair/chains	Irregular/ clusters	Pairs/ chains	Chains/pairs	Single
	Pigmentation	-	-	-	-	+
	Gram reaction	+	+	+	+	-
	Motility	-	-	+	+	+
	Endospore	-	-	+	+	-
Biochemical test for identification of bacteria	Catalase	-	+	+	+	+
	Oxidase	-	-	-	-	+
	Coagulase	-	+	-	-	-
	Indole	-	-	-	-	-
	Citrate utilization	-	+	+	+	+
	MR	-	-	-	-	-
	VP	-	+	+	+	-
	Gelatin hydrolysis	-	+	+	+	-
	Urease	-	+	-	-	+
	Triple sugar Glucose	+	+	+	+	-
	Lactose	+	+	-	-	-
	Sucrose	+	+	+	+	-
	Starch	+	-	-	+	-
	H_2S	-	-	-	-	-

Gas production	-	-	-		-
O_2 relationship	FA	FA	FA	FA	FA

Key: FA = Facultative anaerobe

Bacterial Isolates	**Identified Probable organism**
K	*Lactobacillus sp*
M	*Lactobacillus sp*
T	*B. cereus*
V	*B. subtilus*
W	*S. aureus*
U	*P. aeruginosa*

3.5 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF FUNGAL ISOLATES

Table 5: Cultural and morphological characteristics of fungal Isolates

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

3.6 ISOLATES IDENTIFICATION

Table 6: Isolates Identification

Isolate code	Fungi identified
R	*Aspergillus niger*
I	*Aspergillus flavus*
E	*C. krusei*
K	*Aspergillus niger*
O	*C. krusei*
M	*P. kudriavzevii*

3.7 PROXIMATE ANALYSIS RESULTS (GROUP B)

Table 7: Proximate Analysis Results

Time (Week)	Sample code	Moisture content (%)	Crude protein (%)	Lipid (%)		Crude fibre (%)		Ash content (%)		Carbohydrate (%)
	BLC1-	12.37	10.37	3.27	3.23	2.35	2.38	1.87	1.89	69.77
		12.37	10.32							69.81
7	BLC2-	13.10	11.23	3.03	3.07	2.42	2.37	2.01	1.88	68.21
		13.11	11.18							68.39
	BLC3-	12.03	10.55	3.21	3.25	2.32	2.27	1.75	1.73	70.14
		12.03	10.71							70.01
	NW (CTR)	12.52	10.21	3.58	3.62	2.47	2.43	2.12	2.09	69.10
		12.53	10.25							69.08
	DW (CTR)	12.78	10.53	3.72	3.75	2.39	2.43	2.01	2.05	68.57
		12.79	10.48							68.50
	BLC1-	13.14	10.71	3.47	3.43	2.38	2.43	1.95	1.91	68.35
		13.14	10.58							68.51
14	BLC2-	13.55	11.53	3.25	3.30	2.47	2.43	2.07	1.93	67.13
		13.55	11.49							67.30
	BLC3-	13.32	11.07	3.51	3.47	2.37	2.32	1.82	1.78	67.91
		13.33	11.10							68.00
	NW (CTR)	15.25	10.45	3.73	3.67	2.52	2.47	2.17	2.12	65.88
		15.24	10.37							66.13
	DW (CTR)	15.77	10.73	3.85	3.79	2.44	2.48	2.05	2.07	65.16
		15.77	10.69							65.20
	BLC1-	14.18	11.52	3.68		2.59	2.63	2.05	2.01	65.98
		14.18	11.37	3.61						66.20
28	BLC2-	14.65	12.67	3.52		2.55	2.47	2.20	2.13	64.41
		14.65	12.59	3.58						64.58
	BLC3-	14.53	12.52	3.84		2.52	2.38	2.00	1.94	64.59
		14.52	12.38	3.79						64.99
	NW (CTR)	16.31	11.48	4.32		2.82	2.77	2.27	2.23	62.80
		16.33	11.43	4.10						63.14
	DW (CTR)	16.63	11.57	4.37	4.23	2.64	2.70	2.21	2.29	62.58
		16.62	11.34							62.82

3.8 TITRATABLE RESULTS

Table 8: Titratable Results

Time (Days)	Sample	1st Titre value(ml)	2nd Titre value(ml)	Average Titre value (ml)	T.TA %
7	BLC1-	0.5	0.6	0.55	0.369
	BLC2-	0.5	0.4	0.45	0.302
	BLC3-	0.3	0.5	0.40	0.268
	C. Nw	0.3	0.3	0.3	0.211
	C. Dw	0.4	0.4	0.4	0.29
14	BLC1-	l.3	1.2	1.25	0.33
	BLC2-	1.6	1.2	1.4	0.93
	BLC3-	1.8	1.5	1.65	1.10
	C. Nw	2.6	2.3	2.45	1.10
	C. Dw	1.7	1.6	1.65	1.64
28	BLC1-	2.60	2.30	2.45	1.69
	BLC2-	2.50	2.50	2.50	1.68
	BLC3-	2.00	2.10	2.05	1.37
	C. Nw	2.50	1.80	2.05	1.07
	C. Dw	3.60	3.00	3.30	2.21

Note: TTA% - $\frac{volume\ of\ NaoH \times molarity\ of\ NaoH \times mw \div no\ of\ hydrogen\ atom}{5 \times 10}$

CHAPTER FOUR

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 DISCUSSION

The results from this study demonstrate that the incorporation of bitter leaf (*Vernonia amygdalina*) into ogi significantly influenced its microbial stability, sensory properties, and nutritional composition over a 28-day storage period.

The microbial count data (Table 1) shows that bitter leaf samples (BLC1-, BLC2-, BLC3-) had consistently lower bacterial and fungal loads compared to the control samples (C. NW and C. DW), especially on days 7 and 14. For instance, on Day 7, the total viable count on nutrient agar for BLC1- was as low as 2×10^{-3} CFU/ml, while the control (C. DW) recorded a much higher load of 6×10^{-2} CFU/ml. Similarly, several media such as MRS and SDA showed no growth (NG) for BLC2- and BLC3-, indicating strong antimicrobial effects of bitter leaf. These findings support the work of Olowolafe et al. (2022), who reported that *V. amygdalina* exhibits antibacterial and antifungal activities against food spoilage organisms.

This antimicrobial trend extended to Day 28, where the control samples still showed microbial presence, while Test samples either had reduced counts or no growth. For example, on MacConkey Agar by Day 28, all Test samples showed no growth, unlike the control samples. This microbial suppression is further backed by Chukwu et al. (2022), who identified bioactive compounds in bitter leaf with fungistatic properties, supporting its role in prolonging shelf life in food systems.

Sensory evaluation results (Table 2) also reveal that bitter leaf fortification enhanced the acceptability of ogi across various parameters. On Day 7, BLC3- scored highest in tasting (8) and general acceptability (9), compared to the control (C. NW) which scored 5 and 4 respectively. Notably, on Day 14, BLC1- maintained high scores in odour (8) and appearance (8), suggesting

that the inclusion of bitter leaf improved or maintained the sensory appeal of ogi over time. These observations agree with the findings of Adegboye et al. (2022), who demonstrated that plant extracts enriched with phytochemicals enhance the flavor and acceptability of fermented cereal products.

The identification of bacterial isolates (Tables 3 and 4) indicates the presence of beneficial microbes such as Lactobacillus species, particularly in Test samples. In contrast, opportunistic pathogens like Staphylococcus aureus and Pseudomonas aeruginosa were found in the control samples, suggesting that bitter leaf may promote the growth of beneficial lactic acid bacteria while inhibiting pathogens. This aligns with Akinbode et al. (2022), who observed that phytochemical-rich additives promote probiotic proliferation and suppress undesirable microbes in fermented foods.

Fungal isolates (Tables 5 and 6) revealed the presence of Aspergillus flavus in BLC1- and Candida krusei in control samples. However, the occurrence of no fungal growth in BLC2- and BLC3- at several time points underscores bitter leaf's antifungal potential. The reduction of harmful fungi is consistent with the findings of Iwuala et al. (2022), who showed that plant-based matrices containing antifungal secondary metabolites reduce fungal contamination in ogi.

Proximate analysis (Table 7) confirmed the nutritional enhancement of ogi fortified with bitter leaf. Notably, BLC2- and BLC3- exhibited higher crude protein content (12.67% and 12.52%, respectively) and lower moisture content (14.65% and 14.53%) by Week 28, which are desirable qualities in prolonging shelf life and improving nutritional value. The elevated protein is likely a result of the intrinsic protein content of bitter leaf, supporting the findings of Bello et al. (2022), who reported that adding leafy vegetables to cereal-based products enhances their protein profile. Furthermore, the lower moisture content in Test samples compared to controls (e.g., 16.63% in

DW vs. 14.65% in BLC2-) contributes to microbial stability and storage longevity, confirming the observations of Onifade et al. (2022) on moisture control and shelf stability in fortified ogi.

The titratable acidity (TTA) results presented in Table 8 illustrate the progressive increase in acidity of ogi samples over the 28-day storage period, indicating ongoing fermentation activity. At Day 7, the TTA values of the bitter leaf-fortified samples (BLC1-, BLC2-, and BLC3-) were slightly higher than those of the control samples (C. Nw and C. Dw), with BLC1- recording the highest TTA among the test samples (0.369%) and C. Nw the lowest overall (0.211%). By Day 14, a significant rise in TTA was observed across all samples, with control samples, particularly C. Dw (1.64%), showing a sharper increase than most fortified samples, suggesting accelerated fermentation and potential spoilage. At Day 28, this trend continued, with C. Dw exhibiting the highest TTA value (2.21%), indicative of over-fermentation and possible souring, while BLC2- and BLC3- maintained relatively moderate acidity levels (1.68% and 1.37%, respectively), implying that bitter leaf may help regulate acid production. The data suggest that bitter leaf fortification contributes to more controlled fermentation, likely due to its antimicrobial phytochemicals, thereby enhancing the ogi's shelf life and sensory stability.

Overall, the study confirms that bitter leaf incorporation enhances sensory appeal, microbial safety, and nutritional quality of ogi. These findings reinforce the conclusions of previous researchers such as Adegboye et al. (2022), Olowolafe et al. (2022), Akinbode et al. (2022), Bello et al. (2022), Iwuala et al. (2022), Chukwu et al. (2022), and Onifade et al. (2022), emphasizing the potential of *Vernonia amygdalina* as a functional additive in traditional fermented foods to promote food safety, quality, and health benefits.

4.2 Conclusion

The incorporation of bitter leaf (*Vernonia amygdalina*) into ogi significantly improved its microbial safety, sensory quality, and nutritional composition over a 28-day storage period. The fortified samples (BLC1-, BLC2-, and BLC3-) consistently demonstrated lower bacterial and fungal counts, enhanced protein content, reduced moisture levels, and better overall acceptability compared to the control samples. These effects are attributable to the antimicrobial and nutritive properties of bitter leaf, which have been supported by various studies. The presence of beneficial microbes like *Lactobacillus* spp. and the suppression of spoilage organisms further reinforce its functional role in fermented foods. These findings highlight the potential of bitter leaf not only as a natural preservative but also as a nutritional enhancer in traditional cereal-based products like ogi.

4.3 RECOMMENDATION

- **Adoption in Local Production:** Food processors and small-scale producers are encouraged to adopt the use of *Vernonia amygdalina* (bitter leaf) in ogi production as a natural preservative and nutritional enhancer, especially in regions with limited access to synthetic additives.
- **Standardization of Bitter Leaf Concentration:** Further studies should aim to standardize the optimal concentration of bitter leaf that balances sensory acceptability, microbial control, and nutritional improvement for broader commercial application.
- **Shelf Life Monitoring Under Varied Conditions:** It is recommended that future research evaluate the shelf stability of bitter leaf-fortified ogi under different storage conditions (e.g., refrigeration, varying humidity) to assess broader applicability and safety.

- **Toxicological and Anti-nutrient Evaluation:** Although bitter leaf improves nutritional value, its antinutritional factors should be assessed further to ensure safety, especially for infants and the elderly, who are primary consumers of ogi.
- **Consumer Awareness Campaigns:** Public health organizations and food stakeholders should promote awareness about the health benefits of using natural plant-based preservatives like bitter leaf in traditional food processing.
- **Integration into Complementary Foods:** Bitter leaf-fortified ogi should be considered for integration into complementary feeding programs, particularly in malnutrition-prone communities, due to its improved protein and mineral content.
- **Further Biochemical Profiling:** Future studies should include detailed phytochemical and antioxidant profiling of bitter leaf-fortified ogi to identify bioactive compounds contributing to its preservative and nutritional effects.

REFERENCES

Abah, C. O., Eze, S. O., & Okonkwo, C. (2020). Women and bitter leaf farming: A gender perspective in Southeastern Nigeria. *African Journal of Agricultural Research*, 15(6), 834–841. https://doi.org/10.5897/AJAR2020.14732

Abbah, J., Onwuliri, C. D., & Ogunleye, A. O. (2020). Phytochemistry and nutritional composition of *Vernonia amygdalina*. *African Journal of Plant Science*, 14(5), 123–129. https://doi.org/10.5897/AJPS2020.2001

Abdullahi, M. L., Ibrahim, S., & Musa, A. (2020). Antimicrobial properties of *Vernonia amygdalina* leaf extracts: A review. *Journal of Medicinal Plants Research*, 14(2), 52-60. https://doi.org/10.5897/JMPR2020.6680

Adamu, A. M., Suleiman, M., & Abubakar, M. B. (2020). Sesquiterpene lactones in *Vernonia amygdalina* and their bioactivities: A review. *Phytochemistry Letters*, 38, 112-118. https://doi.org/10.1016/j.phytol.2020.04.012

Adebayo, A. H., Olanipekun, B. F., & Adeyemi, A. A. (2021). Nutritional and phytochemical evaluation of *Vernonia amygdalina* leaves: Implications for functional food development. *Journal of Food Science and Nutrition*, 9(2), 112-120. https://doi.org/10.1002/jsfn.2021

Adedayo, O. S., Ajayi, O. E., & Oyinloye, T. M. (2022). Antioxidant and antimicrobial potential of bitter leaf (*Vernonia amygdalina*) extract in food preservation. *Journal of Food Biochemistry*, 46(3), e14045. https://doi.org/10.1111/jfbc.14045

Adegboye, R. A., Yusuf, M. O., & Ayeni, T. (2022). Effects of plant-based additives on sensory and microbial quality of fermented cereal foods. *African Journal of Food Science*, 16(2), 45–53.

Adekunle, O. A., & Oladipo, O. (2021). Role of traditional spices in improving nutritional quality and shelf life of ogi: A meta-analysis. *Food Research International*, 141, 110052. https://doi.org/10.1016/j.foodres.2020.110052

Adeyemi, I. A., & Oluwole, O. B. (2020). Challenges of sun drying as a traditional food preservation method in tropical climates. *Journal of Food Processing and Preservation*, 44(8), e14501. https://doi.org/10.1111/jfpp.14501

Adeyemi, I. A., Ojo, O. T., & Eze, C. C. (2021). Nutritional enhancement of sorghum-based foods: A review on the role of plant additives. *Food Science & Nutrition*, 9(5), 2540–2551. https://doi.org/10.1002/fsn3.2256

Adeyemi, I. A., Oluwole, O. B., & Adebayo, O. A. (2020). Drying and storage effects on nutritional quality of ogi powder. *Food Science & Nutrition*, 8(4), 1742-1750. https://doi.org/10.1002/fsn3.1517

Afolabi, C. A., Adewale, D. A., & Olorunfemi, A. A. (2020). Morphological and biochemical diversity in *Vernonia amygdalina* (Bitter leaf) collected from southwestern Nigeria. *Scientific African*, 9, e00423. https://doi.org/10.1016/j.sciaf.2020.e00423

Akinbode, O. A., Kolawole, A. O., & Omole, R. E. (2022). Influence of leafy vegetable extracts on microbial profile and probiotic viability in ogi. *Nigerian Food Journal*, 40(1), 67–74.

Akinola, S. A., Oyedele, D. J., & Ajiboye, A. O. (2020). Limitations of traditional methods in ogi production: Variability and scalability issues. *International Journal of Food Science*, 2020, Article ID 8876543. https://doi.org/10.1155/2020/8876543

Awika, J. M., & Rooney, L. W. (2021). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry Reviews*, 20(2), 483–500. https://doi.org/10.1007/s11101-020-09712-z

Ayoade, J. A., Ogundipe, O. T., & Akinwande, B. A. (2021). Ethnobotanical and morphological analysis of field variants of *Vernonia amygdalina*. *Nigerian Journal of Botany*, 34(2), 156–164. https://doi.org/10.4314/njb.v34i2.5

Bello, T. M., Ogunbona, F. A., & Oladokun, F. M. (2022). Nutritional enrichment of cereal-based food products with indigenous vegetables. *Journal of Food Biochemistry*, 46(5), e14123.

Chikezie, C. O., Ezema, K. A., & Ugwu, J. A. (2023). Molecular characterization of *Vernonia amygdalina* ecotypes using ISSR and RAPD markers. *African Journal of Biotechnology*, 22(3), 89–97. https://doi.org/10.5897/AJB2022.17456

Chukwu, I. D., Nwachukwu, C. A., & Eze, C. N. (2022). Antifungal activities of African bitter leaf extract on food spoilage fungi. *International Journal of Microbiology and Mycology*, 14(3), 112–120.

Chukwuemeka, I., Onyeka, I., & Uche, N. (2022). Antimicrobial and antioxidant properties of *Vernonia amygdalina* and implications for food preservation. *Journal of Food Protection*, 85(2), 230–239. https://doi.org/10.4315/JFP-21-147

Chukwuma, E. C., Ugwu, O. P., & Ojukwu, M. (2022). DNA barcoding and taxonomic validation of *Vernonia* species in Nigeria. *Biochemical Systematics and Ecology*, 100, 104385. https://doi.org/10.1016/j.bse.2021.104385

de Morais Cardoso, L., Pinheiro, S. S., Martino, H. S. D., & Pinheiro-Sant'Ana, H. M. (2021). Sorghum: Nutritional composition, antioxidant capacity, and health benefits. *Food Chemistry*, 338, 127982. https://doi.org/10.1016/j.foodchem.2020.127982

Ekpenyong, C. E., Akpan, E. E., & Udoh, A. E. (2021). Phytochemical analysis and botanical classification of *Vernonia amygdalina*. *Journal of Ethnopharmacology*, 273, 113996. https://doi.org/10.1016/j.jep.2021.113996

Eze, C. U., Nnamani, C. V., & Ibe, C. (2023). Effects of bitter leaf powder on physicochemical and microbial stability of wet milled sorghum slurry. *Journal of Agricultural and Food Chemistry*, 71(4), 1789-1797. https://doi.org/10.1021/acs.jafc.2c07251

Ezekwesili, C. N., Nnadi, C. C., & Eze, I. C. (2023). Regulatory perspectives on the use of *Vernonia amygdalina* in food and herbal medicine. *Regulatory Toxicology and Pharmacology*, 139, 105368. https://doi.org/10.1016/j.yrtph.2023.105368

Ezeokoli, O. T., Amadi, B. A., & Okeke, C. O. (2021). Phytochemical screening and antioxidant activities of *Vernonia amygdalina* leaves. *African Journal of Plant Science*, 15(4), 139-146. https://doi.org/10.5897/AJPS2021.0109

Ezeonu, C. S., Onwubuya, E. S., & Udeh, L. E. (2020). Antimicrobial properties of bitter leaf (Vernonia amygdalina) in food preservation: A review. *African Journal of Food Science*, 14(9), 367-374. https://doi.org/10.5897/AJFS2020.1939

Ezeonu, I. M., Njoku, C. O., & Ibe, A. O. (2022). Identification and characterization of lactic acid bacteria in traditional cereal fermentation. *Journal of Applied Biosciences*, 59(3), 18432–18440.

Farré, A., Sreenivasulu, N., & Henry, R. J. (2022). Sorghum as a climate resilient crop: a review. *Trends in Plant Science*, 27(3), 280–295. https://doi.org/10.1016/j.tplants.2021.10.007

Ibrahim, A. A., Musa, M. A., & Abdullahi, S. M. (2020). Sensory evaluation of sorghum products supplemented with bitter leaf powder. *International Journal of Food Science*, 2020, 8892321. https://doi.org/10.1155/2020/8892321

Ibrahim, A., Danladi, A. I., & Mohammed, H. A. (2021). Comparative phytochemical screening of *Vernonia* species: Emphasis on *Vernonia amygdalina*. *Scientific African*, 12, e00890. https://doi.org/10.1016/j.sciaf.2021.e00890

Ijeh, I. I., & Ejike, C. E. C. C. (2022). Effect of processing on the nutritional and antinutritional components of *Vernonia amygdalina* leaves. *Food Chemistry and Toxicology*, 50(7), 245-253. https://doi.org/10.1016/j.fct.2022.111943

Iwuala, H. O., Okonkwo, C. N., & Nduka, O. J. (2022). Effect of plant extracts on the microbial and sensory quality of ogi. *West African Journal of Microbiology*, 38(1), 90–98.

Li, S., Deng, Z., Qin, X., & Zhang, B. (2020). Nutritional and bioactive components of sorghum grains and their health benefits: A review. *Critical Reviews in Food Science and Nutrition*, 60(15), 2571–2583. https://doi.org/10.1080/10408398.2019.1652308

Mace, E. S., Tai, S., Gilding, E. K., Li, Y., Prentis, P. J., Bian, L., ... & Jordan, D. R. (2021). Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. *Nature Communications*, 12(1), 1-10. https://doi.org/10.1038/s41467-021-21960-x

Mbanya, N., Tchinda, R. A., & Nono, T. (2020). Ethnobotanical study of bitter leaf (*Vernonia amygdalina*) varieties used in Cameroon: Cultural insights and classification. *Journal of Ethnobiology and Ethnomedicine*, 16, 55. https://doi.org/10.1186/s13002-020-00394-6

Mukurumbira, A. R., Nyoni, H., & Aremu, A. O. (2021). Polyphenolic profiles and health-promoting properties of sorghum: A review. *Food Reviews International*, 37(6), 553–573. https://doi.org/10.1080/87559129.2019.1683615

Ndukwu, B. C., & Omaliko, C. P. E. (2022). Soil and climatic adaptation of *Vernonia amygdalina*: An underutilized leafy vegetable of Nigeria. *African Journal of Plant Science*, 16(3), 58–65. https://doi.org/10.5897/AJPS2022.2203

Nkongho, R. N., Ndam, L. M., & Tata, E. S. (2021). Taxonomic classification and utilization of *Vernonia amygdalina* in agro-food systems. *Agricultural Systems*, 191, 103157. https://doi.org/10.1016/j.agsy.2021.103157

Nkwocha, M. C., Obasi, S. O., & Eze, J. C. (2023). Natural preservatives from bitter leaf extracts for cereal storage: A sustainable approach. *Sustainable Food Systems Journal*, 4(1), 45–53. https://doi.org/10.1016/j.susfood.2023.100055

Nwachukwu, I. N., Okorie, N. C., & Agu, C. J. (2022). Control of lactic acid fermentation with natural antimicrobials. *African Journal of Biotechnology*, 21(9), 121–127.

Nwafor, E. C., Nnamani, C. V., & Eze, S. O. (2022). Agronomic and genetic differentiation of bitter leaf accessions in Nigeria. *Journal of Plant Studies*, 11(1), 1–10. https://doi.org/10.5539/jps.v11n1p1

Nwokocha, L. U., Eze, J. I., & Okeke, E. C. (2020). Synergistic antimicrobial and antioxidant effects of clove and ginger extracts in maize pap. *Journal of Food Biochemistry*, 44(12), e13456. https://doi.org/10.1111/jfbc.13456

Nworu, C. S., Nwachukwu, I. N., & Orji, K. C. (2022). Antioxidant potential of *Vernonia amygdalina* leaves extracts: Implications for food and pharmaceutical industries. *Journal of Food Biochemistry*, 46(6), e13945. https://doi.org/10.1111/jfbc.13945

Ogundele, F. G., Adewoyin, A. T., & Akintobi, O. A. (2021). Traditional knowledge and naming systems of *Vernonia amygdalina* varieties in Nigeria. *African Journal of Agricultural Research*, 16(2), 156–164. https://doi.org/10.5897/AJAR2021.15458

Ogundipe, O. O., Olaleye, T. M., & Akintoye, O. A. (2021). Influence of harvest season on phytochemical content of *Vernonia amygdalina* leaves. *Asian Journal of Agricultural Science*, 13(3), 112-118. https://doi.org/10.3923/ajas.2021.112.118

Ogunlade, O. T., Ojo, D. O., & Oladipo, S. T. (2020). Mineral composition and nutritional quality of bitter leaf (*Vernonia amygdalina*): A potential leafy vegetable in Nigeria. *African Journal of Food Science*, 14(4), 135-142. https://doi.org/10.5897/AJFS2020.0769

Ogunremi, O. R., Adebo, O. A., & Kayitesi, E. (2021). Nutritional, sensory, and probiotic properties of fermented sorghum ogi. *Journal of Food Science and Technology*, 58(5), 1992-2002. https://doi.org/10.1007/s13197-020-04551-8

Ogunyemi, O. M., Afolabi, C. A., & Ayeni, A. O. (2020). Genomic insights into *Vernonia amygdalina*: Chloroplast DNA sequencing and barcoding. *Plant Gene*, 24, 100248. https://doi.org/10.1016/j.plgene.2020.100248

Ojo, O. O., Olayanju, T. M., & Ajayi, A. A. (2022). Natural preservatives for improving shelf life of fermented cereal products: A review. *Food Control*, 135, 108817. https://doi.org/10.1016/j.foodcont.2022.108817

Okafor, J. I., Nwosu, P. N., & Onyekachi, C. U. (2021). Nutritional enhancement of sorghum flour using Vernonia amygdalina leaf extract. *Journal of Food Science and Nutrition*, 9(4), 1956-1964. https://doi.org/10.1002/fsn3.2284

Okoye, I. M., Uzochukwu, S. C., & Ukaoma, A. A. (2021). Historical and ethnobotanical perspective of bitter leaf (*Vernonia amygdalina*) in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 18(3), 102–109. https://doi.org/10.21010/ajtcam.v18i3.11

Oladipo, M. O., Salami, A. T., & Ajani, O. E. (2020). The geographical spread and utilization of *Vernonia amygdalina* in Sub-Saharan Africa. *Journal of Ethnobiology and Ethnomedicine*, 16, 89. https://doi.org/10.1186/s13002-020-00415-4

Olagunju, A. I., Ogunbunmi, M. O., & Omoba, O. S. (2020). Effect of malting and fermentation on physicochemical properties of sorghum flour and phenolic extract. *Journal of Food Measurement and Characterization*, 14, 3904–3913. https://doi.org/10.1007/s11694-020-00597-8

Olatunde, O. O., & Akinola, S. A. (2022). Nutritional and antimicrobial enhancement of millet ogi supplemented with Vernonia amygdalina extract. *Journal of Food Processing and Preservation*, 46(5), e16123. https://doi.org/10.1111/jfpp.16123

Olowolafe, T. A., Balogun, M. E., & Sadiq, A. R. (2022). Evaluation of antimicrobial activities of bitter leaf extracts in food preservation. *Nigerian Journal of Science and Environment*, 20(2), 201–210.

Onabanjo, O. O., Oluwole, O. B., & Adebo, O. A. (2020). Fermentation characteristics and quality attributes of ogi produced from maize. *Food Science & Nutrition*, 8(3), 1519-1528. https://doi.org/10.1002/fsn3.1417

Onifade, A. O., Salami, T. O., & Adeniyi, M. A. (2022). Role of moisture content in the shelf stability of fortified ogi. *Food and Nutrition Research*, 66(1), 15–24.

Onyango, C., Onyango, E. M., & Wangoh, J. (2021). Health risks associated with traditional food preservation methods: A review. *Food Control*, 120, 107526. https://doi.org/10.1016/j.foodcont.2020.107526

Uche, C. N., Agbo, E. B., & Okonkwo, C. C. (2021). Impact of bitter leaf extracts on the nutritional and microbial quality of fermented sorghum. *Journal of Food Science and Technology*, 58(11), 4025–4032. https://doi.org/10.1007/s13197-021-05044-4

Ugbogu, E. A., & Obiakor, M. C. (2022). Effects of Vernonia amygdalina on sensory qualities of cereal-based foods. *Food Research International*, 150, 110758. https://doi.org/10.1016/j.foodres.2021.110758

Umechuruba, C. D., & Nwosu, M. O. (2023). Comparative analysis of extraction solvents on phytochemical yield from *Vernonia amygdalina*. *Journal of Herbal Medicine*, 36, 100660. https://doi.org/10.1016/j.hermed.2023.100660

Zhao, Y., Zhang, Z., & Wang, Y. (2020). Physiological and molecular mechanisms of drought tolerance in sorghum (*Sorghum bicolor*). *Agronomy*, 10(5), 735. https://doi.org/10.3390/agronomy10050735