Insight into the Beneficial Use of Iru An African Condiment from *Parkia Biglobosa*

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ABSTRACT

The composition and activity of the gut microbiota, which is essential for preserving health, are greatly influenced by the food that humans eat. Iru, a traditional West African fermented condiment, is derived from *Parkia biglobosa* seeds and is a sought-after ingredient in West African cuisine due to its unique aroma, flavor, and texture.

Iru is a spice that adds depth to stews, soups, and sauces in West African cooking, especially in Nigeria and Cameroon. Due to the fermentation process, which transforms complex ingredients into more easily absorbed forms, it has a distinct flavor and fragrance. Beyond only eating, iru has a deep cultural meaning that is frequently emphasized in ceremonies, festivals, and customs. Generations of people have used this fermenting technique. Iru is a traditional condiment from West Africa that showcases the region's culinary heritage and is a favorite condiment in West African cookery. It is made using beneficial bacteria to make distinctive, tasty, and culturally significant food items.

This study investigated the effects of two distinct food components—Maggi Knorr, a chemical seasoning mixture, and Iru (*Parkia biglobosa*), a traditional fermented condiment from West Africa—on the intestinal microbial communities of mice. Using mice as a well-researched animal model, we will assess the short- and long-term effects of food supplementation with Maggi Knorr and Iru on microbial diversity, composition, and functional ability in the gastrointestinal tract. The metabolic activities of the microbial communities will be characterized by means of high-throughput sequencing of 16S rRNA genes in conjunction with metabolism analysis.

This study aimed to investigate how two commonly utilized food additives, namely chemical seasoning (Maggi Star, Knorr and Vedan) and Iru (*Parkia biglobosa*), affect the microbial community residing in the gastrointestinal tracts of rats.

INTRODUCTION

In West Africa, fermented vegetable protein seeds are used to make sauces. African bean locust [*Parkia biglobosa* (Jacq. Benth)].

This species is typically found in gallery forests across various African countries, including Benin Republic, Burkina Faso, Côte d'Ivoire, Cameroon, Nigeria, and Mali. Exhibiting arboreal characteristics, it can reach heights of up to 30 meters. Noteworthy features include red globular inflorescence, variable flat pods, and seeds. *Parkia biglobosa* holds ecological and cultural significance within the regions it inhabits, contributing to the biodiversity of gallery forests in West and Central Africa (Bothon, *et al*., 2023; Balogun, *et al.*, 2018).

The fruit is an unsplit, brown indehiscent pod, measuring 30 to 40 cm in length and 2 to 3 cm in width, yielding up to 20 seeds (Fifame, *et al.*, 2016).

A large amount of African diets consist of condiments, which are used as tasty and culinary ingredients in a variety of cuisines. (Olubunmi, *et al.*, 2022). In Nigeria and Ghana, they are known as iru or dawadawa (Modupe, *et al.*, 2016); afitin and sonru in the Republic of Benin (Bothon *et al*., 2023), in Burkina Faso, as soumbala (Compaore, *et al*., 2020), bikalga, and maari; in Senegal, as nététou; in Sierra Leone, as kinda; in Niger Republic, as dawadawa botso; in Mali, as datou; in Cameroon, and in Sudan, as furundu. (Adelekan, *et al.*, 2017; Parkouda, *et al*., 2010). Additionally, these seeds are valued for their termiticidal properties in oil (Modupe, *et al*., 2017).

In the past, fermented foods were created to boost raw material safety, prolong shelf life, and function as probiotics. Microorganisms are crucial to the production process because they increase the physiochemical, sensory, and safety qualities of the finished goods (Cao, *et al.*, 2023). Iru, a fermented condiment, is produced from African locust bean seeds. (*Parkia biglobosa*).

Primarily used in West Africa, this spice is highly favored by the Yoruba community in Nigeria. It is frequently used to prepare classic meals like okra, melon, and other vegetable soups (Atere *et al.,* 2020).

However, there are regional variations in the vegetable seeds utilized to make these condiments. (Olubunmi, *et. al.*, 2022). Nevertheless, the production process is typically characterized by the spontaneous alkaline fermentation of a solid substrate, leading to an elevation in pH. This process involves the widespread breakdown of proteins into peptides, amino acids, and ammonia, fostering the prevalence of Bacillus species as the primary fermenting organisms. (Atoyebi, *et al*., 2022; Olasupo and Okorie, 2019; Olubunmi, *et. al*., 2022).

Iru, a traditional condiment, is widely accepted in various cultures due to its cultural significance. It is made by fermenting the seeds of the African locust bean tree, *Parkia biglobosa*, which is an indigenous plant crucial to the continent's economy and society. The process involves roasting the seeds over a fire to soften the testa and cotyledons, then pounding the softened seeds with a mortar or foot. The seeds are then boiled for an additional hour to extract the testa. (Olubunmi, *et al.*, 2022).

Iru and ogiri are important condiments used for flavour enhancement in foods and serve as protein substitutes in diets among rural populations across West Africa (Ademola, *et al*., 2018).

Additionally, the craft is practiced in unique, unpredictable environmental settings, producing goods with a range of organoleptic characteristics and quality (Olasupo, and Okorie, 2019; Adamu, *et al*., 2019).

Sorting, washing, dehulling, sieving, short-cooking, draining, and fermentation are some of these procedures (Atoyebi, *et al.*, 2022).

The genus *Parkia* comprises flowering plants that are found across the pantropics and are members of the Fabaceae family (subfamily Mimosoideae) (Saleh, *et al*., 2021). After the Scottish explorer Mungo Park died in January 1805, his name became *Parkia*, and he died in the Niger River in Nigeria (Saleh, *et al.*,

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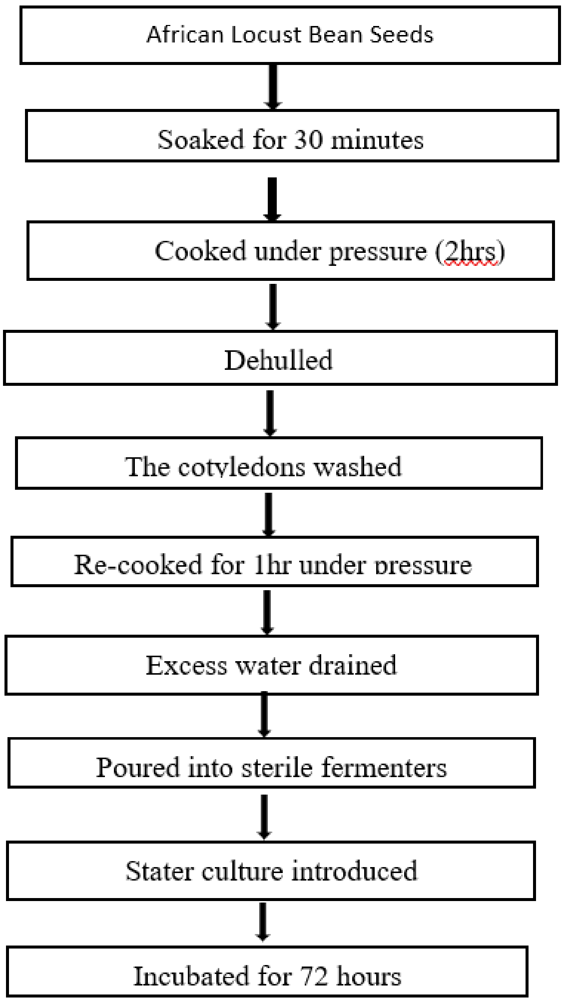
2021). Another four more species were discovered in 2009 (Saleh, *et al*., 2021). There are twenty species in the Neotropics, four in Africa, and ten in Asia. Meanwhile, 80 scientific names from the genus *Parkia* are listed in a 2018 plant list, of which 41 are accepted names and 39 are synonym species (The Plant List, 2018). Pods are the fruits that these plants produce. There are up to 25–30 seeds in each pod. Numerous *Parkia* species have been found to be abundant in minerals, proteins, and carbohydrates (Saleh, *et al*., 2021).

Parkia biglobosa, named after Scottish botanist Robert Brown in 1826, is an African locust bean tree belonging to the legume group. Its family includes species like *Parkia bicolor*, *clappertoiana*, and *filicoidea*. These species can ferment, producing a flavorful condiment rich in vegetable protein. The indigenous people of Africa use *Parkia biglobosa* for culinary and medicinal purposes (Ojewumi, *et al*., 2016).

When compared to several other typical plant meals, iru has the greatest riboflavin level, making it an essential source of riboflavin (Amao, *et al*., 2018).

TRADITIONAL MEDICINAL USES

All tropical nations employ *Parkia* species as remedies for various illnesses. Traditionally, *Parkia* plants have been used for almost all of their components for various therapeutic uses. For the purpose of treating a variety of illnesses, *Parkia* plant components are prepared into paste, decoction, and juice. Almost every known species of *Parkia* is used, in various ways, to treat dysentery and diarrhea (Singh, *et al.*, 2015)



A flowchart that generates iru (Atere *et al.*, 2020).

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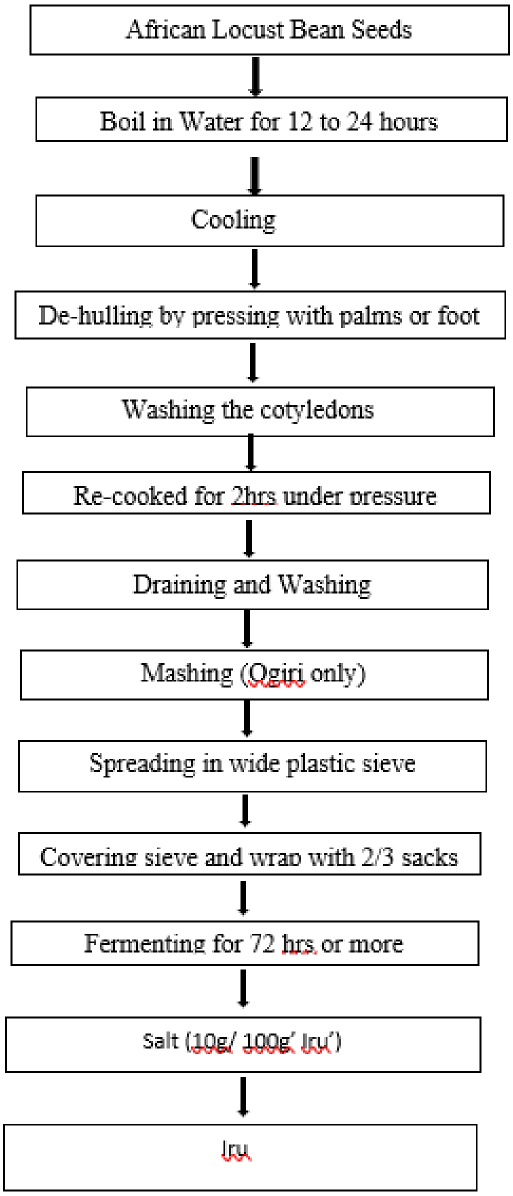

Consulted a woman @ Okinni (Osun State) on Iru Making

FERMENTATION OF IRU

"Foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action" is the definition of fermented foods. Throughout history, an extensive array of foods, including dairy products, fruits, vegetables, meat, fish, soybeans, and various legumes, have undergone the process of fermentation (Marco, *et al.*, 2017).

Making fermented, delicious food condiments has continued to be a family practice in homes with few tools. The techniques employed differ from one place to another based on the customs, beliefs, preferences, and lifestyle of the ancestors who shared the same line of work. These modifications in processing techniques result in differences in Iru quality (Adamu, *et al.*, 2019).

Food fermentation has long been employed as a preservation method because it produces antimicrobial metabolites (such organic acids, ethanol, and bacteriocins) that reduce the likelihood of infection with pathogenic microorganisms. Furthermore, food's organoleptic qualities—taste and texture—are enhanced

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with fermentation; without fermentation, bitter phenolic chemicals are removed from food, rendering some items inedible, like olives. There are two main methods for fermenting food. To begin with, certain foods have the ability to naturally ferment; these are referred to as “wild ferments” or “spontaneous ferments,” and they take place when there are microorganisms in the raw food or in the processing environment. These foods include kimchi, many fermented soy products, and sauerkraut. Second, foods like kefir, kombucha, and natto may be transformed into “culture-dependent ferments” by introducing starter cultures (Rezac, *et al* ., 2018).

There are various benefits to fermented foods. (Melini, *et al.*, 2019, and Sanlier, *et al*., 2019):

(1). Ones that are fermented last longer on the shelf than unfermented ones.

(2). The improvement of organoleptic qualities is exemplified by cheese, which has more refined flavor compared to its raw substrate, milk.

(3). The elimination of undesirable or hazardous components from raw materials; for instance, the toxic cyanide level of cassava is decreased during the manufacture of garri, and fermentation eliminates the stomach-producing agents in soybeans.

(4). Increased nutritional value brought on by the presence of fermenting microorganisms. For example, the nutritional value of bread and garri is improved by the yeast and lactic acid bacteria, respectively.

(5). Fermentation reduces the amount of time food needs to cook. For instance, soybean products and West African cuisine such as Ogi, which is made from fermented maize.

(6). In vitro, the fermented products have higher antioxidant activity. Yogurt and fermented milk, for example, have higher antioxidant content than milk because, after breaking down, they form biopeptides, which are mostly α-casein, α-lactalbumin, and β-lactoglobulin.

Traditional fermented foods are an important part of the diet in Africa. (El Sheikha and Montet 2014). West Africans consume a lot of fermented sauces made from African Locust Beans (*Parkia biglobosa*) in their diet (Adelekan, *et al.*, 2017).

The terms “Iru,” “sonru” in Benin, “afitin” (Bothon, *et al*., 2023), “soumbala” (Ojewumi, *et al*., 2016), and “dawadawa” (Olasupo and Okorie, 2019; El Sheikha and Montet 2014) are used to refer to them. They are used to enhance the flavor of many meals, including soups and sauces, because to their sensory properties and high nutritional content (Bothon, *et al*. 2023; Olasupo and Okorie, 2019). They are significant low-cost sources of protein and fat because they contain high amounts of free amino acids such as cysteine, methionine, leucine, isoleucine, tyrosine, phenylalanine, and even lysine, which is rare in plant diets (Agbobatinkpo, *et al*. 2011).

The local environment and the needs and expectations of the client influence how long the fermentation process takes (Bothon, *et al*., 2023). As a matter of fact, the temperature of the food matrix has a significant impact on the proliferation of *Bacillus* species, which in turn affects the success of fermentation.

An immense number of West African women earn a living by producing this fermented locust bean, which takes little capital to produce in small-scale enterprises (Olaoye, 2016). The process of making iru requires many unit activities following the bean’s harvest, depodding, and depulping (Atoyebi, *et al.*, 2022). In Benin, the fermentation process takes place on a wooden tray with a layer of dehulled African Locust Beans (ALB) spread out around 2 cm thick. This allows the product to ferment at a temperature of between 30 and 36 C. The primary bacteria in ALB fermentation, *Bacillus spp*., may grow at their ideal temperature in a fermentation tray with a thick coating of ALB cotyledons (Olasupo and Okorie, 2019), improving the fermented product's safety.

Traditional starters were created to enhance the fermentation process of locally produced fermented goods. Examples of these starters for ALB fermentation include yanyanku and iku-iru (Bothon**,** *et al.* 2023), as well as ikpiru (Agbobatinkpo, *et al*., 2011).

Olasupo and Okorie (2019) stated that yanyanku helped with the fermentation process by softening the dehulled ALB, and that the rate of softening depends on the quality of the yanyanku and the ratio of yanyanku to dehulled ALB (Agbobatinkpo, *et al*., 2011). In fact, the softening of ALB cotyledons is one of the quality criteria that is typically used to assess the effectiveness of fermentation. Agbobatinkpo, *et al.* (2011) El Sheikha (2018) stated that the optimal conditions for fermentation include time, temperature, pH, substrate pretreatment, and inoculum-substrate ratio.

Iru fermentation, according to Olubunmi, *et al.* (2022), is a solid-state, exothermic process in which the fermenting seed's temperature gradually rises from ambient temperature to around 30-45 EC1.

African locust bean (*P. biglobosa*), as it is called in Yoruba, is fermented in an alkaline way to provide iru, a flavorful condiment that brightens soups and stews and balances a diet low in protein. It enhances taste and provides protein to the diets of low-income households. (Olanbiwoninu, *et al*., 2017).

The locust bean seeds are extracted from the pods, which are huge, uneven, and flat clusters (Olanbiwoninu, *et al.*, 2017). According to Olanbiwoninu *et al.* (2017), it has around 40.4% protein, 31.5% fat, 3.1% fiber, and 15% carbohydrates. The primary reason people eat African locust beans is because of their flavor.

Food that has undergone fermentation is safer, more palatable, and more nutrient-dense. African locust beans are not appetizing when cooked, but they transform in texture, composition, and nutritional value when they are fermented to make Iru, a condiment (Olanbiwoninu, *et. al*., 2017).

The fermentation is caused by many strains of *Bacillus subtilis*. Many strains of *B. subtilis* have been discovered from iru samples that were collected from different locations throughout the Southwest. Depending on the country, different amounts of IRU are consumed. (Olanbiwoninu, *et al.*, 2017).

While the general estimated consumption for parts of Nigeria ranges from 1 to 17 g per person per day, the Yorubas of southwest Nigeria consume 10 g of iru per person per day (Olanbiwoninu, *et al.*, 2017). In Ghana and Togo, the average daily consumption of iru per person is 2 g and 4 g, respectively.

The biological process by which different microorganisms like bacteria and fungi transform complex substrates like starch or sugar into simpler molecules is known as fermentation. (Eze, Onwuakor, Ukeka. 2014).

Due to the "pre-digestion" that fermentation imparts on dietary substrates, fermented foods have the potential to be rich sources of nutrients since they increase the bioavailability of the related nutrients. Toxins, antinutritional substances, and allergies, including cyanogenic glycosides, mycotoxins, tannins, phytate, lectins, protease inhibitors, saponins, alkaloids, and oxalate, can occasionally be eliminated by fermentation (Samtiya, 2021).

Furthermore, iru adds calories, protein, and minerals to meals; it also acts as a flavoring and seaner for soups and sauces. Legumes and oilseeds are fermented by allowing microbes to operate on the substrate to make iru. The microorganisms break down the components of the substrate during this process, producing compounds that enhance the nutritional content and flavor of the finished food (Olubunmi, *et al.*, 2022).

It is clear that different microbial flora, which eventually becomes gram positive flora, facilitates the fermentation of vegetable proteins into condiments; nevertheless, it is unclear how much of a role this microbial flora has in the features of the final products (Amao, *et al.*, 2018).

According to Ogunshe *et al.* (2007), the total sugar level increased during the first 24 hours of fermentation, decreased on the second day, and peaked on the third and sixth days. However, the total amino acid level increased during the entire fermentation period. According to Uaboi-Egbenni *et al*. (2009), the highest mean value for decreasing sugar on the fourth day of fermentation was 1.25 mg. (Amao, *et al.*, 2018).

MICROBIAL DIVERSITY IN IRU

In the natural world, bacteria, viruses, protozoa, protists, archaea, and fungus are constantly interacting with one another. According to Neelakanta and Sultana (2013), these microorganisms are frequently discovered in dynamic “consortia” composed of populations of several microbial species. They do not exist in isolation.

Knowledge of the dynamics of the microbial community inside a consortium will be beneficial for the genetic information of all coexisting members.

Metagenomics, also known as genomic studies of microorganisms, is a technique of examining sets of genomes from a diverse population of bacteria that is not relied on culture.1. Handelsman and his associates initially used the term “metagenomics” in their investigation of natural compounds derived from soil bacteria. Metagenomics is frequently used interchangeably with community genomics, environmental genomics, and population genomics (Neelakanta, and Sultana, 2013).

Pace’s 1985 concept provided the initial spark for the discipline of metagenomics. It prompted other investigations, the first of which included direct random shotgun sequencing of ambient DNA after cloning DNA directly from environmental materials in a phage vector (Neelakanta, and Sultana, 2013).

Metagenomics has also provided valuable insights into the “changes” in the microbial community. For example, studies using a metagenomic approach have elucidated changes in the microbial composition in subjects with different diets. In a similar vein, metagenomics has shed light on differences in the microbial makeup of ticks collected from various geographical locations (Neelakanta, and Sultana, 2013).

According to Neelakanta and Sultana (2013), there are four categories into which genomic studies may be separated according to different methods of screening:

(a) Using bulk genome sequencing, a shotgun approach;

(b) Investigations motivated by genomic activity and targeted at determining specific microbial roles;

(c) Investigations of genomic sequences using functional or phylogenetic gene expression analysis; and

(d) Detecting the whole gene content in environmental sample data using next generation sequencing technology.

Neelakanta and Sultana (2013) classify these four methods as targeted metagenomics (activity-driven and sequence-driven research) and unselective metagenomics (shotgun analysis and next generation sequencing).

In order to ascertain the actual microbial makeup of an environment, metagenomics aims to directly isolate DNA from a complicated environmental sample that comprises a range of microbiota. This eliminates the need for conventional culturing methods and enables researchers to investigate the genetically rich resources of uncultured microbiota (Ngara, and Zhang, 2018, and Ahmad, *et al.*, 2019).

We are grateful for Next Generation Sequencing (NGS), which uses precisely selected amplified genomic DNA areas, such as 16S amplicon sequencing, targeted metagenomics has become more accessible for these metagenomic research (Amrane, 2018).

Processing of Samples for Metagenomic Analysis

The first step in every metagenomic project is sample preparation. All of the cells in the sample should be represented by the DNA used for metagenomic research, and it should be perfect for creating genomic libraries. Gene counts between nanograms and micrograms are necessary for metagenomic analysis. (Neelakanta, and Sultana, 2013).

Metagenomic Sequencing

In recent times, metagenomic research has made substantial use of a number of techniques, including shotgun sequencing. In shotgun metagenomics, DNA is isolated from an environmental sample, randomly sheared, sequenced into short segments, and then assembled into consensus sequences. Using this technique, it was possible to successfully discover a number of microorganisms in environmental samples that would normally go undetected in culture procedures. (Neelakanta, and Sultana, 2013).

Taxonomic classification of microbes found in Iru.

The growth of *Bacillus spp*., which is highly influenced by the temperature of the food matrix, is one of the many factors that determine whether the fermentation process is successful.

Because most food-associated microflora cannot be grown on conventional laboratory medium, estimations of “true” microbial diversity in food systems remain challenging (Adelekan, *et al.,* 2017).

With the development of new instruments, it became feasible to examine the molecular details of eukaryotic gene structure and function. Genetic engineering, or the practical modification of genetic material, produces hundreds of valuable goods nowadays. Genes from various sources, frequently different species, are routinely combined in test tubes and then transferred into live cells so that the recombinant DNA may be produced and duplicated. Given its ease of growth and well-understood biology, Escherichia coli is frequently employed as a host (Adelekan, *et al.,* 2017).

Microbial detection may be made much more precise, sensitive, and fast by using molecular biology-based identification techniques for the biodiversity of dietary microbes. It is possible to anticipate the evolutionary relationships between the microorganisms in fermented food and get molecular characterisation using these molecular techniques. It has been observed that *Bacillus subtilis* is the predominant microbe present. The use of the 16S rRNA gene for microbial diversity identification results from its stability and conservativeness throughout the evolutionary era (Adelekan, *et al*., 2017).

The typical microflora of the fermenting beans was dominated by *Staphylococcus* and *Bacillus species*. Yet, the composition of the substrate and the sanitary practices used throughout the production process determine how much the accompanying flora of the fermenting substrate contributes (Amao, *et al.,* 2018).

The usual microflora of the fermenting beans was dominated by species of *Staphylococcus* and *Bacillus* (Amao, *et al.,* 2018). Nonetheless, the nature of the substrate and the sanitary practices implemented during manufacturing dictate the role played by the substrate’s associated flora during fermentation (Olubunmi, *et al.,* 2022).

Alkaline fermentation of vegetable condiments was attributed primarily to *Bacillus species*, however a number of studies have also identified other microorganisms involved in iru production, Olubunmi, *et al*., 2022; Ogunshe, *et al*., 2008) include Micrococcus, Leuconostoc, and Enterobacteriaceae, with *Bacillus* and *Staphylococcus species* making up the bulk of the microflora (Amao, *et al*., 2018).

Numerous *Bacillus species* and a few *Staphylococci species*, including *S. saprophyticus*, were among the microbial flora found in laboratory-prepared anyifermented Samanea saman. These bacteria did not seem to be significantly involved in the fermentation process. (Okonko, 2008).

Microbial dynamics in the course of fermenting

Process management requires an understanding of the microorganisms that contribute to the unique characteristics of food during the fermentation process, which includes using specified starter cultures and choosing the right conditions for the technology. Since the introduction of DNA sequencing and the polymerase chain reaction in molecular biology during the past 20 years, there has been a resurgence of interest in microbial ecology (Cocolin and Ercolini, 2009). These techniques aid in the identification of various microbial populations, such as those that proliferate under lab conditions, those that do not, and those that are both active and dormant in the environment. With this innovative approach, molecular biology techniques are directly applied to profile the diversity of microbes without the need for cultivation (culture-independent methods) and molecular methods are applied to isolated strains during fermentation to identify and characterize them (culture-dependent methods), (Atere, *et al.*, 2020).

It is widely known, according to Atere, *et al*. (2020), that plate culture techniques only reveal a tiny portion of the true microbial population in natural habitats.

Numerous studies have examined the succession of microbes participating in the fermentation of African locust bean seeds using culture-dependent methodologies, and their findings indicate that *Bacillus spp*. are the predominant bacteria present. The bacteria involved in the fermentation of African locust beans (*Parkia biglobosa*) have been widely investigated, and oil seeds are often processed to produce sauces. (Atere *et al*., 2020).

Beneficial Functions of Microbes in Iru

The waxy substance known as cholesterol is produced by the liver of animals and is also consumed through animal-based foods such dairy, meat, poultry, and fish. The body needs cholesterol to build cell membranes, insulate neurons, and create certain hormones. It is also a crucial lipid in various membranes. However, high cholesterol affects human heart function and is a key risk factor for cardiovascular disease (CVD), which includes coronary heart disease and stroke. (Atere, *et al.*, 2020). According to the World Health Organization, 17.3 million deaths worldwide in 2008 were related to CVD. An estimated 23.6 million people will die from CVD every year by 2030, according to estimations (Kobayashi, *et al*., 2012; Napoli, *et al*., 2012). Studies have demonstrated that elevated blood levels of either total cholesterol (TC) or low-density lipoprotein (LDL) raise the risk of a new or recurrent coronary event (Aronow, 2013).

Microorganisms perform critical functions in the production process, enhancing the physiochemical, sensory, and safety properties of finished goods. (Atere, *et al.*, 2020).

Synthetic medications called statins are typically used to reduce hypercholesterolemia; yet, they come with a high price tag and frequently cause adverse effects. Muscle pains, altered liver function, allergic response (rash), heartburn, disorientation, stomach discomfort, constipation, and decreased desire for sexual activity are among the adverse effects of cholesterol-lowering medications. (Atere, *et al.*, 2020).

The nutritional significance of the fruit pulp is notable, attributed to its richness in carbohydrates, proteins, and minerals (Termote, *et al*., 2022; Olalude, *et al*., 2021). Fruit husk extracts have been used as a bonding agent between locally produced clay tiles and the soil underneath (Bothon *et al*., 2023). Additionally, the fruit pulp infusion has been used in West Africa to improve the longevity of earth structures, room walls, and flooring (Banakinao, *et al*., 2016; Abagale, *et al*., 2020). It also helps produce laterite blocks for structures, which offer long-lasting defense and waterproofing (Aguwa, *et al*., 2016; Sorgho, *et al*., 2014).

Lactic acid bacteria's probiotic potential in Iru.

Traditional fermentation is a food preparation method that makes use of microorganisms, particularly yeast and lactic acid bacteria (LAB). Despite being an antiquated method of food preservation, it remains a customary practice among indigenous tribes in Africa and the majority of the developing world, typically carried out on a local or household basis (Mokoena, *et al*., 2016).

The generation of organic acids and a broad range of antimicrobial compounds by lactic acid bacteria (LAB), which have been found from a variety of fermented foods, is responsible for the preservation of the quality and palatability of fermented foods. African societies use lactic acid bacteria (LAB) to produce a variety of fermented foods throughout the continent, such as beverages, fruits and vegetables (such roots or tubers), fermented milks, and fermented meats. The two types of fermentations that are most frequently employed to create fermented foods and beverages are lactic acid and alcohol fermentations. Two other types of fermentation are those using alkaline and amino acids (Mokoena, *et. al*., 2016).

Despite the growing interest in Lactic Acid Bacteria (LAB) as a potential probiotic, there is a lack of research on its innovative uses, particularly in Africa, despite its proven benefits in reducing diarrhea, enhancing antibacterial properties, and extending fermented food shelf life. (Mokoena, *et. al*., 2016).

Lactic Acid Bacteria (LAB) fermentation of food and beverages

Using microorganisms and its enzymes, lactic acid food fermentation is a method that turns fermentable sugars in food substrates primarily into lactic acid and other restricted products. Both in homes and the food industry, fermentation is frequently used to preserve food and create a range of food products (Mokoena, *et al*., 2016).

Traditional food fermentation is mostly done by LAB, which has been granted the "generally regarded as safe" (GRAS) designation. Foods that have undergone fermentation have better organoleptic qualities and are more palatable. In Africa, sour porridges, fermented milks, and alcoholic and non-alcoholic drinks are common fermented food items. A staple meal in many parts of the world, cereals can be fermented to change their flavor, acidity, taste, and food digestibility in ways that are desired. (Mokoena, *et. al*., 2016).

Probiotic bacteria typically do not permanently colonize the human digestive system, however certain strains have the ability to do so temporarily and modify the local microbiota (Shinde, 2012).

According to Mokena *et al*. (2016), LAB are extremely helpful organisms for humans, and their consumption should be encouraged for overall health.

Though not alone, the majority of probiotic foods include LAB from the Lactobacillus genus (39). Probiotic strains can activate and control several components of the immune response, both acquired and natural. However, there have been reports of variations in the immune system functioning of *Bifidobacterium* and *Lactobacillus* strains. These probiotics' interactions and special metabolic properties are essential to their capacity to colonize the gastrointestinal tract and influence the immune system (Mokoena, *et. al*., 2016).

Orwa

Factors influencing the microbial dynamics in Iru.

The high rainfall in tropical and subtropical climates is one of their most notable climatological characteristics, since it contributes significantly to the discharge of nutrients from agricultural areas into surface waterways. Furthermore, the high temperatures and year-round primary productivity in these locations make the surface water sources especially prone to cultural eutrophication, in contrast to temperate environments when spring and summer are the more productive seasons (Cunha, *et al.*, 2013; Gradilla-Hernández, *et al.*, 2018, 2019, 2020).

Additionally, changes in the lakes' water quality have an impact on the microbial communities that regulate the biogeochemical processes in lakes, such as the sulfur, nitrogen, phosphorus, and carbon cycles (Liu, *et al* ., 2018; Wang, *et al*., 2018; Yao, *et al*., 2018); likewise, the metabolic activity of microorganisms through various biochemical processes has a significant impact on the water quality of lakes (Díaz-Torres, *et al*., 2022).

Metagenomic approaches have shown rapid advancement and power in investigating microbial ecology and linking microbial dynamics to biogeochemical processes (Díaz-Torres et al., 2022). Alternatively, phylogenetic analysis of communities by reconstruction of unobserved states (PICRUSt), which utilizes 16S rRNA gene sequences and a reference genome database, has frequently been used to determine the functional profile of bacterial communities (Langille, *et al*., 2013; Wilkinson, *et al*., 2018).

Microbial Safety and Quality Control

In South and Northern Nigeria, the fermented cotyledon of the locust bean (*Parkia biglobosa*) is consumed as iru or dawadawa. The meal known as "okpehe," or fermented Prosopis africana seeds, is popular in the eastern region of Nigeria (Oguntoyinbo, 2012).

When food is processed in an unhygienic environment using antiquated machinery and disregarding Good Manufacturing Practices (GMP), the product may become contaminated and pose a serious health risk (Oguntoyinbo, 2012).

To remain competitive with standardized bouillon cubes and ensure product acceptance and consistency, a strategy to increase condiment safety must be developed, since customer preferences for high-quality, safe food items are growing. A comprehensive approach that enhances food safety by identifying dangers and developing preventative measures without unduly relying on end-product testing is the Hazard Critical Control Points Program (HACCP), (Cormier, *et al*. 2007).

Implementing HACCP will thereby increase the safety of condiments in West Africa by identifying risks, assessing risks, and providing techniques to reduce, mitigate, and manage risks. This meets with the HACCP implementation mandate issued by the WHO/FAO. It has been determined that the endorsement and support of the national government, WHO, and FAO are necessary prerequisites for the successful implementation of (Williams, *et al*., 2003; WHO 1995).

Potential Health Benefits of Consuming Iru (locust beans), (Nwokolo, 2023; and Ikhimalo, 2019)

- Facilitates lowers cholesterol levels

One of the most natural ways to lower cholesterol is to regularly consume locust beans. The natural condiment's hypolipidaemic and antioxidant characteristics disrupt the body's ability to control cholesterol levels, which explains why. Lowering cholesterol is the benefit of locust bean gum.

- Treats hypertension

When the blood pressure on the arteries is higher than usual, it results in hypertension, also referred to as high blood pressure. This disease has the characteristic of being a quiet killer, so unless you get frequent checks, you could not notice anything wrong for years.

Because fermented locust beans are abundant in antioxidants, they have a blood pressure-lowering impact.

Consuming locust beans has been associated with both the management and prevention of hypertension. This is because it has been shown that the seeds in the condiment have antihypertensive properties. Another component of locust bean leaves, procyanidin, has been shown to reduce blood pressure in people (Ikhimalo, 2019).

- Gum health is improved.

There are oral microorganisms everywhere. While some are safe, others could result in dental and gum problems.

However, one of the best methods to cure or prevent gum infections is to consume locust beans.

These natural condiments have qualities that soothe receding gums, treat mouth ulcers, and stop the growth of germs that cause tooth decay.

You can reduce your chance of acquiring eye conditions including xerophthalmia, cataracts, and night vision simply by eating more locust beans.

Locust beans contain vitamin A, which reduces the risk of developing glaucoma, myopia, minus eyes, eye infections, and other eye conditions.

- Treatment for diarrhea or remedy for diarrhea

When your feces abruptly increase in volume, looseness, and frequency, you have diarrhea. Peptin and tannins found in locust beans are often recommended for individuals experiencing diarrhea.

According to recent studies, the African locust bean’s leaves, roots, bark, and fruit may all be prepared as a home remedy for a range of conditions, such as fever, diarrhea, stomach problems, boils, and burns.

Medicinal uses for locust bean bark include local treatment for diarrhea, skin irritations, pneumonia, leprosy, inflammation, bronchitis, parasitic worms, and many other conditions.

- Preventing and treating diabetes

Eating locust beans is one way to avoid diabetes. There is a strong association between cholesterol levels and locust bean extracts, both methanolic and aqueous, according to recent research. Stated differently, the naturally occurring condiment increases levels of “good” HDL cholesterol and decreases levels of “bad” LDL cholesterol.

Locust beans not only help avoid diabetic complications but also lower the risk of ischemic heart disease and help people lose weight

- Fantastic during the pregnancy

A baby’s proper growth and development depend on a variety of nutrients, which African locust beans provide in plenty. These minerals include calcium, iron, and protein. It may be possible to prevent anemia and gestational diabetes during pregnancy by ingesting dawadawa (iru). It is especially suggested for vegetarians and vegans as a high-protein and calcium source.

- Aids in the prevention of anemia

Fatigue and pallor are symptoms of anemia, a blood condition caused by a deficiency of red blood cells ness hemoglobin. Including fermented locust beans in your diet can help prevent anemia since they are rich in iron and other essential elements.

Additionally, a research discovered that feeding African locust beans to children together with other native foods helps prevent malnourishment and anemia.

- Possesses a high antioxidant content.

Antioxidants are substances that protect your body from free radicals, which are linked to a number of illnesses, including diabetes and heart disease. Antioxidants including flavonoids and phenols (catechins) abound in awadawa (Iru). These compounds' antioxidant properties aid in preventing metabolic illnesses.

- Fertility and locust beans

On the internet, a lot of people have questioned if locust beans and fertility are related. The idea that locust beans can improve fertility is not well supported by scientific research. Fertility can be increased by eating foods high in protein, particularly plant-based protein like locust beans.

Nwokolo, C., (2023). Major Health Benefits of Locust Beans (Iru). HealthGuide.ng.

DISCUSSION

In West Africa, traditional fermented condiments are frequently utilized to boost protein intake, improve nutritional value, and add taste characteristics to a range of cuisines. They constitute a significant portion of the people's daily protein requirements.

The bacterium's genesis in iru is partly attributable to the salt that is added as a preservative at the end of the fermentation process. Undoubtedly, the presence of *B. thuringiensis*, *B. cereus*, and *Staphylococcus species* raises questions regarding the microbiological safety of the fermented condiment. Data about the food-borne pathogen's potential for toxicity in African traditional fermented meals are available (Obafemi, *et al*., 2022).

Based on the results of 16S rRNA gene sequencing and culture-dependent analysis, Iru is composed of clonally related Bacillus species, which have been identified as *B. subtilis, B. amyloliquefaciens, B. cereus, B. licheniformis,* and *Brevibacillus formosus*, in order of frequency of occurrence. By combining ARDRA, ITS-PCR, ITS-PCR-RFLP, and RAPD-PCR, it was also possible to identify a significant degree of variation among the *Bacillus* isolates and confirm that *B. subtilis* and *B. amyloliquefaciens* were the predominant species. The results clearly showed that autochthonous clonally related bacilli may be subtyped, and that dominant groups are determined by genetics. This is in line with previous findings from fermentation studies of related vegetable proteins in West Africa and India (Olasupo, and Okorie, 2019; Kim, *et al*., 2010; Oguntoyinbo, *et al*., 2010).

The intricate microbiological process of fermentation dynamics in food or condiments like iru involves interactions between several bacteria. The role of the accompanying flora of the fermenting substrate is determined by the type of the substrate and its cleanliness during the manufacturing process. The *Bacillus, Micrococcus*, and *Staphylococcus species* that were isolated at the species level from the iru samples were numerous. Because of its capacity for proteolysis and oil breakdown, *Bacillus* is the most common kind. Iru fermentation has been linked to *B. subtilis* (Olanbiwoninu, *et. al.*, 2017).

There are species in the B. subtilis group that are generally regarded as safe (GRAS), according to the US Food and Drug Administration. Food fermentation techniques, according to Olanbiwoninu, *et al*. (2017), mainly depend on the coculturing of bacteria that cooperate to produce the necessary product attributes.

The 16S rRNA genes of the bacteria that produce “Iru” may be amplified and sequenced as an effective way to identify the microbial population associated with the fermentation of African locust beans. It is undeniable that the use of molecular methods in microbiology is improving our knowledge of the ecology of food fermentations (Adelekan, *et al*., 2017).

Micediets supplemented with fermented *P. biglobosa* seeds showed a discernible decrease in cholesterol levels. This decrease is far less than the decrease that the common medication, statins, recorded. This finding is consistent with that of Ayo-Lawal, *et al.* (2014), who found that rats given fermented *P. biglobosa* seed had lower cholesterol levels. Linoleic acid (C18:2) has been shown to be the most abundant fatty acid in fermented P. biglobosa seed. There may have been a drop in TC in the treated groups because this fatty acid is said to slightly lower blood lipid levels. (Aremu, *et al.*, 2015).

The groups fed fermented iru all had varying degrees of reductions in cholesterol levels as compared to the untreated group. The condiment may have contributed to the observed decline in cholesterol levels after the 28-day treatment period, as shown by the higher cholesterol levels in the untreated group (Atere, *et al.*, 2020).

LDL (low density lipoprotein) levels were lower in the treated group compared to the untreated group. The liver forms low-density lipoproteins (LDLs) by packaging triglycerides (TGs) and cholesterol (Atere, *et al.*, 2020).

The Iru fermented with *Lactobacillus plantarum* demonstrated the best hypocholesterolaemic benefit in this study. Ultimately, creating iru with this specific bacterial strain may provide a cutting-edge approach to treating hypercholesterolemia. The study’s findings also demonstrated that, while cholesterol reductions were seen in the group treated with iru fermented with different cultures, they were still significantly less than those seen with statins, the conventional anticholesterol medication. Regular ingestion of premium fermented *P. biglobosa* seeds may help protect the body from high levels of cholesterol, LDL, and TG. The benefits of this condiment include its affordability, naturalness, and easy accessibility. It could offer a cutting-edge therapeutic alternative for disorders of lipid metabolism, taking the place of pricy and sometimes troublesome synthetic drugs (Atere, *et al.,* 2020).

Legume seeds that are going to be used to make condiments need to be kept dry and pest-free. They have to be free of stones and not mushy. Without any color or scent, the water utilized for cooking, cleansing, and soaking ought to be sourced from neutral and secure outlets. Using an easy-to-use, inexpensive dry dehuller is recommended as it will limit contamination, cut down on water content, and save time. (Oguntoyinbo, 2012).

Rather than using calabash as the fermentation vessel, plastic bowls are a cost-effective alternative that can be completely cleaned with detergent after each fermentation procedure. To ensure the safety of the final product, post-fermentation contamination must be avoided throughout processing. Food handlers will need to follow proper hygiene procedures for this. Gloves and packaging are two ways to avoid post-fermentation contamination of condiments into bags made of polypropylene rather than leaves (Oguntoyinbo, 2012).

The use of high riboflavin producers as starters in the fermentation of locust beans would not only increase the nutritional content of the iru produced but also improve diets poor in riboflavin, hence lowering the incidence of riboflavin deficiencies among rural inhabitants. Iru fermentation is an exothermic solid substrate fermentation process, according to Nie, *et al*., (2023), in which the fermenting seed's temperature gradually rises from room temperature to between 30 and 45 degrees Celsius. The discovery that Bacillus and Staphylococcus species grew best at temperatures between 35 and 40°C indicates that the bacteria isolated from iru had the ability to ferment iru substrate (Olanbiwoninu, *et al.*, 2017).

CONCLUSION

The use of salt as a preservative affects the growth of isolates as well as the preservation of riboflavin levels in the final condiment, Iru.

The results show, in summary, that a combination of culture-dependent and culture-independent techniques may be used to efficiently define the bacterial microbiota and investigate the diversity and kinetics of naturally fermented foods. of summary, our study has revealed that the bacterial composition of Iru is far more varied than previously reported, highlighting the necessity of implementing uniform protocols throughout manufacturing facilities to improve safety, uniformity, and quality.

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