

Review Article

# Fermented African Locust Bean (Iru), a Potential Dietary Prebiotic and Probiotic

Paulina Oludoyin Adeniyi\*

Wageningen Center for Development Innovation, Wageningen University and Research, Wageningen, Netherlands

## Abstract

The fact that indigenous foods in Nigeria are always forgotten as possible dietary prebiotics and probiotics cannot be overemphasized, hence, there is need to bring to limelight the potentials of our indigenous foods that are potent prebiotics and probiotics. This is therefore a review of prebiotic and probiotic concept, food sources with emphasis on iru, fermented African locust bean. The search engines used for this study are; Google Scholar, AGORA and HINARI. The Galactose-oligosaccharide and arabinogalactan which are the prebiotics in African locust bean (*Parkia biglobosa*) can be partially hydrolyzed in the course of fermentation of the bean into iru while the unhydrolyzed part acts as a prebiotic when the condiment is consumed. This makes iru a possible source of prebiotic. In the same vein *Bacillus* spp and *Lactobacillus* spp which are probiotics dominate the fermentation procedure in the production of iru and they were affirmed to be acid tolerant, thermotolerant, bile salt tolerant with appreciable or notable antibacterial activity against gastrointestinal pathogens. Fermented African locust bean, Iru, indeed has potentials of dietary prebiotic and probiotic, hence, its consumption should be optimally and maximally encouraged, popularized and publicized in order to harness the nutritious, aromatic and health benefits of this indigenous culinary condiment.

## Keywords

African Locust Bean, Fermentation, Prebiotics, Probiotics

## 1. Introduction

Locust bean is native to West Africa where it grows as a forest tree. Botanically it is named *Parkia species* most especially *Parkia biglobosa*. It is a nutritious bean but the bioavailability of the nutrients in it is impaired by the presence of anti nutritional factors such as tannin, phytates, saponin, trypsin inhibitors, oxalates, etc [1]. These anti nutrients adversely affect different body functions such as digestion, absorption, nutrient bioavailability, toxicity effect to the body and affect body metabolic activities and rate [2], hence, fermentation is commonly employed on locust bean processing to reduce these anti nutritional factors and also improve its

nutritional constituents [2, 3]. Some of the benefits of fermentation of locust bean are that it effectively reduces anti nutritional factors, improves bioactive performance of the bean, exposes hydrophobic regions thus beneficially altering techno-functionality and microbial enzymes are produced to enhance digestion [4].

The culinary use of fermented locust bean is quite versatile in soups, stews, sauces and other dishes. It is commonly used as a condiment to enhance flavor and taste of meals and even as meat replacement in poor and low income households. It is called 'Iru' in Yoruba (Southwestern Nigeria). Dawadawa in

\*Corresponding author: [doyinadeniyi@yahoo.com](mailto:doyinadeniyi@yahoo.com) (Paulina Oludoyin Adeniyi)

**Received:** 1 May 2024; **Accepted:** 20 May 2024; **Published:** 13 June 2024



Copyright: © The Author(s), 2024. Published by Science Publishing Group. This is an **Open Access** article, distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Hausa (Northern Nigeria) and Ugba in Igbo (Southeastern Nigeria). It has specific name that it is being referred to in other West African countries where it is being consumed and used.

In recent times there is a new trend of attention being given to prebiotics and probiotics in public health. A prebiotic is a non-digestible food ingredient or constituents of foods that promotes the growth of beneficial microorganisms in the gastrointestinal tract, that is, the indigestible part of food that serves as food or feed for the normal flora in the intestine and as a result improves the health of the host. A probiotic is a live microorganism which exerts health benefits to the host when consumed or administered to the host. Examples of dietary prebiotics include; banana, whole grain cereals, almonds, cabbage, flax, soybeans, etc while examples of probiotics are; yoghurt, cheese, pickles, etc which are thriving sites or substrates for probiotic lactic acid bacteria (LAB), Gram positive microbes that have been in use for ages in food processing and production. Other examples of probiotic foods include; tempeh, kefir, miso, sauerkraut etc.

The prebiotic components in foods include: Fructo-oligosaccharides (FOS) in onion, chicory, garlic, asparagus, banana, artichoke; Galacto-oligosaccharides (GOS) present in lentils, chickpeas and beans [5-7]; and Trans-galacto-oligosaccharides (TOS) present in some mammals' milk such as the milk of marsupials but human milk contains negligible proportion [8, 9]. When these are fermented by gut microbiota or normal flora, short chain fatty acids (SCFAs) are produced such as lactic acid, butyric acid, propionic acid, etc. These SCFAs exert some health benefits to the host such as; anti-inflammatory, anti-obesity, anti cancer, antidiabetes, cardiovascular protective, immunoregulatory, hepatoprotective and neuroprotective activities [10]. The mechanisms of action of probiotics include; increased adhesion to intestinal mucosa, enhancement of epithelial barrier with resultant and accompanying effect of inhibition of pathogen adhesion, production of antimicrobial substances, competitive exclusion of pathogenic microorganisms and modulation of the immune system [11, 12].

In general, many legumes and legume-based beverages which were fermented with lactic acid bacteria (LAB) had been reported to be potential carriers of probiotics and prebiotics [13]. As a legume subjected to natural and wild fermentation it is most pertinent that fermented locust bean is rich in prebiotics as well as probiotics. As a bean it is expected to be rich in Galactose-oligosaccharides (GOS) and as a fermented food it is most probable that it is rich in gut loving beneficial microorganisms. Indigenous food ingredients are mostly forgotten when in the discourse of prebiotics and probiotics, hence, the need to bring these food ingredients to limelight in this discourse is an evergreen necessity. This study hereby reviews the potentials of fermented locust bean as a dietary prebiotic and probiotic for improved public health of Nigerians, both home and in diaspora as well as that of West Africans who consume the nutritious and aromatic food

condiment.

## 2. Fermented Locust Bean as a Nutritious Foodstuff

Locust bean or specifically African locust bean (*Parkia biglobosa*) is native to West African countries and it is commonly fermented before it is put to use in cuisines. This is because the bean is hard and cannot be softened by the conventional cooking processes. Also the flavor, taste and aroma develop during the fermentation process for it to be suitable as a condiment in West African cuisines. More still, the anti nutritional factors that the bean contains are drastically reduced in the process of fermentation and digestibility is also improved [2].

In Nigeria fermented locust bean is called Iru in Yoruba, Dawadawa in Hausa and Ugba in Igbo. However, it has unique and specific name it is called in other West African countries. In Benin Republic it is called sonru, afitin or iru; In Senegal it is called netetou or soumbala while in Guinea and Mali it is referred to as soumbala. It is called Kpalugu in Kusasi and Dagomba languages (Northern Ghana and Southern Burkinafaso), Kainda in Krio (Sierra Leone) and Soulou in Central Togo [14, 15].

African locust bean is a forest tree but has been domesticated in some African countries. The fruit is a long pod in which is dispersed seeds (beans) with yellow sweet tasting pulp that can be consumed raw as a snack or made into drinks [16]. The seeds are commonly referred to as African locust beans which are fermented into food condiment. Fermented locust bean is a highly nutritious food condiment. When dried at 50°C, the proximate composition was as follows: Moisture-12.76%; protein-32.51%; carbohydrate-32.42%; crude fat- 9.01%; fiber-8.65% and ash- 4.65% while in fresh (wet state), moisture, protein, carbohydrate, crude fat, fiber and ash contents were 38.76%, 31.88%, 7.47%, 8.98%, 7.88% and 5.03% respectively [1]. Calcium, magnesium, sodium, copper and zinc content of wet and dried (at 50°C) fermented locust bean were (in mg/100g): Calcium- 745.61 and 745.19; magnesium-85.43 and 83.13; sodium- 13.44 and 13.27; copper-21.40 and 21.09; zinc- 294.39 and 293.85 [1]. Titratable acidity in wet and dried iru samples were 0.042 and 0.042mg lactic acid/g and pH is 4.50 and 4.50 respectively [1]. The anti nutritional factors in mg/100g of wet /dried locust bean samples were found to be 0.36/0.35 (saponin), 0.74/0.71 (tannin) and 1.12/ 1.05 (oxalate) [1].

The pH, crude fat, crude fiber, crude protein, ash, moisture and carbohydrate in unfermented locust bean peeled using the traditional hand peeling method were: 6.00, 17.39%, 5.27%, 30.43%, 3.39%, 18.39% and 19.13% respectively. Here the locust bean was boiled for 12 hours before peeling using the traditional hand peeling method [17]. However, after 2 hours of boiling and peeling using mechanical dehuller, the pH and proximate composition of unfermented locust beans were as

follows: pH-5.77; crude fat- 20.87%; crude fiber- 4.47%; crude protein- 35.50%; ash- 5.13%, moisture- 8.05% and carbohydrate- 23.40% [17]. In the same vein the mineral composition of unfermented locust beans peeled using the traditional hand peeling method ( in mg/100g) is as follows: Phosphorous-40.03; sodium- 2.95; potassium- 0.20; calcium- 13.55 and magnesium- 36.50 while in the sample boiled for 2 hours and peeled using mechanical dehuller the mineral composition (in mg/100g) is as follows: phosphorous- 63.25; sodium- 4.17; potassium- 0.37; calcium- 17.60 and magnesium- 47.60 [17]. All these show that locust bean is a nutritious food stuff either in fermented or unfermented state, hence, its consumption should be encouraged especially in fermented form since the digestibility and bioavailability of the nutrients are enhanced in the fermented form of the bean [2]. Consumption of fermented locust bean could be a cost efficient and cheap source of protein and other vital nutrients to combat food insecurity and nutrient inadequacies most especially among the poor and low income group in Nigeria and other countries where fermented locust bean is being consumed.

#### *Production of Fermented Locust Bean*

Fermented locust bean, iru, is commonly prepared using the traditional method of preparation. Since it is a local, indigenous condiment it is usually processed in rural areas. African locust bean (*Parkia biglobosa*) is sorted and cleaned by hand picking to remove any extraneous matter. This is then boiled for 12 hours after which it is dehulled using hand or mortar and pestle. The seed coats are separated from the cotyledons by sieving with water. After this it is poured in calabash lined with banana leaves and later covered with banana leaves then kept in dark cupboard for 3 or more days. Sometimes it is brought to boil again after the cotyledons have been separated before pouring the cotyledons in the calabash. After 3 days it is ready for use and it is called 'iruworu'. If allowed to ferment for longer periods (7 days) the flavor and taste become stronger and it becomes more pungent, then it is called 'iru-pete'.

In the course of fermentation oligosaccharides which are most abundant in the unfermented locust bean (stachyose, raffinose and sucrose) decreased in the first 24 hours while the reducing sugars (galactose, glucose and fructose) in the unfermented and fermented samples were similar [18]. The activity of alpha-galactosidase was highest at 24 hours of fermentation while the peak for sucrose activity was 36 hours [18]. It is used as condiments to improve flavor, aroma and taste in mainly savory dishes such as soups, stews and sauces as well as in some traditional one pot dishes. It is also used as a meat replacer for health reasons (people on low animal protein diets) and among the poor and low income groups [19, 20].

### 3. Prebiotics

Prebiotics are non-digestible food components that serve as food substrates for intestinal microbiota and in the process

provide some health benefits for the host. They can not be digested in human gut but can be degraded by gut microbiota in the process of feeding on them thus fermenting them into short chain fatty acids which are beneficial not only for gut health but also distant organs in the host. A prebiotic should be resistant to the stomach low pH, cannot be digested or hydrolyzed by mammalian digestive enzymes, cannot be absorbed in the digestive system but can be degraded or fermented by the gastrointestinal microbiota or micro flora for growth, multiplication and activation of the microbiota to concomitantly improve the host's health [21]. Most prebiotics are non-digestible carbohydrate sources but there are few of these that are not carbohydrates. Types of prebiotic include:

- 1) Fructans such as inulin and fructo-oligosaccharides (FOS) while food sources are onion, chicory, garlic, asparagus, artichoke, banana etc. The degree of polymerization of inulin is up to 60 while that of fructo-oligosaccharide is less than 10, however, fiber are considered to be carbohydrates with the degree of polymerization of greater than or equal to 3 [22]. Lactic acid bacteria, butyrate-producing bacteria, bifidobacteria and other gut microbiota can degrade or ferment fructans to enhance their growth and activity while the host's health is also improved [23].
- 2) Galacto-oligosaccharides (GOS): these are products of lactose extension. Bifidobacteria and Lactobacilli as well as Enterobacteria, Bacteroidetes and Firmicutes are greatly stimulated by GOS [22] and food sources include dairy products, beans and certain root vegetables, legume seeds, lentils, peas, mustard, chickpea etc.
- 3) Trans-galacto-oligosaccharides (TOS): these are GOS with excess galactose at C<sub>3</sub>, C<sub>4</sub> or C<sub>6</sub> and are produced from the enzymatic trans glycosylation of lactose and lactulose [21].
- 4) Resistant starch: this type of starch is resistant to digestion in the small intestine and the activities of microbiota on it during its degradation or fermentation produces high level of butyrate through which it improves host's health [24]. In human colon, even though *Ruminococcus bromii* is the key determinant of the degradation of resistant starch, other microflora like *Bifidobacteri-aadolescentis* have been involved to an appreciable extent and to a lesser extent *Eubacterium rectal* and *Bacteroides thetaiotaomicro* [25]. Food sources include green banana, plantains, whole grains such as barley, oats, cooked rice or potatoes that have been cooled in the freezer or fridge etc.
- 5) Pectic oligosaccharides (POS): These are derived from pectin which is a polysaccharide. It is present in appreciable quantity in mango, sugar beet and citrus.
- 6) Xylo-oligosaccharides: these are non digestible oligosaccharides that are made up of mainly xylose units and they have high prebiotic potentials. Food sources include bamboo shoots, fruits, vegetables, milk and honey.
- 7) Non-carbohydrate oligosaccharides: these are not car-

bohydrates but are being classified as prebiotics such as flavanols derived from cocoa which stimulate lactic acid bacteria [26].

#### *Mechanisms of Action of Prebiotics*

Prebiotics serve as nutrient substrates for microflora or microbiota. These microbiota derive energy and other components they need for survival from the fermentation or degradation of prebiotics, hence, growth of gut microbiota is sustained [27, 28]. The byproducts of the fermentation of a complex prebiotic can serve as a substrate for another microorganism in a process called cross feeding, for instance, the byproducts of fermentation of resistant starch by *Ruminococcus bromii* serve as substrates for different species of microbiota [27, 28]. The activities of gut microbiota on prebiotics can also have butyrogenic effect on the gut by lowering the pH since most of the byproducts of the fermentation process are acids. This pH lowering effect may alter the population of the acid sensitive microbiota such as *Bacteroids* in a reducing trend but favor the multiplication of butyrate forming microbiota such as *Firmicutes* [29]. In these three mechanisms prebiotics are able to alter and maintain gut microbiota.

The maintenance of host health as well as prevention of disorders by prebiotics is mainly owing to the short chain fatty acids (SCFAs) that are produced as a result of their degradation or fermentation by gut microbiota [30]. These SCFAs are micromolecules and can easily diffuse from the gut into the blood circulation via the gut enterocytes thus exerting their health beneficial effects within the gastrointestinal tract as well as other distant organs and systems where they are needed [31]. Poor or low population of colonic microbiota such as *Bifidobacteria*, *Faecalibacterium*, *Bacteroides* and *Firmicutes* have been implicated in Irritable Bowel Syndrome and Crohn's Disease (any relapsing inflammatory bowel disease that involves any part of digestive tract from mouth to anus). This means that the effect of prebiotic on maintaining microbiota growth and multiplication goes a long way in preventing these disorders in human [32]. More still, prebiotic fermentation byproducts, such as butyrates, protect the host against the risk of colorectal cancer through apoptosis induction [33]. A symbiotic therapy of microbiota and prebiotic (such as *Lactobacillus rhamnosus* and *Bifidobacterium lactis* with inulin) can efficiently reduce the risk of colorectal cancer via inducing colonic cells necrosis and reducing cancer cell proliferation in colorectal (33-35).

Prebiotic is capable of enhancing the immune system and functions by increasing the population of protective microbiota and reducing the population of harmful or pathogenic microorganisms as can be seen in the destructive or inhibitory effect of mannose on *Salmonella* as well as its ability to induce expression of pro and anti inflammatory cytokines, hence, it is able to stimulate the immune system [36]. The byproducts of prebiotics such as propionate and butyrate (SCFAs) have been reported to improve lipid profile in human by lowering cholesterol and low density lipoprotein with a

beneficial effect in modulating dyslipidemia [37] while propionate inhibited production of lipid from acetate which is another byproduct of prebiotic degradation or fermentation. Hence, prebiotics are able to improve cardiovascular health [38].

Some prebiotics have also been reported to enhance calcium absorption in humans. These include lactulose, Trans-galacto-oligosaccharides (TOS), inulin + oligofructose [39]. Prebiotics can also modulate the nervous system since the gut-brain axis connects the gastrointestinal tract to the central nervous system [40]. The products of prebiotic fermentation by gut microbiota can influence the brain via the vagus nerve [41] and FOS and GOS have been reported to have regulatory effects on neurotransmitters, synaptic proteins as well as brain-derived neurotrophic factors in rats [42]. Prebiotics modulate the endocrine system since the growth of microbiota induced corticosterone and adrenocorticotrophic hormone in mice [43] and other hormones such as plasma peptide YY [44].

Simply put, a prebiotic is a substrate that is selectively utilized by host micro flora conferring health benefits [45]. Microbiota in human colon is dominated by bacteria of the genera *Bifidobacterium* and *Lactobacillus*, hence, the increase in the population of these bacteria could be used as a benchmark for a healthy gut microbiota [46]. There are other microflora within the colon that cross feed on the lactate and acetate produced by the activities of *Lactobacillus* and *Bifidobacterium* on prebiotics to exert their health promoting effect, hence, beneficial bacteria in the colon other than these two also benefit from the presence of a prebiotic [47].

## 4. Probiotics

Probiotics are live microorganisms that exert health benefits when consumed or applied to the body and are found in yoghurt, fermented foods, beauty products and dietary supplements [48]. They are mainly *Bifidobacterium species* and *Lactobacillus species* while some species of *Pediococcus*, *Streptococcus*, *Enterococcus*, *Bacillus* and yeasts have also been classified as probiotics. Available commercially are the following probiotic microorganisms: *Bifidobacterium adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, *B. essecis*, *B. infantis*, *B. lactis*, *B. longum*, *Bacillus lactis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. fermentum*, *L. helveticus*, *L. lactis*, *L. paracasei*, *L. rhamnosus*, *L. plantarum*, *L. reuteri*, *L. salivarius*, *Saccharomyces boulardii* [49-52]. Lactic acid bacteria are also commonly identified as probiotics [53]. *Bifidobacterium species* are rod-shaped, anaerobic, non-gas producing, non-spore forming gram positive, non-motile and catalase -ve microorganisms with morphology similar to that of bifidobacteria and were isolated from the feces of breast-fed infants [54]. However, *Bifidobacterium* were not found in isolation in the feces of breast-fed infants but also strains of *Lactobacilli* have been reported to be present there [55] and strains of strains of



*Lactobacillus* and *Enterococcus* were isolated from human saliva and feces [56] as well as human colostrums [57]. All these clearly evidenced the fact that human milk and gut are indeed thriving sites for microflora and probiotics are found existing in them. Lactic acid bacteria (LAB) are highly well pronounced as probiotics both in foods and their hosts because they improve food flavor and quality and prevent various inflammatory illnesses caused by oxidation in the host. The main focus of all LAB in this concept are the genera *Lactobacillus* and *Bifidobacterium*.

#### 4.1. Mechanisms of Action of Probiotics in the Body

One of the mechanisms by which probiotic LAB exerts their healthy and beneficial effect in human host is by exhibiting notable antioxidant activity. Reactive Oxygen Species (ROS) such as superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and highly reactive hydroxyl radical ( $HO^\cdot$ ) are commonly produced during body metabolism and stress. The accumulation of these ROS leads to oxidative stress causing damages to protein, lipids, DNA, cells and many lead to cell death causing aging and other forms of diseases [58, 59]. The possible mechanism through which probiotic LAB exert antioxidant activity is by promoting production of antioxidant enzymes which neutralizes and removes the ROS in the host intestine, activating antioxidant enzymes or modulating and reducing circulatory oxidative stress thus protect cells from oxidative stress-induced damage [60, 61]. Other possible mechanisms of action include; improved intestinal barrier function via enhanced intestinal integrity and permeability [62]; modulation of the mucosal immune system via direct stimulation of immune cells residing in the gastrointestinal tract (GIT) such as T-regulatory cells, effector lymphocytes, natural killer T cells, B cells, dendritic cells and epithelial cells; modulation of enzyme activity by changing the microbial metabolism in order to combat any strange elements or form of intrusion [63]. The immune cells are stimulated via regulation of gene expression and signaling pathways in the host cells which results in macrophage activation and enhanced phagocytosis [63]. Another mechanism by which probiotics exert their health benefits on the host is by the production of antimicrobial agents or compounds such as bacteriocins which exert competitive exclusion of pathogens [64]; improving the digestion and absorption of food [65, 66]; as well as alteration of the intestinal microflora [67, 68]. As earlier mentioned, probiotics are live microorganisms which when administered in adequate quantity exert or confer a health benefit on the host [69] and *Lactobacillus* and *Bifidobacterium* species are the most commonly used.

#### 4.2. Food as a Potent Carrier of Probiotics

Some foods which are commonly referred to as probiotics foods because they contain live microorganisms that are

beneficial to host health are; yoghurt, sauerkraut, miso, kefir, soft cheeses, acidophilus milk, naturally fermented sour pickles (without use of vinegar), tempeh, cultured cottage cheese, labneh, kimchi, kambucha, certain aged cheese, tepache, kvass, natto, namashoyu, gochujang, fermented olives, sour pickles, fermented seaweed, fermented hot sauce, umeboshi (pickled plum), etc,

**Yoghurt and yoghurt drinks:** This is made by inoculating and fermenting pasteurized and cooled milk with live cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and allowing it to ferment. Some yoghurt may contain other strains of probiotics and these are normally listed among the ingredients on the package.

**Kefir:** In the same vein kefir is a fermented milk drink made with kefir grains and milk. Bacteria and yeasts co exist in kefir grains and these act as natural starter culture in the fermentation process [70]. Kefir grains act as the source of the starter culture in which LAB, yeasts and acetic acid bacteria coexist in a symbiotic relationship [71]. Kefir is produced by inoculating pasteurized and cooled ruminant milk (mostly cow's) with kefir grains and allowed to ferment at room temperature for 24 to 48 hours after which the grains are drained off and the resultant beverage (kefir milk) can be chilled and consumed. The LAB and yeasts species (probiotics) predominating in kefir are *Lactobacillus kefirianofaciens*, *Lacticaeibacillusparacasei*, *Lactiplantibacillusplantarum*, *Lactobacillus acidophilus*, *Lactobacillusdelbrueckii* (bacteria) as well as *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Candida kefyr* and *Kluyveromycesmarxianus* (yeasts) [72].

**Sauerkraut:** This is the product of naturally fermented shredded salted cabbage. Lactic acid bacteria (LAB) predominate in the fermentation process. The production process involves cleaning of the cabbage followed by shredding, addition of salt at 3% w/w, kneading for a few minutes to allow the release of the juice and covering to ferment at ambient temperature for 2, 5 or 7 days. The length of days could be more [73]. The probiotics which predominate in sauerkraut are *Lactobacillus spp* and *Leuconostoc spp* which are LAB [73].

**Miso:** This is a common seasoning in Japanese cuisine. It is fermented soybean paste. Rice, barley, soybean or the mixture of two or more of these can be used. Soybean (or rice) koji which is soybean (or rice) fermented with a mold (*Aspergillusoryzae*) is used as the starter culture. This is then mixed with cooked and salted soybean paste and allowed to ferment and age (for 2 to 12 months at ambient temperature [74]. In the commercial production of miso the lactic acid bacterium-*Tetragenococcusshalophilus* and the yeast- *Zygosaccharomycesrouxii* are commonly introduced as pure starter cultures at the beginning of the fermentation process [75].

**Naturally fermented sour pickles:** When vegetables are allowed to ferment naturally to produce pickles without the addition of vinegar or acetic acid, probiotics are multiplied in the pickles. For instance, in the naturally fermented sour pickled cucumbers lactic acid bacteria, which predominate,

produce several bacteriocins as well as antimicrobial peptides which inhibit the invasion and thriving of spoilage bacteria to exert desired nutritional quality, flavor and appealing sensory properties [76].

All these dietary probiotics are not indigenous to Nigeria but they are always the ones mentioned when this concept is being discussed.

### 4.3. Probiotic Foods Indigenous to Nigeria

Fermented foods in Nigeria that have been identified as possible probiotic foods are; kunu-zaki, nunu, gari, fufu, lafun, ogi/akamu/koko, etc but fermented locust bean is not always mentioned as a possible probiotic food.

**Kunu-zaki:** This is a fermented, non-alcoholic cereal beverage which is popular in Northern Nigeria but is now consumed all over the country. It is commonly produced from locally available cereal grains such as millet, sorghum, rice, acha (hungry rice), maize etc, but mostly millet and sorghum are used. It is prepared by cleaning the cereals, washing it, soaking or steeping it for 1 to 2 days, washing, wet-milling (optionally with spices), pre gelling half of the paste with boiling water and leaving the 2 portions (cooked and uncooked) to ferment for 1 or 2 days after which the 2 portions are mixed together, diluted with water to desired consistency and sieved to produce the final product which is then sweetened with sugar or honey as desired. The fermentation process in kunu is achieved mainly by yeasts (*Saccharomyces cerevisiae*) and LAB in which *Lactobacillus spp* predominate [77, 78]. *Saccharomyces rouxii* and *Penicillium spp* may also be involved [79].

**Nunu:** This is a locally fermented milk beverage indigenous to Nigeria and a few other West African countries. It is yoghurt-like in taste. The processing method is usually by natural fermentation in which fresh cow milk is allowed to ferment for 1 or 2 days in calabash or rubber buckets. Starter culture which is a portion of previously prepared nunu may be introduced into the fresh cow milk. Nunnu may be consumed alone or with fura which is a cereal-based meal. The fermentation process is basically by lactic acid bacteria with *Lactobacillus spp* predominating among which are *Lactobacillus casei*, *L. bulgaricus*, *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *L. thermophilus*, *L. reuteri*, *L. helveticus*, *L. delbrueckii*, *L. brevis*, *L. cellobiosus*, *L. fermentum* with *L. acidophilus* occurring the most and *L. reuteri* the least [80]. However, nunu contains both LAB and yeasts. The LAB in Nunnu are identified as *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus* while the yeasts are *Saccharomyces pastorianus*, *Candida stellata*, *Kluyveromyces fragilis*, *Zygosaccaromyces bisporus*, *Zygosaccaromyces rouxii* [81]. Enterobacteriaceae were present at the early stage of fermentation but were eliminated as fermentation progressed. These were *Enterobacter*, *Klebsiella*, *Escherichia*, *Proteus vulgaris* and *Shigella*. This means that the pathogenic microorganisms were not able to withstand the fermentation

process conditions, hence, they were eliminated thus making nunu safe for consumption [81].

**Gari:** This is fermented cassava flake that is a common staple in Nigeria and other West African countries. In its processing, cassava is peeled, washed, grated, pressed and fermented (for 3 to 5 days or more), sieved and pan roasted to yield Gari. Only 4 strains of *Lactobacillus fermentum* out of the 47 bacteria strains isolated from Gari were resistant to acidic pH and bile salt medium and showed antibacterial activity towards microbial pathogens such as Methicillin-resistant *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella enteritidis*, *Escherichia coli*, *Escherichia coli* (0157) and *Yersinia enterocolitica* owing to the production of bacteriocin, thus presenting gari as a potent dietary probiotic [82]. The four isolates were different strains of the same species of *Lactobacillus fermentum*. However, in another study only strains of *Lactobacillus plantarum* and *Lactobacillus acidophilus* were able to withstand high temperature of 50 to 60°C for 1 hour after LAB isolated from gari were tested for their thermo tolerance, and these also exhibited inhibitory activity against food borne pathogens such as *Salmonella enterica*, serovar *Typhimurium*, *Escherichia coli*, *Staphylococcus aureus* as well as spoilage microorganisms *Listeria monocytogenes* [83]. This implies that even when gari is made into gelatinized meal (eba) with boiling water, it can retain its probiotic activity since some of the probiotics in it are thermo tolerant.

Fufu and lafun are also fermented cassava meals in which LAB predominate and are gelatinized by cooking fermented cassava paste (for fufu) and fermented cassava flour (for lafun) with constant stirring in the pot on the fire. It is most probable that what is obtainable in gari is the same in fufu and lafun since their fermentation is also by LAB.

**Ogi:** This is fermented cereal gruel and is a staple meal in northern and southwestern Nigeria. It is produced by cleaning and washing of cereal grains (maize, sorghum or millet) after which it is steeped or soaked for 2 to 3 days. This is then washed again with water and wet-milled. The paste is diluted with water and sieved followed by leaving the mixture to precipitate or allowed to settle. The supernatant (omidun) is then decanted. To cook it the ogi slurry is mixed with boiling water to gelatinize it to form the gruel. Spices like ginger and cloves may be added during wet milling to enhance the flavor. The gruel may be sweetened with sugar, honey or supplemented with milk. Accompaniments like akara (bean cake), moinmoin (steamed bean pudding), robo (melon cake) etc may be consumed with it. Traditionally, raw ogi slurry is commonly administered or given to diarrhea patients to lower frequency of stooling. The fermentation process in the production of ogi is a lactic acid fermentation involving LAB *Weissella paramesenteroides*, *Lactobacillus brevis*, *L. rossiae*, *L. fermentum*, *L. plantarum*, *Acetobacter pasteurianus*, *Paeinibacillus spp* and *Bacillus spp* with ogi prepared from yellow maize having the highest LAB count ( $4.8 \times 10^{11}$ ), that from red sorghum was  $3.8 \times 10^{11}$  while that from white maize was least ( $20 \times 10^{10}$ ) [84]. The uncooked ogi maintained a steady count

of  $10^{10}$  cfu/ml for 5 days of fermentation while the cooked ogi (the gruel) was  $10^9$  cfu/ml meaning that some of the LAB thermo tolerant being able to withstand and resist the high cooking temperature [84]. *Lactobacillus* spp dominated the 27 LAB strains that were isolated and identified since *L. plantarum* was 36%, *L. fermentum* was 24%, *L. brevis* 20%, *Acetobacterpasteurianus* 8%, *Weisellaparamesenteroides* 5% and *L. rossiae* 4% [84]. A total of 21 LAB out of the 27 isolated exhibited antibacterial activity against *Shigella* spp, *Salmonella* spp and *Escherichia coli* [84]. Also the antibacterial activity of LAB in ogi has been ascertained and reported by other scientific reports [85-87]. It is clearly evident that ogi, in cooked or raw form is a potent probiotic food.

These are some of the indigenous fermented foods in Nigeria that are possible dietary probiotics. It is of interest to note here that fermented locust bean, iru, is not always mentioned in the discourse of fermented foods as possible dietary probiotics. However, iru is not only a possible probiotic food but also has potentials to be a prebiotic. It is therefore pertinent and important that this concept be well understood and iru be brought to limelight as a possible prebiotic and probiotic food.

## 5. Iru as a Dietary Probiotic

As earlier mentioned iru is a product of fermentation of African locust bean. It is mostly consumed as a condiment to enhance the flavor and aroma of soups, stews, sauces and other indigenous dishes. It can also be used as a meat replacer in meals most especially among the poor and low income groups. It is indigenous to West African countries including Nigeria.

*Bacillus* spp have been identified as the microorganisms that dominate the fermentation process in iru and the strains that have been isolated are; *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, *B. polymyxa*, *B. alvei* and *B. badius* [88]. About 90% of these exhibited antibacterial activity, 55% inhibited the growth of the pathogen- *Listeria monocytogenes* while *B. subtilis* and *B. licheniformis* survived high acid and bile environments [88]. The alkaline pH medium established by the *Bacillus* spp which predominate in the fermentation of African locust bean to produce iru is well favorable for the growth and multiplication of the *Bacillus* spp. The pH of iruworo (with lesser period of fermentation) was 8.4 while that of irupete (with longer period of fermentation) was 8.1 [88]. The proportion of the *Bacillus* spp isolated from iru were; *Bacillus subtilis*- 44%, *B. licheniformis*- 28%, *B. megaterium*- 24% and *B. coagulans*- 4% [89]. However, *Bacillus* spp, *Pediococcus* spp, *Streptococcus* spp and *Lactobacillus* spp were isolated and identified from iru samples collected from different regional markets in Nigeria [90]. *Bacillus cereus* strains isolated from fermented locust bean, iru, exerted inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*, were acid tolerant, more tolerant to bile salt, hence, can serve as a potent probiotic and iru may be a possible probiotic food [91].

It is of interest to note that even LAB are involved in the fermentation process during the production of iru. *Lactobacillus fermentum* and *L. plantarum* isolated from fermented locust bean samples were observed to tolerate low pH and bile salt concentration while also exerted antimicrobial activity on pathogens with *L. plantarum* showing the highest potency [92]. It has also been affirmed that the fermentation process in iru production is dominated by salt tolerant *Bacillus subtilis* with mild presence of *Micrococcus* spp and *Pseudomonas* spp [93]. The health benefits derivable from the consumption of iru was also ascertained by its ability to lower blood cholesterol in hypercholesterolemic rats, hence, it may be useful in the prevention and management of coronary heart diseases in human beings [94]. Fermented locust bean may also exert a protective effect against breast cancer in humans [95].

Having established the fact that iru has a high potential as a dietary probiotic, it is imperative that the foodstuff or condiment be popularized and publicized as a probiotic food. It should not be forgotten when probiotic foods are being mentioned and there is need for public awareness to emphasize this because people mostly think far away when we are talking about food sources of probiotics. It is high time we valued our indigenous foods with great potentials which can be harnessed in this discourse; hence the benefits derivable will be adaptable and sustainable.

### 5.1. Iru as a Prebiotic Food

The Galacto-oligosaccharides (GOS) and arabinogalactan are the prebiotics in African locust bean. During fermentation raffinose and sucrose in African locust bean are totally hydrolyzed while arabinogalactan, Galacto-oligosaccharides and stachyose are partially degraded or hydrolyzed thus making them available in iru as prebiotics [96]. Even though amylase activity was not detected during the fermentation of African locust bean into iru the reducing sugar level increased from 63mg/g to 134mg/g during the first 24 hours of fermentation but later decreased [97]. This shows that the fermentation medium was not favorable for carbohydrate-hydrolyzing microorganisms after 24 hours, hence, it is most probable that non-digestible oligosaccharides (which are suitable prebiotics) in the bean would escape the hydrolysis process. They would therefore be present in the final fermented product, iru. However the environment favoured protein hydrolyzing fermentative microorganisms since there was active proteinase activity which resulted in increased number and quantity of amino acids since African locust bean is a protein-rich seed [97].

There is an absolute paucity or dearth of available scientific reports on the non-digestible oligosaccharide constituents of fermented African locust bean, but, as a bean it contains galactose-oligosaccharide specifically arabinogalactan which is non-digestible and a prebiotic. The process of fermentation only partially hydrolyzed this leaving some in the fermented product as a prebiotic [96]. It is also possible that some of



these could be present in the fermented product, iru, since carbohydrate hydrolyzing microbes are hindered or inhibited after 24 hours of fermentation. Hence, some of the arabinogalactan and other Galactose-oligosaccharides escaped being hydrolyzed in the course of fermentation which makes them available in iru as a prebiotic [96]. However, the paucity of available scientific reports on this demands that more scientific investigation be carried out in this area of discourse.

## 6. Conclusion and Recommendation

It is clearly evident that some of our indigenous foods and foodstuffs in Nigeria are potent prebiotics and probiotics among which is iru. Hence it is imperative that Nigerians, both home and abroad, should consume iru to derive maximum or optimal health benefits derivable from prebiotics and probiotics, since this is not far from us but available with us.

## Abbreviation

DNA	Deoxyribonucleic Acid
FOS	Fructo-Oligosaccharide
GIT	Gastrointestinal Tract
GOS	Galacto-Oligosaccharide
LAB	Lactic Acid Bacteria
POS	Pectic-Oligosaccharide
ROS	Reactive Oxygen Species
SCFAs	Short Chain Fatty Acids
TOS	Trans-Galacto-Oligosaccharide

## Author Contributions

Adeniyi Paulina Oludoyin is the sole author. The author read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

## References

- [1] Famuwagun A. A. and Taiwo K. A. (2023). Drying characteristics, nutritional and anti nutritional properties of locust bean seed. *Cogent Food and Agriculture*; 9(1): 2202276 <https://doi.org/10.1080/23311932.2023.2202276>
- [2] Yakubu C. M., Sharma R. and Sharma S. (2022a). Fermentation of locust bean (*Parkia biglobosa*): modulation in the anti nutrient composition, bioactive profile, in vitro nutrient digestibility, functional and morphological characteristics. *International Journal of Food Science + Technology*; 57(2): 753-762.
- [3] Oluwaniyi O. O. and Bazambo I. O. (2016). Nutritional and amino acid analysis of raw, partially fermented and completely fermented locust bean (*Parkia biglobosa*) seeds. *African Journal of Food, Agriculture, Nutrition and Development*; 16(2): 2016 <https://10.18697/ajfand.74.15025>
- [4] Yakubu C. M., Sharma R., Sharma S. and Singh B. (2022b). Influence of alkaline fermentation time on in vitro nutrient digestibility, bio- and techno-functionality, secondary protein structure and macromolecular morphology of locust bean (*Parkia biglobosa*) flour. *LWT-Food Science and Technology*; 161: 113295 <https://10.1016/j.lwt.2022.113295>
- [5] Rastall R. A. (2006). Galacto-oligosaccharides are prebiotic food ingredients. In: *Prebiotics: Development and Application*. Gibson G. R. and Rastall R. A (Eds). John Wiley & Sons Ltd, USA.
- [6] Sims I. M. and Tannock G. W. (2020). Galacto-and Fructo-oligosaccharides utilized for growth by co-cultures of Bifidobacterial species characteristic of the infant gut. *Applied and Environmental Microbiology*; 86(11): <https://doi.org/10.1128/AEM.00214-20>
- [7] Ambrogi V., Bottacini F., Cao L., Kuiper S. B., Schoterman M. and Sinderen D. (2021). Galacto-oligosaccharides as infant prebiotics: production, application, bioactive activities and future perspectives. *Critical Reviews in Food Science and Nutrition*; 63(6): 753-766.
- [8] Vijayasathya M., Prabhu Y. A., Pavithra S., Prabha S. J. and Rao T. J. M. (2022). Immune response of Fructo and Galacto-oligosaccharides. In: *Prebiotics and Probiotics in Disease Regulation and Management- Kesharwani, Rao T. J. M. and Keservani R. K. (Eds). WILEY online library* <https://doi.org/10.1002/9781394167227.ch2>
- [9] Mei Z., Yuan J. and Li Dandan (2022). Biological activity of galacto-oligosaccharides: A review. *Frontiers in Microbiology*; 13 <https://doi.org/10.3389/fmicb.2022.993052>
- [10] Gowrishankar S., Kamaladevi A. and Pandian S. K. (2021). Prebiotics mechanisms of action: An overview. In: *Advances in Probiotics Microorganisms in Food and Health*. Chapter 9 pgs 137-148. Academic Press, USA.
- [11] Zyl W. F., Deane S. M. and Dicks L. M. T. (2020). Molecular insights into probiotic mechanisms of action employed against intestinal pathogenic bacteria. *Gut Microbes*; 12(1): <https://doi.org/10.1080/19490976.2020.1831339>
- [12] Mazziota C., Tognon M., Martini F., Torreggiani E. and Rondono J. C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*; 12(1): 184 <https://doi.org/10.3390/cells.12010184>
- [13] Cichouska P. and Ziarno M. (2022). Legumes and legume-based beverages fermented with lactic acid bacteria as a potential carrier of probiotics and prebiotics. *Microorganisms*; 10(1): <https://doi.org/10.3390/microorganisms10010091>
- [14] Campbell-Pratt G. (1980). African locust bean (*Parkia* species) and its West African fermented food product, dawadawa. *Ecology, Food and Nutrition*; 9: 123-132.
- [15] Ariyo O. C. (2023). Economic analyses of traditional processing of African locust beans (*Parkia biglobosa*, Jacq. Benth) seeds in Kaduna metropolis, Kaduna state, Nigeria. *Ethiopian Journal of Environmental Studies and Management*; 16(4): 415-426.



- [16] Olatoye K. K., Ironde E. A., Awoyale W. and Adeyemo O. I. (2023). Nutrient composition, antioxidant properties and sensory characteristics of instant kunu from pearl millet supplemented with African locust bean pulp. *Journal of Ethnic Foods*; 10(21): <https://doi.org/10.1186/s42779-023-00188-1>
- [17] Olaniran A. F., Okonkwo C. E., Erinle O. C., Owolabi O. A., Ojediran J. O. and Olayanju T. A. (2020). Optimum boil duration and its effect on nutritional quality and acceptability of mechanically dehulled unfermented locust bean seeds. *Preventive Nutrition and Food Science*; 25(2): 219-224.
- [18] Odunfa S. A. (1983). Carbohydrate changes in fermenting locust bean (*Parkia filicoidea*) during iru preparation. *Plant Foods for Human Nutrition*; 32(3-10); <https://doi.org/10.1007/BF01093924>
- [19] Ikhimalo O. P. (2019). African locust beans: more than just a condiment. *Journal of Underutilized Legumes*; 1(1): 99-111.
- [20] Chinma C. E., Ezeocha V. C., Adedeji O. E., Inyang C. U., Enijiugba V. N. and Adebo O. A. (2023). African legume, pulse and oil seed-based fermented products. In: *Indigenous fermented foods for the tropics*. Academic Press, USA. Chapter 5 pp 73-84.
- [21] Gibson G. R., Scott K. P., Rastall R. A., Tuohy K. M., Hotchkiss A., Dubert-Ferranden A., Gareau M., Murphy E. F., Saulnieriloh G. et al., (2010). Dietary prebiotics: Current status and new definition. *Food Science and Technology Bulletin*; 7: 1-19.
- [22] Louis P., Flint H. J. and Michel C. (2016). How to manipulate the microbiota: Prebiotics. In: *Microbiota of the human body*. Springer, Switzerland, pp 119-142.
- [23] Scott K. P., Martin J. C., Duncan S. H. and Flint H. J. (2014). Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. *FEMS Microbiology Ecology*; 87: 30-40.
- [24] Fuentes-Zaragoza E., Sanchez-Zapata E., Sendra E., Sayas E., Navarro C., Fernandez-Lopez J. and Perez-Alvarez J. A. (2011). Resistant starch as prebiotic: A review. *Starch-Starke*; 63: 406-415.
- [25] Ze X., Duncan S. H., Louis P. and Flint H. J. (2012). *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *International Society for Microbial Ecology Journal*; 6: 1535-1543.
- [26] Tzounis X., Rodrigue-Mateos A., Vulevic J., Gibson G. R., Kwik-Urbe C. and Spencer J. P. (2011). Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *American Journal of Clinical Nutrition*; 93: 62-72.
- [27] Lordan C., Thapa D., Ross R. P. and Cotter P. D. (2020). Potential for enriching next generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes*; 11(1): 1-20.
- [28] Fei Y., Chen Z., Han S., Zhang S., Zhang T., Lu Y., Berglund B., Xiao H. Li L. and Yao M. (2023). Role of prebiotics in enhancing the function of next-generation probiotics in gut microbiota. *Critical Reviews in Food Science and Nutrition*; 63(8): 1037-1054.
- [29] Wang S., Xiao Y., Tian F., Zhao J., Zhang H., Zhai Q. and Chen W. (2020). Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. *Journal of Fermented Foods*; 66: 103838 <https://doi.org/10.1016/j.jff.2020.103838>
- [30] Ashaolu T. J., Ashaolu J. O. and Adeyeye S. A. O. (2021). Fermentation of prebiotics by human colonic microbiota in vitro and short-chain fatty acids production: a critical review. *Journal of Applied Microbiology*; 130(3): 677-687.
- [31] Bedu-Ferrari C., Biscarrat P., Langella P. and Cherbuy C. (2022). Prebiotics and the human gut microbiota: from breakdown mechanism to the impact on metabolic health. *Nutrients*; 14(10): 2096 <https://doi.org/10.3390/nu14102096>
- [32] Wilson B. and Whelan K. (2017). Prebiotic inulin-type fructans and galacto-oligosaccharides: Definition, specificity, function and application in gastrointestinal disorders. *Journal of Gastroenterology and Hepatology*; 32: 64-68.
- [33] Mahdavi M., Laforest-Lapointe I and Masse E. (2021). Preventing colorectal cancer through prebiotics. *Microorganisms* 9(6): 1325 <https://doi.org/10.3390/microorganisms9061325>
- [34] Candela M., Guidotti M., Fabbri A., Brigidi P., Franceschi C. and Florentini C. (2011). Human intestinal microbiota: Cross-talk with the host and its potential role in colorectal cancer. *Critical Reviews in Microbiology*; 37: 1-14.
- [35] Kim S. H. and Lim Y. J. (2022). The role of microbiome in colorectal carcinogenesis and its clinical potential as a target for cancer treatment. *Intestinal Research*; 20(1): 31-42.
- [36] Li C., Niu Z., Zou M., Liu S., Wang M., Gu X., Lu H., Tian H. and Jha R. (2020). Probiotics, prebiotics and synbiotics regulate the intestinal microbiota differentially and restore the relative abundance of specific gut microorganisms. *Journal of Dairy Science*; 103(7): 5816-5829.
- [37] Xu D., Feng M., Chu Y., Wang S., Shete V., Tuohy K. M., Liu F., Zhou X., Kamil A., Pan D., Liu H., Yang X., Yang C., Zhu B., Lv N., Xiong Q., Wang X., Sun J., Sun G. and Yang Y. (2021). The prebiotic effects of oats on blood lipids, gut microbiota and short-chain fatty acids in mildly hypercholesterolemic subjects compared with rice: A randomized controlled trial. *Frontiers in Immunology*; 12: <https://doi.org/10.3389/fimmu.2021.787797>
- [38] Zhang D., Jian Y. P., Zhang Y. N., Li Y., Gu L. T., Sun H. H., Liu M. D., Zhou H. L., Wang Y. S. and Xu Z. X. (2023). Short-chain fatty acids in diseases. *Cell Communication and Signaling*; 21(212): <https://doi.org/10.1186/s/2964-023-01219-9>
- [39] Carlson J. L., Erickson J. M., Lloyd B. B. and Slavin J. L. (2018). Health effect and sources of prebiotic dietary fiber. *Current Developments in Nutrition*; 2(3): nzy005 <https://doi.org/10.1093/cdn/nzy005>
- [40] Gaman A. and Kuo B. (2008). Neuromodulatory processes of the brain-gut axis. *Neuromodulation*; 11: 249-259.

- [41] Forsythe P., Bienenstock J. and Kunze W. A. (2014). Vagal pathways for microbiome-brain gut axis communication. In: *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*. Springer, New York, USA. pp115-133.
- [42] Williams S., Chen L., Savignac H. M., Tzortzis G., Anthony D. C. and Burnet P. W. (2016). Neonatal prebiotic (bgos) supplementation increases the levels of synaptophysin, glun2a-subunits and bdnf proteins in the adult rat hippocampus. *Synapse*; 70: 121-124.
- [43] Sudo N., Chida Y., Aiba Y., Sonoda J., Oyama N., Yu X. N., Kubo C. and Koga Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology*; 558: 263-275.
- [44] Savignac H. M., Corona G. and Burnet P. W. (2013). Prebiotic feeding elevates central brain derived neurotrophic factor, n-methyl-d-aspartate receptor subunits and d-serine. *Neurochemistry International*; 63: 756-764.
- [45] Gibson G. R., Hutkins R., Sanders M. E., Prescott S. L., Reimer R. A., Salminen S. J., Scott K., Swanson K. S., Cani P. D., et al., (2017). Expert Consensus Document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) Consensus statement on the Definition and scope of Prebiotics. *Nature Review on Gastroenterology and Hepatology*; 14: 491-502.
- [46] Roberfroid M., Gibson G. R., Hoyle L., McCartney A. L., Rastall R., Rowland I., Wolvers D., Watzi B., Szajewska H., Stahl B., et al., (2010). Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition*; 104: S1-S63.
- [47] Louis P. and Flint H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*; 19: 29-41.
- [48] Reid G. (2016). Probiotics: definition, scope and mechanisms of action. *Best Practice and Research Clinical Gastroenterology*; 30(1): 17-25.
- [49] Crismondo M. R., Drago L. and Lombardi A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agents*; 12: 287-292.
- [50] Chow J. (2002). Probiotics and prebiotics: A brief overview. *Journal of Renal Nutrition*; 12: 76-86.
- [51] Shah N. P. (2007). Functional cultures and health benefits. *International Dairy Journal*; 17: 1262-1277.
- [52] da Silva M. N., Tagiliapietra B. L., Flores V. A. and Richards N. S. P. S. (2021). In vitro test to evaluate survival in the gastrointestinal tract of commercial probiotics. *Current Research in Food Science*; 4: 320-325.
- [53] Feng T. and Wang J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*; 12(1): <https://doi.org/10.1080/19490976.2020.1801944>
- [54] Desan R., Mudana S. O., Julyanto C. M. P., Purnana E. T., Sugata M., Jo J. and Tan T. J. (2024). Isolation and identification of *Bifidobacterium* species from human breast milk and infant feces in Indonesia. *Biodiversitas Journal of Biological Diversity*; 25(1): <https://doi.org/10.13057/biodiv/d250139>
- [55] Demirok N. T., Durak M. Z. and Arici M. (2022). Probiotic lactobacilli in faeces of breastfed babies. *Food Science and Technology*; 42: <https://doi.org/10.1590/fst.24821>
- [56] Bazirch H., Shariati P., Jamlkandi S. A., Ahmadi A. and Boroumand M. A. (2020). Isolation of novel probiotic *Lactobacillus* and *Enterococcus* strains from human salivary and fecal sources. *Frontiers in Microbiology*; 11-2020 <https://doi.org/10.3389/fmicb.2020.597946>
- [57] Liu w., Chen M., Duo L., Wang J., Guo S., Sun H., Menghe B. and Zhang H. (2020). Characterization of potentially probiotic lactic acid bacteria and bifidobacteria isolated from human colostrums. *Journal of Dairy Science*; 103(5): 4013-4025.
- [58] Lin H. W., Lee H. L., Shen T. J., Ho M. T., Lee Y. J., Wang I., Lin C. P. and Chang Y. Y. (2024).  $Pb(NO_3)_2$  induces cell apoptosis through triggering of reactive oxygen species accumulation and disruption of mitochondrial function via SIRT3/SOD2 pathways. *Environmental Toxicology*; 39(3): 1294-1302.
- [59] Chen P. H., Lu H. K., Renn T. Y., Chang T. M., Lee C. J., Tsao Y. T., Chuang P. K. and Liu J. F. (2024). Plumbagin induces reactive oxygen species and endoplasmic reticulum stress-related cell apoptosis in human oral squamous cell carcinoma. *Anticancer Research*; 44(3): 1173-1182.
- [60] Li B., Pan L. L. and Sun J. (2022). Novel probiotic lactic acid bacteria were identified from healthy infant feces and exhibited anti-inflammatory capacities. *Antioxidants*; 11(7): 1246 <https://doi.org/10.3390/antiox11071246>
- [61] Li W., Gao L., Huang W., Ma Y., Muhammed I., Hanif A., Ding Z. and Guo X. (2022). Antioxidant properties of lactic acid bacteria isolated from traditional fermented yak milk and their probiotic effects on the oxidative senescence of *Caenorhabditis elegans*. *Food and Function*; 13(6): 3690-3703.
- [62] Zheng Y., Zhang Z., Tang P., Wu Y., Zhang A., Li D., Wang C. Z., Wan J. Y., Yao H. and Yuan C. S. (2023). Probiotics fortify intestinal barrier function: a systematic review and meta-analysis of randomized trials. *Frontiers in Immunology*; 14-2023 <https://doi.org/10.3389/fimmu.2023.1143548>
- [63] Begum J., Buyamayum B., Lingaraju M. C., Dev K. and Biswas A. (2021). Probiotics: Role in immunomodulation and consequent effects. *Letters in Animal Biology*; 1(1): 53 <https://doi.org/10.62310/liab.v1i1.53>
- [64] Fijan S. (2023). Probiotics and their antimicrobial effect. *Microorganisms*; 11(2): 528 <https://doi.org/10.3390/microorganisms11020528>
- [65] Cappello C., Tlais A. Z. A., Acin-Albiac M., Junior W. J. F. L., Pinto D., Filannino P., Rinaldi F., Gobetti M. and Cagno R. D. (2023). Identification and selection of prospective probiotics for enhancing gastrointestinal digestion: application in pharmaceutical preparations and dietary supplements. *Nutrients*; 15(6): 1306 <https://doi.org/10.3390/nu15061306>

- [66] Varvara R. A. and Vodnar D. C. (2024). Probiotic-driven advancement: exploring the intricacies of mineral absorption in the human body. *Food Chemistry*; X; 21: 101067 <https://doi.org/10.1016/j.fochx.2023.101067>
- [67] Zhang Y., Zheng T., Ma D., Shi P., Zhang H., Li J. and Sun Z. (2023). Probiotics Bifidobacteriumlactis M8 and Lactobacillus rhamnosus M9 prevent high blood pressure via modulating the gut microbiota composition and host metabolic products. *Msystems*; 8(6): e00331-23.
- [68] Dasriya V. L., Samtiya M., Ranver S., Dhillon H. S., Devi N., Sharma V., Nikam P., Puniya M., Chaudhary P., Chaudhary V., et al., (2024). Modulation of gut microbiota through probiotics and dietary interventions to improve host health. *Journal of the Science of Food and Agriculture*: <https://doi.org/10.1002/jsfa.13370>
- [69] FAO/WHO (2006). Probiotics in food- Health, nutritional properties and guidelines for evaluation. FAO Food and Nutrition paper 85 [www.fao.org/3/a0512e/a0512e.pdf](http://www.fao.org/3/a0512e/a0512e.pdf)
- [70] Caballero V., Maughan L., Bolton D. and Celayeta J. M. F. (2024). Modelling and dynamics of microbial populations and Salmonella spp. in milk kefir. *Food and Bioproducts Processing*; 145: 217-225.
- [71] Garofalo C., Ferrocino I., Reale A., Sabbatini R., Milanovic V., Alkic-Subasic M., Boscaino F., Aquilanti L., Pasquini M., Trombetta M. F., Tavoletti S., Coppola R., Coccolin L., Blesic M., Saric Z., Clementi F. and Osimani A. (2020). Study of kefir drinks produced by backslipping method using kefir grains from Bosnia and Herzegovina: microbial dynamics and volatile profile. *Food Research International*; 137 <https://doi.org/10.1016/j.foodres.2020.109369>
- [72] Prado M. R., Blandon L. M., Vandenberghe L. P. S., Rodrigues C., Castro G. R., Thomaz-Soccol V. and Soccol C. R. (2015). Milk kefir: composition, microbial cultures, biological activities and related products. *Frontiers in Microbiology*; 6 <https://doi.org/10.3389/fmicb.2015.01177>
- [73] Touret T., Oliveira M. and Semedo-Lemsaddek T. (2018). Putative probiotic lactic acid bacteria isolated from sauerkraut fermentations. *PloS ONE* <https://doi.org/10.1371/journal.pone.0203501>
- [74] Saeed F., Afzaal M., Shah Y. A., Khan M. H., Hussain M., Ikram A., Ateeq H., Noman M., Saewan S. A. and Khashroum A. O. (2022). Miso: a traditional nutritious and health-endorsing fermented product. *Food Science and Nutrition*; 10(12): 4103-4111.
- [75] Allwood J. G., Wakeling L. T. and Bean D. C. (2021). Fermentation and the microbial community of Japanese koji and miso; 86(6): 2194-2207.
- [76] Zielinski H., Surma M. and Zielinska D. (2017). Frias J., Martinez-Villaluenga C. and Penas E. (Eds). The naturally fermented sour pickled cucumbers. In: *Fermented foods in Health and Disease Prevention*. Academic Press. Chapter 21, pp 503-516. USA.
- [77] Aboh M. I. and Oladosu P. (2014). Microbiological assessment of kunu-zaki marketed in Abuja Municipal Area Council (AMAC) in the Federal Capital Territory (FCT), Nigeria. *African Journal of Microbiological Research*; 8: 1633-1637.
- [78] Ndukwe J. K., Aduba C. C., Ughamba K. T., Chukwu K. O., Eze C. N., Nwaiwu O. and Onyeaka H. (2023). Diet diversification and priming with kunu: an indigenous probiotic cereal-based non alcoholic beverage in Nigeria. *Beverages*; 9(1): 14 <https://doi.org/10.3390/beverages9010014>
- [79] Ekwem O. H. and Okolo B. N. (2017). Microorganisms isolated during fermentation of sorghum for production of Akam (a Nigerian fermented gruel). *Microbiological Research Journal International*; 21: 1-5.
- [80] Chukwuemeka A. E., Igwillio U. C. and Aigboje O. (2019). Identification and characterization of Lactobacillus isolates recovered from locally fermented milk (nunu) consumed within Lagos Metropolis. *International Journal of Recent Research in Life Sciences*; 6(1): 38-45.
- [81] Akabanda F., Owusu-Kwarteng J., Glover R. L. K. and Tano-Debrah K. (2010). Microbiological characteristics of Ghanaian traditional fermented milk product, Nunu. *Nature and Science*; 8(9): 178-187.
- [82] Ayodeji B. D., Piccirillo C., Ferraro V., Moreira P. R., Obadina A. O., Sanni L. O. and Pintado M. M. E. (2017). Screening and molecular identification of lactic acid bacteria from gari and fufu and gari effluents. *Annals of Microbiology*; 67: 123-133.
- [83] Fossi B. T. and Ndjouenkeu R. (2017). Probiotic potential of thermotolerant lactic acid bacteria isolated from 'Gari', a cassava-based African fermented food. *Journal of Applied Biology and Biotechnology*; 5(4): <https://doi.org/10.7324/JABB.2017.50401>
- [84] Afolayan A. O., Ayeni F. A. and Ruppitsch W. (2017). Antagonistic and quantitative assessment of indigenous lactic acid bacteria in different varieties of ogi against gastrointestinal pathogens. *The Pan African Medical Journal*; 27: 22 <https://doi.org/10.11604/pamj.2017.27.22.9707>
- [85] Oyetayo V. O. and Osho B. (2004). Assessment of probiotic properties of a strain of Lactobacillus plantarum isolated from fermenting corn (ogi). *Food, Agriculture and Environment*; 2(1): 132-134.
- [86] Afolayan O. A. and Ayeni F. A. (2017). Antagonistic effects of three lactic acid bacterial strains isolated from Nigerian indigenous fermented Ogi on E.coli EKT004 in co-culture AKJournals. <https://doi.org/10.1556/066.2017.46.1.1>
- [87] Kwasi R. E., Aremu I. G., Dosunmu Q. A. and Ayeni F. A. (2019). Viability of lactic acid bacteria in different components of ogi with anti diarrhoeagenic E.coli activities. *The North African Journal of Food and Nutrition Research*; 3(6): <https://doi.org/10.51745/najfnr.3.6.206-213>
- [88] Olanbiwoninu A., Deborah E., Ayooluwa O., Awotundun T. and Fasiku S. (2022). Probiotic capability of Bacillus spp. isolated from Iru- fermented African locust bean (Parkia biglobosa). *EC Microbiology*; 18(6): 18-30.
- [89] David O. M., Olagunju J. I., Adebayo A. A., Oluwaniyi T. T. and Olajide M. O. (2016). Probiotic properties and antibiotic resistance pattern of Bacillus spp. isolated from two types of fermented locust bean (iru). *British Biotechnology Journal*; 10(4): 1-12 <https://doi.org/10.9734/BBJ/2016117698>

- [90] Osho A., Mabekoje O. O. and Bello O. O. (2010). Comparative study on the microbial load of Gari, Elubo-isu and Iru in Nigeria. *African Journal of Food Science*; 4(10): 646-649.
- [91] Nwagu T. N., Ugwuodo C. J., Onwosi C. O., Inyima O., Uchendu O. C. and Akpuru C. (2020). Evaluation of the probiotic attributes of *Bacillus* strains isolated from traditional fermented African locust bean seed (*Parkia biglobosa*), “dad-dawa”. *Annals of Microbiology*; 70(20): <https://doi.org/10.1186/s13213-020-01564-x>
- [92] Kuti K., Hussaini A., Usman A. and Isa A. (2021). Isolation of *Lactobacillus* species from fermented *Parkia biglobosa* seed and screening for their probiotic activity. *Agricultural Science and Technology*; 13(2): 212-216.
- [93] Oguntimehin M. O., Fagbemi S. A., Ojo O. D., Adisa A. M., Rotifa O. J. and Enujiugha V. N. (2023). Response of *Bacillus subtilis* to salt and acid stresses and the optimal effect on fermentation of locust bean (*Parkia biglobosa*) seeds into iru (a soup condiment). *IPS Journal of Applied Microbiology and Biotechnology*; 2(1): 20-28.
- [94] Atere A. V., Adedeji A., Akinmoladun A. C., Oyetayo Y. O. and Akinyosoye F. A. (2020). Local condiment, iru, obtained from the fermentation of *Parkia biglobosa* seed substantially reduced the serum cholesterol level of Wistar rats. *Preventive Nutrition and Food Science*; 25(2): 153-157.
- [95] Ayo-Lawal R. A., Osoniyi O., Ilevbare O. E. and Ekpo O. (2020). Cytotoxic effects of fermented African locust bean seeds on a breast cancer cell. *Innovare Journal of Health Sciences*; 8(3): 1-4.
- [96] Ouoba L. I. I., Diawara B., Christensen T., Mikkelsen J. D. and Jakobsen M. (2007). Degradation of polysaccharides and non-digestible oligosaccharides by *Bacillus subtilis* and *Bacillus pumilus* isolated from Soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. *European Food Research and Technology*; 224: 689-694.
- [97] Odunfa S. A. (1985). Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during iru fermentation. *International Journal of Food Science + Technology*; 20(3): 295-303.