



# Traditional and Artisanal Beverages in Nigeria: Microbial Diversity and Safety Issues

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**Abstract:** A review of up to 90 articles on the microorganisms associated with important artisanal or traditional beverages in Nigeria was carried out. This resulted in an overview of the prevalent microorganisms associated with soymilk, nono (fermented cow milk), tiger nut milk, yoghurt, kunu, zobo, palm wine and the local beers pito and brukutu. The bacteria genera, namely Bacillus, Escherichia, Lactobacillus, Staphylococcus, and Streptococcus, were detected in all nine beverages. On the contrary, this survey resulted in finding that the genera Saccharomyces, Aspergillus, Candida, and Penicillium were the eukaryotic microorganisms isolated in all beverages. The occurrence of fungal isolates, which can be responsible for producing mycotoxins, is a concern and shows the need for post-production tests. Overall, there is a low prevalence of bacteria associated with hygiene, especially the *Escherichia* genus in alcoholic beverages such as palm wine, pito and burukutu, which may be due both to a low acidity and high ethanol content. However, the prevalence of hygiene indicator genera was higher in nonalcoholic drinks, probably because of incorrect practices during processing. The magnitude of the production and sales of unregulated local beverages in Nigeria has reached the stage where significant regulation and food safety standards are required to safeguard public health. An opportunity exists to monitor and characterize the microbial flora of the artisanal beverages using molecular methods at all stages of production and storage.

**Keywords:** food safety; artisanal; bacteria; yeasts; fungi; traditional beverages; hygiene indicator organisms

# 1. Introduction

Unregulated beverages are commonly referred to as artisanal, unrecorded, illicit, or illegal drinks in a community. A common attribute of these kinds of products is that they are produced outside government regulation without any rules or regard to standard food safety guidelines. They could be home-made, surrogate, and counterfeit alcoholic beverages that predispose people to hazardous substances [1]. An unrecorded beverage can also be nonalcoholic and a country such as Nigeria has its fair share among other products found in different parts of the world. These kinds of products are generally untaxed by the government and anybody can become a manufacturer. A feature of unregulated beverages is that they are prone to food fraud and sometimes producers often claim that



their products with substituted components give more nutrients than regulated beverages. It has been pointed out that beverages such as fruit juices, coffee, tea and alcoholic beverages are likely to be targets of food fraud by adulteration through practices, which may involve mixing or substituting the original components [2]. The packaging could also be compromised because, in Nigeria, many producers sell their products in used plastics that may not be sanitary.

Some illegal local energy drinks are high in caffeine and caffeine intoxication may result in tachycardia, vomiting, cardiac arrhythmias, seizures, or death [3]. The abuse of alcohol using local drinks may lead to unfavourable health conditions because a high consumption is linked with stroke, coronary heart disease and risk of death from aortic aneurysm [4]. Apart from obesity and diabetes, sugar-sweetened beverages are associated with hyperuricaemia and gout [5] and an increased mortality [6]. The lack of extensive regulations for most local products in developing countries such as Nigeria can affect public health because these products often contain compounds that are fermented by microorganisms, which may lead to food infection, intoxication, renal failure and other chronic illnesses. Sometimes a product may be regulated at the production stage but not during sales where the products can be displayed and sold under direct sunlight or inappropriate temperatures.

Tamang et al. [7] have emphasised that culturable and nonculturable microorganisms naturally ferment the majority of global fermented food and beverages and that traditional food fermentation represents an extremely valuable cultural heritage in most regions, and harbours a huge genetic potential of hitherto undiscovered strains. They recommended that holistic approaches for the identification and complete profiling of microorganisms in global fermented foods are beneficial. In other reports [8] it was pointed out that spontaneous food fermentations pose potential risks to human health and that the spontaneous fermentation by autochthonous microorganisms in artisanal beverages requires a careful understanding and controlled processes to avoid food poisoning [9,10]. Hence, the need for a transdisciplinary assessment to determine the actual trends and risk avoidance has been suggested [11].

In Nigeria, there are several local unregulated drinks produced by microbial fermentation that are important and tied to the socio-economic lives of many people. A good number of families rely on home-made beverages and sell them in the streets to provide an additional source of income. Some drinks, such as fermented palm wine [12], pito [13] and burukutu [14], are alcoholic whereas others, such as nono [15], are milk-based. Local yoghurts may be from animal or plant-based milk [16]. Other plant-based milk products include tiger nut milk [17] and soymilk [18]. The beverages kunu [19] and zobo [20] maybe sugar-sweetened during production with table sugar and there are many variants of these drinks. Epidemic disease outbreaks from the consumption of contaminated products in Nigeria are not well monitored due in part to the reluctance by public health authorities to rapidly confirm and respond to outbreaks [21].

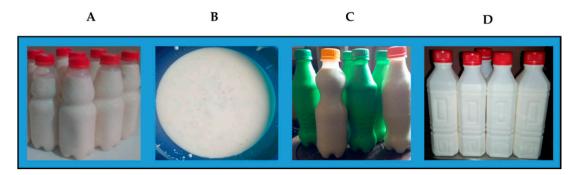
The World Health Organization has reported that the prevalence of heavy episodic drinking (HED), which is defined as consumption of 60 or more grams of pure alcohol, remains as high as 60% in some sub-Saharan African countries [22]. The HED prevalence could be much higher if all unregulated spirits, which sometimes lead to fatalities, are taken into consideration. Fatalities from the consumption of unregulated beverages, especially distilled gin from palm wine in Nigeria, have been reported. The case of methanol poisoning [23], which killed 89 persons and caused a ban on the local distillation of gin from palm wine, was highlighted.

A concern which may lead to the microbial contamination of unregulated artisanal local drinks in Nigeria is poor hygiene. Poor hygiene organisms can occur at any point during the production or storage of finished products and public sales. Around the world, it is known that poor hygiene in developing countries contributes to the proliferation of food pathogens in several local beverages. The origin of bacterial or fungal contaminations during the processing of many local food and beverages in Nigeria is unclear and the prevalence of microorganisms needs further characterization. Besides, microbial fermentation products of local drinks in Nigeria are poorly studied while it has been shown that the microorganisms responsible for the fermentation of traditional drinks may produce other compounds or derivatives, which are harmful to health [24]. Therefore, this work aims to review different microbial genera already associated with some unregulated beverages in Nigeria and the impact it may have on public health. Future microbiological needs and regulatory requirements are highlighted.

To generate queries for the search activities, the name of the local drink and Nigeria were used for a search on the databases Web of Science, Pubmed, Google Scholar and Scopus such as so, "Pito and Nigeria" and so on. The drinks searched were yoghurt, nono, soymilk, tiger nut milk, zobo, kunu, pito, burukutu, and palm wine due to their popularity. Duplicate reports were removed and no less than 10 studies carried out in Nigeria for each drink that detected organisms were selected and analyzed to get an overview of the microorganisms present. The microbes were recorded to the genus level and the most prevalent genera that appeared in the studies were highlighted.

# 2. Milk and Milk-Like Products

The milk beverages (cow and plant-based) that could be sold unregulated are shown in Figure 1. Apart from nono (Figure 1B) which is mainly sold in bowls, the other milk products—soymilk (Figure 1A), tiger nut milk (Figure 1C), and yoghurt (Figure 1D)—are usually marketed with plastic bottles in cities. Sometimes the yoghurts marketed may just be fermented nono that are packaged and sold [25] without regulation.



**Figure 1.** Traditional milk products found in Nigeria. Soymilk (**A**)—plant-based, nono (**B**)—animal-based, and tiger nut milk (**C**)—plant-based. The unregulated yoghurt (**D**) could be any type of milk.

#### 2.1. Plant-Based Milk Products

## 2.1.1. Soymilk

In Nigeria, the consumption of soybeans (*Glycine max*) and their products, such as soymilk, has increased in the last two decades because of their high protein content (39%) and the affordability compared to animal protein [26]. Unregulated soymilk sold in the streets of Nigeria is promoted for this reason and it currently supplements the food of many households that are not able to source enough protein from meat and poultry. Traditional home-made soymilk is normally prepared from soaked soybeans by grinding, heating, and filtering with a mesh screen to get soymilk [27]. The soymilk is then packed in sachets or plastic bottles (Figure 1A) and sold in the streets with or without regulation. Sometimes the milk is dried and sold as powders for patrons to reconstitute.

Several microbial studies [28–37] have been carried out on soymilk in Nigeria and up to 22 genera of bacteria and fungi have been identified (Table 1). For bacteria, the most prevalent genus among the studies examined was *Staphylococcus* whereas *Aspergillus* was the most prevalent fungi. Other genera found in three or more studies include *Bacillus*, *Escherichia*, *Pseudomonas*, *Saccharomyces*, *Penicillin*, and *Rhizopus*. The prevalence of *Staphylococcus* indicates hygiene problems whereas the presence of *Aspergillus* raises the issue of possible contamination with aflatoxins. Aflatoxins have been found in many species of *Aspergillus* [38,39] and the occurrence in both branded and unbranded soymilk

powder [28] is a big concern. It has been reported that the soybean used for making the soymilk is likely to be the main source of *Aspergillus*-harbouring aflatoxins [40] and its occurrence is influenced by the method of growing, harvesting, storage and processing of crops [41].

#### 2.1.2. Tiger Nut Milk

The interest in tiger nut milk (TNM) also known as *Kunun-Aya*, has increased over the years possibly because of its sensory, nutritional and probiotic prospects [42]. TNM (Figure 1C) beverages are made from the extract of tiger nuts. According to Sanchez-Zapata et al. [43], the tiger nut (*Cyperus esculentus* L.) is an edible perennial grass-like plant of tropical and Mediterranean regions with sweet almond-like tubers that are highly appreciated for their health benefits and nutritive value. Its attributes include a high content of fibre, proteins, sugars, oleic acid, glucose, phosphorus, potassium, and vitamins. The preparation of TNM has been previously described [44]. It involves removing nut nodules after which a cleaning, washing, and soaking in water for 5 h is then conducted. The water is drained off, followed by homogenization with a blender and then filtered with a cheesecloth to get the milk-like supernatant, which is the TNM.

Studies looking at the microorganisms in TNM have been carried out elsewhere, and Enterobacteriaceae, yeasts and moulds were detected [45]. It was concluded that there exists a rather high contamination level in home-made tiger nut beverages indicating the need to apply correct and strict hazard analysis and critical control point (HACCP) systems during manufacturing and storage. In Nigeria, many workers [46–55] have carried out a microbiological analysis on TNM and *Aspergillus, Candida*, and *Saccharomyces* were found to be the main fungal isolates, whereas *Bacillus, Escherichia* and *Salmonella* were the main bacterial isolates (Table 1). *Salmonella* can transmit typhoid fever and could occur from the water used for processing. The predominant fungi *Aspergillus* has been linked with aflatoxins in tiger nuts from Kaduna city [56] but the concentration was still within the safe limits prescribed by the national regulatory body.

#### 2.2. Animal-Based Milk Products

## 2.2.1. Nono

The food drink nono in the Hausa language in northern Nigeria is the name for fermented cow milk. It is made by fermenting fresh cow milk for up to 48 h and then sieving to get the final product [57]. It is common to see the product hawked in the street by the so-called milkmaids. Sometimes the quantity required is measured out in bowls (Figure 1B) and then consumed straight away by the patron for instant refreshment. The proximate values of the product from different locations can vary greatly in their physicochemical and microbiological quality. The microorganisms isolated from nono, investigated in different cities [58–67], are summarised in Table 1. Overall, the bacteria genus *Escherichia* had the highest prevalence, followed by *Staphylococcus* and *Klebsiella*. Among the other organisms reported, the genera *Enterobacter* and *Salmonella* were detected in up to three studies. More bacteria than fungi species were reported and the most prevalent fungal genus was *Candida* spp.

The predominance of *Escherichia* spp. over the natural *Lactobacillus* milk organism suggests serious hygiene issues. It has been emphasised [64] that the microbiological safety of nono is not guaranteed due to unhygienic practices during production and product contamination from the vendors. The isolation rate of *Escherichia* varied among locations. The examination of 420 samples [62] found that 4.5% of the samples were contaminated, whereas it was 40% of 209 samples in another study [61]. Another investigation in Kaduna city [65] had 27% of 100 samples contaminated with *Escherichia*. The regular occurrence of *Escherichia* may encourage the spread of antimicrobial resistance because some strains from nono have shown resistance to ampicillin [59].

#### 2.2.2. Yoghurt

Yoghurt is obtained from cow milk after fermentation and may be produced at various levels of viscosity. Popular brands sold in Nigeria are sometimes watery or milk-like and can be distinguished from milk by the sour taste. *Lactobacillus* plays a big role in yoghurt fermentation [68] and other predominant organisms involved include *Lactococcus lactis, Streptococcus thermophilus, Bifidobacterium,* and *Leuconostoc* species [69]. Registered commercial yoghurts sold in Nigeria have a variety of physicochemical attributes and, amid the popular brands, there exists imitations or unregulated brands sold in sachets or plastic bottles (Figure 1D). These products fall short of labelling laws and the ingredients used are not known. Unregulated sales in Nigeria are rampant and these products are often sold under uncontrolled temperatures in the street.

Several investigators in the Nigerian cities of Onitsha, Kano, Keffi, Enugu, Awka, Kaduna, Abuja, and Port Harcourt have reported the microorganisms found in yoghurts sold in Nigeria [70–79]. The summary in Table 1 shows that *Staphylococcus* spp. is the most prevalent among the studies carried in the aforementioned cities but the organism was not isolated from a sample in the study of Kano city [70]. This emphasizes that the species is from post-process contaminations, as is generally believed, rather than a starter during yoghurt production. Other species reported in three or more studies include *Escherichia, Bacillus, Streptococcus*, and in particular, *Lactobacillus*, which is known for its role in milk fermentation for yoghurt production. The microbiological quality of the product may differ based on the good manufacturing practices of the manufacturer during and after production. To ascertain the cleanliness of a production process, a test for *Escherichia* is sometimes carried out. Some investigators have detected the organism in local beverage products after production [79], whereas other workers did not isolate any [71] in the samples analysed.

Up to 11 fungi genera were seen in the studies reviewed. The most prevalent was *Aspergillus* spp. *Rhizopus, Mucor,* and *Saccharomyces* species (Table 1). The pH modulation by fungi which enables them to secrete alkalis and acids [80] can enable them to survive during the storage of yoghurts and may have enabled several fungal species to grow because the conditions or stress environment became adaptable.

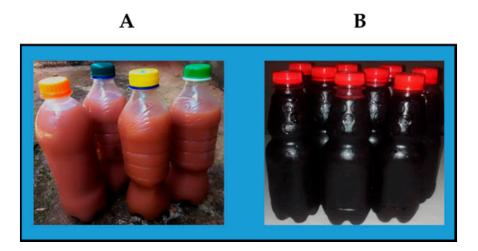
# 3. Soft Drinks

Two home-made soft drinks that are very popular in all regions and across all social strata in Nigeria are kunu and zobo. Despite its consumption by millions of people, it has yet to be properly regulated and no high volume industrial-scale production of the products has been carried out.

## 3.1. Kunu

The beverage kunu or kunu-zaki is a cheap traditional nonalcoholic fermented beverage, with a sweet–sour taste that originated from Northern Nigeria and is presently consumed across the country regardless of age [81]. The production steps for kunu have been outlined in a survey report [82]. It involves the use of millet (*Pennisetum typhoideum*), and most of the time sorghum (*Sorghum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*) and *ocha acha* (*Digitalis exilis*) can also be used. In the report, it was explained that the grains can be used singly or combined. First, the grain is steeped in water for 12–72 h, and then dry- or wet-milled with or without spices before cooking it into a thin free-flowing gruel. Further dilutions may be carried out before packing into plastic bottles (Figure 2A) for private consumption or unregulated sales to members of the public.

Some studies carried out on the product in Nigeria [83–92] show that the beverage has serious hygiene concerns because the prevalence of *Escherichia* species was highest in this product when compared to others reviewed (Table 1). A comparison between freshly processed and street-hawked kunu [86] showed that the fresh samples were free of coliforms whereas products found on the street were not. This shows that kunu is very susceptible to post-process contamination. The predominance of *Saccharomyces* (Table 1) indicates the sugar availability for fermentation and the main fungal isolates *Aspergillus, Penicillium*, and *Fusarium* suggest the possibility of mycotoxin presence.



**Figure 2.** Popular unregulated soft drinks in Nigeria. Production of kunu (**A**) and zobo (**B**) is usually carried out from the home or other unregistered facilities.

# 3.2. Zobo

The drink zobo is one of the locally made beverages in Nigeria that is prepared from the plant *Hibiscus sabdariffa* [93]. The beverage is an aqueous extract with a characteristic intense red colouration [94] that is loved by many. To make the drink, calyces are washed, soaked overnight, and then boiled for 15 min before filtering [95]. It may be sweetened with sugar or fruit extracts for a healthier drink after which its filling into plastic bottles is carried out (Figure 2B) for private consumption or unrecorded sales.

Microbial studies [96–105] have been carried out on the product and the bacterial and fungal species prevalent are *Bacillus* and *Penicillium* among other genera. A high prevalence of *Escherichia* and *Staphylococcus* (Table 1) suggest the hygiene issues present in other beverages.

#### 4. Alcoholic Beverages

# 4.1. Palm Wine

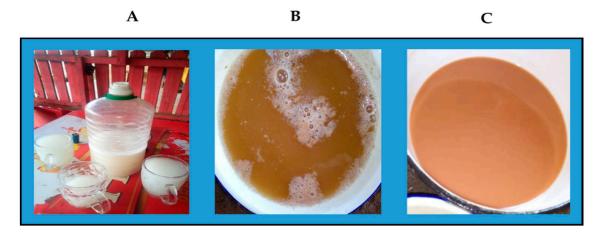
Palm wine (Figure 3A) is a milky white alcoholic drink obtained from different species of palm trees around the world. Unregulated sales are rampant in Nigeria and despite some food safety hazards associated with the drink [24], it is believed to have oenological potentials [106] and maybe be suitable as a probiotic [107] or functional beverage [108]. The drink is obtained by making incisions on the tree to collect the sap, which predisposes the drink to environmental contamination. Palm wine has a short shelf life because, after its collection from the field, the beverage undergoes a rapid microbial fermentation in a few days which yields several secondary metabolites due to microbial activity. The sale of this drink in most regions of Nigeria is vastly unregulated except for a few commercial producers that bottle and sell the product locally or internationally.

Yeasts, especially *Saccharomyces*, dominate palm wine among other non-*Saccharomyces* yeasts, bacteria and fungi and it is possibly the most studied local or traditional beverage in Nigeria for its microbial content. After the pioneering microbial work of Okafor [109], Faparusi and Bassir [110], several investigators [111–120] have reported different microbial genera from the drink in the last 10 years. There is a lower prevalence of bacteria associated with hygiene (Table 1) possibly because of the presence of ethanol, a low pH, and the acidic conditions of the drink.

## 4.2. Pito

Pito (Figure 3B) is an artisanal alcoholic beer widely consumed in West Africa, especially in Nigeria, and it provides a source of income for many in rural areas. The beverage is made from malted sorghum or millet grains or a combination of both [121] and the production process involves malting,

fermentation and then maturation. The beer has a very short shelf life and is normally consumed within a day after production. The bacteria and fungi associated with the drink in different Nigerian cities have been reported by several investigators [122–131]. Similar to palm wine, there was a low prevalence of hygiene organisms, which may be due to the inhibitory constituents, and the yeast *Saccharomyces* was the dominant genera among the fungi species in the studies reviewed (Table 1).



**Figure 3.** Three main artisanal alcoholic beverages sold in Nigeria. Palm wine (**A**), pito (**B**) and burukutu (**C**) are sometimes sold in makeshift open bars.

## 4.3. Burukutu

Another local beer consumed mainly in the northern and middle belt region of Nigeria is fermented burukutu. The drink is made similarly to pito, with the same materials but with slight variations in the production process [123]. Contrary to some of the aforementioned beverages, which have acceptance among all social classes, burukutu consumption is much higher among low-income earners in Nigeria. The microorganisms harboured by the drink have been characterised [132–141]. Unlike the alcoholic beverages palm wine and pito, which have a low prevalence of hygiene indicator organisms, burukutu showed a high prevalence of *Escherichia* and *Staphylococcus* among the studies reviewed (Table 1) possibly because of its physicochemical properties that allow bacteria to thrive. The prevalence of *Saccharomyces* is expected because of the sugar from the raw material available for fermentation. Again, *Aspergillus* prevalence may be a source of aflatoxins and it has been demonstrated to be present in the drink [142].

S/n

1

2

3

4

5

6

7

8

Bacteria

Bacillus

Enterobacter

Escherichia

Klebsiella

Lactobacillus

Lactococus

Micrococus

Enterococcus

Nono [58–67]	Soymilk [28–37]	Tiger Nut Milk [46–55]	Zobo [96–105]	Kunu [83–92]	<i>Pito</i> [123–132]	Burukutu [132–143]	Palm Wine [123–132]
2	4	6	8	7	2	5	4
3	2	1	3	1		1	
7	5	4	7	9	2	5	1
4	2	2	2	1	2		
2	1	3	3	5	3	5	3
		1		1		1	
	1	2	5	2	1	1	1
		1		1			
2	1	2	3	1	1		
3	2	4	3	2	1	1	
2		2	1	2		1	
4	6	4	7	6	2	6	3
1	3	2	3	5	3	4	3
1						1	
2	3	2	5	3			1
							1
					1	1	

**Table 1.** Summary of the prevalence of bacteria and fungi in Nigerian beverages after an analysis of up to 90 studies. The prevalence was calculated by recording the number of times a particular genus was reported in a product.

Yoghurt

[70-79]

5

3

2

4

1

9	Proteus		2	1	2	3	1	1		
10	Salmonella		3	2	4	3	2	1	1	
11	Shigella		2		2	1	2		1	
12	Staphylococcus	6	4	6	4	7	6	2	6	3
13	Streptococcus	4	1	3	2	3	5	3	4	3
14	Pediococcus		1						1	
15	Pseudomonas	2	2	3	2	5	3			1
16	Zygomonas									1
17	Acetobacter	1						1	1	
18	Acinetobacter				1					
19	Citrobacter		1		1		1			
20	Yersinia		1							
21	Serratia				1	1				1
22	Leuconostoc						1			
23	Corynebacterium					1	1		1	
24	Pediococcus						1		1	
25	Flavobacterium							1		
26	Vibrio					1				
27	Veillonella					1				
28	Aeromonas					1				
29	Alcaligenes							1		
30	Listeria								1	

S/n	Bacteria	Yoghurt [70–79]	Nono [58–67]	Soymilk [28–37]	Tiger Nut Milk [46–55]	Zobo [96–105]	<i>Kunu</i> [83–92]	<i>Pito</i> [123–132]	Burukutu [132–143]	Palm Win [123–132]
	Fungi									
1	Saccharomyces	2	1	3	3	1	5	4	7	5
2	* Torula yeast						1			
3	Candida	1	2	1	3	2	2	2	1	3
4	Aspergillus	3	1	7	4	3	4	2	5	1
5	Penicillium	1	1	3	2	4	4	1	1	1
6	Rhizopus	2		4	1	2	2	2	2	
7	Mucor	2	1	1		1	1	2	1	
8	Acremonium	1								
9	Alternaria	1		1						
10	Fusarium	1		1	1		3	1	1	
11	Debaryomyces	1								
12	Rhodotorula	1								
13	Cladosporium			1		1				
14	Neosartorya			1						
15	Neurospora			1						1
16	Pichia				1			1		2
17	Monilia						1			
18	Torulopsis					1				
19	Trichosporon									1
20	Rhodotorula							1		1
21	Schizo-saccharomy	ces								1
22	Blastomyces							1		
23	Sporotrichum							1		
24	Kluyveromyces							1		
25	Geotrichum		1			2	1	1		

S/n = serial number; \* Torula yeast, genus name not reported in ref [89]. Could be Candida.

## 5. Significance of Organisms Found

# 5.1. Hygiene Indicators, Pathogens and Toxic Compound Producers

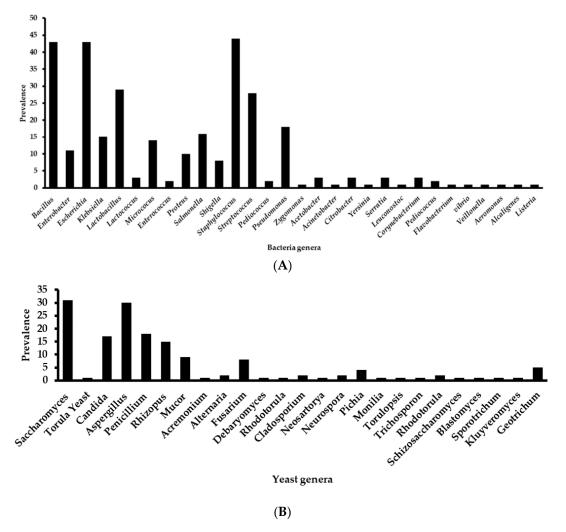
The hygiene indicators and pathogens in this section are mainly with reference to the microbial criteria outlined in the work of other investigators [143,144]. In total, 30 bacteria and 25 fungi genera (Table 1) were found in the 90 studies reviewed. The bacteria genera namely *Bacillus, Escherichia, Lactobacillus, Staphylococcus,* and *Streptococcus* were detected in all nine beverages. Additionally, only the fungi genera *Saccharomyces, Aspergillus, Candida,* and *Penicillium* were found in all the beverages. Overall, these bacterial and fungal species were the most prevalent (Figure 4).

It is worrying that the three most prevalent bacteria (Figure 4A) are Bacillus, Escherichia, and *Staphylococcus*. These species are known hygiene indicators and suggest very poor hygienic conditions during the preparation and storage of beverages. In particular, the roles of Escherichia and other *Enterobacteriaceae* as hygiene markers are well-reported [143] and high counts of these species mean that the food or drink did not follow normal food safety procedures. The concern is that the hygiene indicator organisms could also be pathogenic. Most of the studies reviewed declared the beverage unsafe once hygiene indicator organisms were detected without any further tests for pathogenicity. There were no tests to confirm whether the *Bacillus* species observed were pathogenic *B. cereus* or B. subtilis, which could have probiotic potential. Additionally, a confirmation assay to determine if the E. coli strain found was the shiga toxin-producing E. coli O157:H7 or other E. coli serotypes with pathogenic potential was not carried out. The yoghurt and nono Staphylococcus strains have been found to harbour the mecA gene [145] that encodes methicillin resistance but, in most cases, the isolates were not probed to determine if they are methicillin-resistant strains. It has been reported that the microorganisms associated with unhygienic processing conditions are mainly environmental contaminants with a few of faecal origin [146]. Hence, it can be concluded that most of the indicator organisms found in the studies reviewed were due to process contamination and they may not be pathogenic strains.

The most prevalent yeasts are the genus *Saccharomyces* and *Candida* (Figure 4A). The dominance of *Saccharomyces* is expected because it is known as a dominant organism in many beverage fermentations. There are no immediate concerns for adverse toxic metabolites from *Saccharomyces* fermentation but the risk could exist due to methanol production when the product is distilled into gin [23]. Some clinical strains of *Candida* have been implicated in many infections even though many nonpathogenic strains are part of the environmental flora. The occurrence of three fungal genera, namely *Aspergillus, Penicillium* and *Fusarium*, which are responsible for producing the majority of the mycotoxins that are toxic to humans, animals and plants [147] may mean that post-production tests of the beverages are required to assure food safety.

#### 5.2. Molecular Characterization

Since 99% of species level identifications in the papers reviewed were based on classical biochemical methods only, it may be erroneous to report to the species level. In line with current taxonomy methods, it is important that reclassification based on molecular methods is carried out in order to obtain the actual species associated with the beverages reported. The problem regarding the resources required to perform molecular characterizations contributed to the minimal investigation of the pathogenicity of microorganisms isolated in the beverages reviewed. For some of the beverages, such as pito, studies on the molecular characterisation of organisms in the product are rare in the literature. In most studies where a molecular identification was carried out, it was facilitated by the collaboration with other laboratories outside of the country. These collaborations are desirable because they have led to the sequencing and discovery of novel new species or actual strains involved in the fermentation of some beverages. Different yeasts [148,149] and bacteria [150] species have been identified in palm wine and specific *Lactobacillus* strains have been found [151] in nono after its molecular characterization.



**Figure 4.** Summary of 30 bacteria (**A**) and 25 yeast (**B**) genera associated with nine local beverages reported in 90 articles. The most commonly reported species for bacteria, yeast and filamentous fungi are *Staphylococcus, Saccharomyces* and *Aspergillus,* respectively. Overall prevalence was calculated as the actual number of times a genus was isolated across the 90 studies.

To help understand the microbial populations of these products and to design specific starter cultures and manufacture safer foods, Ezekiel et al. [152] used a high-throughput sequence analysis to investigate the bacterial communities in kunu and found potential human enteric pathogens and other organisms not associated with kunu processing. In another metagenomics study [153] of Nigerian and other African beverages, dominant species were highlighted and it was found that lactobacilli were less abundant in fermentations performed under laboratory conditions when compared to artisanal or commercial fermentations.

# 5.3. Practical Implications of the Genera Found in Beverages

Three out of the nine genera found in all beverages (Section 5.1), namely *Bacillus, Escherichia* and *Staphylococcus*, are well known for foodborne outbreaks which can occur due to post-process contamination. The screening of foods for the prevalence of these bacterial species, in particular *Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus*, is always expected to be carried out in line with national or regional regulations. For fungi, most isolates originate from the raw material used for making the beverage and they are often isolated during spoilage, hence they are not discussed in this section.

# 5.3.1. Bacillus

According to Cote et al. [154], the genus Bacillus has over 260 species and is a diverse group of spore-forming bacteria responsible for food poisoning resulting in diarrheal syndromes. These syndromes are suspected to be caused by the various cytotoxins and degradative enzymes produced by bacteria under the control of the virulence regulator PlcR. However, it has been highlighted that most strains that produce these factors are not generally pathogenic. Species regarded as nonpathogenic include *B. thuringiensis* strains that are used as biopesticides [155] and *B. coagulans*, which have been reported [156] to promote intestinal digestion, inhibit the growth of pathogenic bacteria and normalize immune cells. Contrastingly, the species Bacillus cereus forms spores, biofilms, emetic toxins and causes diarrhoea [157]. It can also produce cereulide, a potent toxin which is highly heat- and acid-resistant [158]. The median Bacillus cereus outbreak incubation periods have been reported to be up to 4 h in a study that covered 16 years [159], but knowledge of the mechanistic basis of spore germination and outgrowth is still a difficult challenge [160]. In some countries, *Bacillus cereus* has been proposed as the main causal agent of foodborne outbreaks [161] and outbreaks around the world are still common especially after celebration parties [162] and in schools, as exemplified in previous reports [163–165]. The proper identification of the *Bacillus* species in a beverage from a region or location will ensure a targeted and effective control.

#### 5.3.2. Escherichia

The organisms in the genus *Escherichia* are well known enteric pathogens. One of the species *E. coli* O157:H7, is one of the most dangerous foodborne pathogenic bacteria around the world [166]. Some species harbour extended-spectrum  $\beta$ -lactamase and cause multidrug resistance which is influenced by various factors such as environmental sources, and international travel [167] and this may be why the most frequent route of transmission for *E. coli* O157:H7 infections is through the consumption of contaminated food and water [168]. A proposed primary prevention strategy in developing countries and for travelers to these regions is the continuous development of new vaccines [169]. Foodborne disease outbreaks due to the presence of *E. coli* toxins in food is common [170] and the aetiology of some shiga toxin *E. coli* is trains has been reported to be up to 87 h [154], which may be helpful for medical interventions when it is suspected that it has been ingested. The implications of persistent foodborne diarrheagenic *E. coli* in the agricultural and food production environment for food safety and public health has been reviewed. It was highlighted that bacterial processes such as initial attachment, biofilm formation, horizontal gene transfer and responses to environmental stresses enable the emergence of potentially more virulent strains within the agricultural and food production environment [171].

#### 5.3.3. Staphylococcus

*Staphylococcus* is widely distributed in nature and is a part of normal flora among humans, animals, plants and the environment, hence the possibility of food contamination with the genus is very high. A huge concern in the last couple of decades is the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA). These strains were first reported in humans in the 1960s as important nosocomial pathogens, and subsequently emerged in food-initiated MRSA outbreaks [172]. Similar to *Bacillus*, the median outbreak incubation period is believed to be up to 4 h [159]. The presence of MRSA in the food chain has implications for food safety and surveillance programs are required for rapid MRSA detection and control [173]. Use of a state-of-the-art biosensor method for the detection and quantification of *S. aureus* is increasingly becoming popular [174], and a preventive control approach may include the removal of *Staphylococcal* biofilms which serve as a virulence factor, allowing MRSA strains to adhere to surfaces and other materials used in the food industry [172]. An improved understanding of household transmission dynamics and the relationship between the transmission in healthcare and household settings [175], where food and beverages are consumed, is important in the control of MRSA.

#### 5.3.4. Positive Aspects of Beverage Fermentation

Issues associated with the organisms detected due to poor hygiene or the presence of the organism in the beverage fermentation process can be positive and not only negative, as mentioned above. Processes such as adding lactic acid bacteria as a probiotic during fermentation have been shown to acidify tiger nut milk and reduce the growth of undesirable microrganisms [176]. Another probiotic bacteria is *Bacillus coagulans* whose genetic model has been proposed [177] for use in the evaluation of risk-related gene traits, combining a whole genome sequencing analysis with updated bioinformatics tools and standard phenotypic assays. The local beverages can be screened for this organism and the model can be used to establish the safety of all bacteria found in traditional beverages. Other fungi, such as *Aspergillus oryzae*, can produce fungal biomass for feed, fatty acids and glycerol from fat-rich dairy substrates [178]. This suggests that wastes from the local beverage productions can be valourised for new product development. Sometimes products can be improved by the co-inoculation of some genera mentioned in this study. Tufariello et al. [179] found that a multistarter formulation using *Candida zemplinina, Saccharomyces cerevisiae* and *Lactobacillus plantarum* for industrial wine production was able to enhance the organoleptic wine features to different extents. It would be interesting to examine the effect of co-inoculating these organisms in the aforementioned local beverages.

Another concern for beverage fermentation is the production of bioamines, which can be good or not beneficial. According to previous reports [180,181], biogenic amines can be found in all foods containing proteins or free amino acids and are found in a wide range of fermented and nonfermented food products. The reports also highlighted that they play an important role as a source of nitrogen and precursor for the synthesis of hormones, alkaloids, nucleic acids, proteins, amines and food aroma components. In contrast, it was pointed out that food containing high amounts of biogenic amines may have toxicological effects.

## 6. Future Perspectives

There are many "farm to cup" opportunities for more microbiological studies in several aspects of the beverages mentioned in this review. The dominant species that occur at each stage of the production process and storage needs to be determined with molecular methods. Data on the mechanism of the formation and types of secondary metabolites associated with fermentation are critical for public health and would be beneficial if obtained. Furthermore, folklore health claims for the products from the microbial fermentation of the beverages needs further investigation. The regulation for the production and sales of these traditional beverages needs standardization with set production thresholds. Additionally, basic food safety training and licensing for producers and sellers should be compulsory. The costs of these must be very affordable or subsidized and training materials in the language of the person concerned may be used.

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