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Anyogu, Amarachukwu ORCID: <https://orcid.org/0000-0001-9652-7728>, Olukorede, Ayomide, Anumudu, Christian, Onyeaka, Helen, Areo, Esther, Adewale, Obadina, Odimba, Joyce N. and Nwaiwu, Ogueri (2021) Microorganisms and food safety risks associated with indigenous fermented foods from Africa. *Food Control*, 129. p. 108227. ISSN 0956-7135

<http://dx.doi.org/10.1016/j.foodcont.2021.108227>

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1 **Microorganisms and food safety risks associated with indigenous fermented foods from**
2 **Africa**

3 Amarachukwu Anyogu^{a*}, Ayomide Olukorede^b, Christian Anumudu^c, Helen Onyeaka^c, Esther Areo^d,
4 Obadina Adewale^d, Joyce N. Odimba^e, Ogueri Nwaiwu^f

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13 ***Corresponding author:** Amarachukwu Anyogu, PhD, Applied Biotechnology Research Group,
14 College of Liberal Arts and Sciences, University of Westminster, 115 New Cavendish Street, London
15 W1W 6UW, United Kingdom.

16 **Email:** amara.anyogu@gmail.com

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26 **Abstract**

27 Indigenous fermented foods (IFFs) have a long history in Africa and are embedded in cultural norms
28 and practices. Typically, these foods are produced at small or household scale using indigenous
29 processing technologies. In addition, limited knowledge of good manufacturing and handling
30 practices can lead to production under unhygienic conditions. This results in variations in the quality
31 and safety attributes of IFFs, as spoilage and pathogenic bacteria can be introduced at any stage of
32 the value chain. These foods have an important role in the African diet and can contribute to food
33 security by increasing the availability of cheap, nutritious food and supporting livelihoods. However,
34 the presence of foodborne pathogens and antibiotic-resistant bacteria in IFFs may constitute a
35 health risk to consumers. Therefore, this review presents an overview of the microorganisms
36 associated with IFFs from Africa, focusing on microbial food safety hazards. African indigenous
37 fermented foods offer a vast genetic potential of undiscovered strains that possess valuable
38 technical characteristics. However, IFFs may also serve as vehicles of pathogenic and antibiotic-
39 resistant bacteria and genetic determinants. Significant research and data gaps exist regarding the
40 microbiological safety of these food products, which warrant urgent attention. We propose practical
41 solutions for improving the safety of African IFFs requiring action and collaboration from all
42 stakeholders, including researchers, producers, governmental regulatory bodies, and consumers.

43 **Keywords:** Africa; antibiotic resistance; fermented foods; food safety; lactic acid bacteria; pathogens

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56 **1. Introduction**

57 Indigenous fermented foods (IFFs) have a long history in Africa and are embedded in the cultural
58 norms and practices. They make a valuable contribution to the continent's effort in achieving its
59 sustainable development goals focused on food security, poverty alleviation and gender equality
60 (Franz et al., 2014). Fermentation adds value, improving the organoleptic characteristics, variety,
61 nutritional quality, digestibility, and safety of foods (FAO, 2010; Ganzle, 2020). As a low-cost
62 technology, indigenous fermentations improves access to local, inexpensive, nutritious food. In
63 addition, IFFs contribute to the livelihoods of many, especially women, through income generation
64 via small-scale enterprise (Misihairabgwi & Cheikyoussef, 2017; Rolle & Satin, 2002).

65 Fermentation is a processing technique where desirable changes occur in a food product due to the
66 metabolic activities of microorganisms (Caplice and Fitzgerald, 1999). There has been increasing
67 interest from researchers to understand the diversity and technological properties of the microbial
68 community in IFFs from Africa. This knowledge is required for producing local, well-characterised
69 starter cultures to improve the quality and safety of IFF products (Akabanda et al., 2013; Anyogu et
70 al., 2014; Bayili et al., 2019). Despite these efforts, IFF production in Africa largely remains a cottage-
71 level technology, mainly reliant on spontaneous fermentation processes or backslopping, which may
72 introduce spoilage or pathogenic organisms (Benkerroum and Tamime, 2004). Bacteria of public
73 health interest have been isolated from IFFs, and some investigators have noted concerns about the
74 safety of these foods (Ahaotu et al., 2013; Omemu et al., 2014; Samet-Bali et al., 2016; Walsh et al.,
75 2017).

76 Data from the World Health Organisation (WHO) estimate that diarrhoeal diseases account for 70%
77 of mortalities associated with foodborne disease in the African region (WHO, 2015). Also, antibiotic
78 resistance (AR) has emerged as one of the utmost global public health concerns. Fermented food
79 products have diverse microbial ecosystems, yet their ability to serve as vehicles for transferring AR
80 microorganisms and genes remains unclear.

81 Reviews discussing IFFs from Africa have mainly focused on the microbial diversity of fermenting and
82 technologically important bacteria (Achi and Ukwuru, 2013; Jans et al., 2017; Parkouda et al., 2009;
83 Tamang et al., 2016). Recently, Nwaiwu et al. (2020) reviewed spoilage and pathogenic microbiota
84 associated with indigenous fermented beverages but only concentrated on one country. Paudyal et
85 al. (2017) analysed the prevalence of foodborne pathogens in foods from selected African countries;
86 however, their focus was on raw and ready-to-eat foods and not IFFs specifically.
87 A comprehensive overview of the microbiological safety of IFFs from Africa is lacking. The purpose of
88 this review is to summarise current knowledge on the microbiology of IFFs with a focus on
89 pathogenic and AR bacteria. It also discusses approaches to improve the safety of these foods and
90 highlights data gaps that could be explored in further research.

91 **2. Indigenous African fermented food products**

92 Fermentation has long been used as a preservation technology for extending the shelf life of various
93 substrates. The characteristic flavour, texture and colour of IFFs due to the metabolic activities of
94 fermenting microorganisms has guaranteed their widespread acceptability by consumers, thereby
95 establishing a major role for IFFs in the African diet (Mokoena et al., 2016). Olasupo et al. (2010)
96 classified IFFs from Africa into five major groups based on their raw materials. These include (1)
97 fermented non-alcoholic cereals, (2) alcoholic beverages, (3) fermented animal proteins, (4) starchy
98 root crops, and (5) fermented vegetable proteins.

99 Cereal grains comprising maize, sorghum, millet, and wheat are important staple crops in Africa,
100 accounting for as much as 50% of the total daily calorific consumption (OECD/FAO, 2016). These
101 cereals are common starting materials for lactic acid-fermented beverages and porridges known by
102 different names such as *ogi* in Nigeria (Oguntoyinbo et al., 2011), *togwa* in Tanzania (Mugula et al.,
103 2003), *koko* in Ghana (Lei and Jakobsen, 2004) and *hussuwa* in Sudan (Yousif et al., 2010).

104 Fermented non-alcoholic cereal-based products have an important role in the diet as
105 complementary foods for infants or breakfast meals (Byakiya et al., 2019; Soro-Yao et al., 2014). The
106 extensive use of cereal-based complementary foods makes them an attractive target in efforts

107 towards combating infant malnutrition in Africa. For example, *koko*, made from fermented corn
108 dough, is the most commonly consumed complementary food in Ghana. However, it has been noted
109 to be inadequate in meeting dietary protein and micronutrient needs (Suri et al., 2014). To improve
110 the nutritional profile of *koko*, a legume-based supplement was developed recently, with preliminary
111 studies suggesting good acceptability by consumers (Tano-Debrah et al., 2019).

112 Fermented alcoholic beverages are consumed across the continent. The majority of these are
113 produced from cereals, e.g., sorghum, millet, and maize. These include *dolo*, *burukutu*, or *pito* in
114 West Africa (Sawodogo-Lingani et al., 2007; Onyenekwe et al., 2015), *borde* in Ethiopia (Abegaz,
115 2007), and *sesotho* in South Africa (Cason et al., 2020). Fruit fermentation for alcohol production is
116 uncommon. However, plantain and banana can be fermented to produce *agadagidi* (Sanni and
117 Lonner, 1993). Palm wine is a popular alcoholic drink in many West African countries. Palm wine is
118 obtained from the fermentation of sap obtained from palm tree species such as *Elaeis guineensis*,
119 *Raphia hookeri*, *Borassus aethiopum*, and *Borassus akeassii*. The production of ethanol, lactic acid
120 and acetic acids by yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) are the most
121 significant activities contributing to the distinctive organoleptic characteristics and stability of the
122 product (Amoa-Awua et al., 2007; Karamoko et al., 2012; Nwaiwu & Itumoh, 2017). The gradual
123 accumulation of ethanol at the early stages of fermentation correlates with the increase in the AAB
124 population, as AAB can use ethanol as a carbon source (Amoa-Awua et al., 2007). Organic acids
125 produced by LAB & AAB contribute to the sour taste, aroma and colour, although AAB are often
126 considered as spoilage organisms. The low pH and alcohol content control the growth of undesirable
127 bacteria, including *Enterobacteriaceae* (Djeni et al., 2020; Ouoba et al., 2012).

128 Cassava (*Manihot esculenta*, Crantz) ranks third after rice and maize as a source of calories in
129 tropical countries and is the most consumed starchy root crop in Africa. Total annual cassava
130 consumption has tripled from 20 million tonnes in 1970 to just over 60 million tonnes in 2013
131 (FAOSTAT, 2020a; Szyniszweska et al., 2020). Fermentation practices involve either submerged
132 fermentation for the production of *lafun* and *chikwangue* (Miambi et al, 2003; Padonou et al., 2009)

133 or solid-state to produce *attieke* and *garri* (Ahaotu et al., 2017; Djeni et al., 2015). Fermentation is
134 essential for preventing the rapid post-harvest deterioration of cassava tubers. It is also an
135 important processing technique for decreasing the amount of cyanogenic glucosides (CGs), which
136 occur naturally in cassava. These CGs can be hydrolysed to produce hydrogen cyanide when the
137 plant tissue is damaged, but it should be noted that the concentration of CGs can be cultivar specific
138 (Abiodun et al., 2020). Cyanide can inhibit cellular respiration by binding to cytochrome oxidase in
139 the electron transport chain, and high exposure to cyanide can lead to severe illness and, in some
140 cases, death (Akintowa et al., 1994; Kimaryo et al., 2000).

141 Consequently, the WHO has recommended a safe limit of 10 mg cyanide/kg of cassava (FAO/WHO,
142 1995). Although the breakdown of cyanide has been attributed to the activity of endogenous
143 enzymes, Obilie et al. (2004) observed that the highest loss of cyanogenic glucosides, from 72.4 mg
144 cyanide/kg to below detectable limits, occurred during fermentation compared to other processing
145 stages. These results are corroborated by Kivunde et al. (2000) during controlled fermentations using
146 LAB as a starter where the total cyanide content reduced from 176.3 mg/kg in raw cassava to 8.2
147 mg/kg in *kivunde*. Their observation of higher cyanide content in spontaneously fermented and
148 backslopped samples was attributed to β -glycosidase activity of the starter strains, suggesting an
149 additional advantage of controlled fermentations in guaranteeing the safety of fermented cassava
150 products. Another demerit of cassava is its low nutritional content, specifically protein, vitamins and
151 minerals and studies on fermented cassava products biofortified with protein (Ahaotu et al., 2017)
152 and Vitamin A (Abiodun et al., 2020) have recently been reported.

153 In Africa, milk has a long historical connection with pastoral communities, e.g., the Berbers, Fulani
154 and Maasai ethnic groups in North, West and East Africa. Milk is a significant source of nutrients, and
155 total milk production in Africa was 49 million tonnes in 2019 (FAOStat, 2020). Fermentation is an
156 essential processing technology for extending the shelf life of milk, a highly perishable product.

157 African fermented dairy products comprise of naturally fermented milks, e.g., *gariss* and *mabisi*;
158 yoghurt-based products, e.g., *kindirmo* and *amasi*, and cheeses, e.g., *wara*, *jben* and *klilia*

159 (Abdelgadir et al., 2008; Mourad & Bettache, 2015; Moonga et al., 2020; Nyambane et al., 2014;
160 Omemu et al., 2014; Osvik et al., 2013; Sudi et al., 2011) are widely consumed across Africa.
161 Despite consumer preferences for fresh meat and seafood, the combination of a warm, tropical
162 climate and variable access to refrigeration facilities, particularly in rural areas, means that meat and
163 seafood products are usually available in processed form. Smoking, salting, and drying are common
164 preservative strategies for meat and seafood products in Africa (El-Sheika et al., 2014). Compared to
165 other parts of the world, meat and fish fermentation is not widespread across Africa. However,
166 fermented meats, e.g., *sujuk* and *boubanita*, are consumed in different North African countries
167 (Benkerroum, 2013). Fermented fish products serve as both flavouring agents and a source of
168 protein (Anihouvi et al., 2007) and can be produced from a wide variety of fish species.
169 A significant amount of protein intake in the African diet comes from plant sources. The alkaline
170 fermentation of proteinaceous oil seeds such as locust bean, melon, sesame, and castor oil have
171 widespread use as flavour enhancers and meat substitutes (Ahaotu et al., 2013; Ezeokoli et al.,
172 2016). These seeds are often inedible in their natural state as they contain anti-nutrients such as
173 indigestible oligosaccharides and phytates, which are metabolised during fermentation (Parkouda et
174 al., 2009). Plant leaves also serve as substrates for fermentation. *Kawal* and *ntoba mbodi* are
175 produced from the fermentation of the leaves of *Cassia obtusifolia* and *Manihot esculenta*,
176 respectively (Dirar, 1985; Voudibio Mbozo et al., 2017).

177 **3. Fermenting and technologically important microbiota of African indigenous fermented** 178 **foods**

179 Accurate identification and characterisation of the microbiota of IFFs is a critical first step for the
180 selection of multi-functional starter cultures. Starter cultures are required for the controlled and
181 large-scale production of IFFs with improved quality and safety attributes (Ahaotu et al., 2017;
182 Edema and Sanni, 2008; Soro-Yao et al., 2014). The last four decades have seen an increase in
183 research studies aimed at elucidating the microbial consortia present in IFFs from Africa. These
184 studies have provided data on the vast diversity of microbial species, including lactic acid bacteria

185 (LAB), *Bacillus*, and yeasts that dominate the fermentation of cereals, legumes, dairy, and root crops
186 (Table 1).

187 Lactic acid bacteria dominate the fermentation of cereals, dairy products, and starchy root tubers.
188 Of the LAB, species belonging to *Lactobacillus*, *Lactococcus*, *Weissella*, *Leuconostoc*, *Streptococcus*,
189 and *Enterococcus* are frequently isolated (Diaz et al., 2019; Oguntoyinbo et al., 2011).

190 Previously, the beneficial effects of fermented foods were focused mainly on preservation and
191 desirable organoleptic characteristics. Nowadays, a better understanding of the microorganisms
192 involved in fermentation processes has drawn more attention to the various health benefits
193 associated with IFFs. Lactic acid bacteria are among the most studied microorganisms in this regard,
194 given their interesting technological characteristics. During fermentation, LAB metabolise sugars to
195 produce a wide range of organic acids, including lactic, acetic and propionic acid, which produce a
196 low pH environment that is detrimental to the growth and survival of spoilage and pathogenic
197 organisms (Caplice & Fitzgerald, 1999).

198 Other antimicrobial metabolites associated with LAB metabolism include ethanol, hydrogen
199 peroxide, diacetyl, and bacteriocins showcasing the diversity of LAB in utilising different metabolic
200 pathways and substrates (Oguntoyinbo and Narbard, 2015; Mokoena et al., 2016; Soro-Yao et al.,
201 2014). As LAB species are important members of the gut microbiome, their contribution to human
202 health as probiotics has been extensively studied. Positive actions have been reported to include
203 restoration of gut microbiota after antimicrobial therapy, vitamin production, and stimulating the
204 immune system (Markowiak and Slizewska, 2017). A role for IFFs from Africa as delivery vehicles for
205 probiotics is receiving significant attention (Achi & Ukwuru, 2015; Mokoena et al., 2016). Recently,
206 two cereal-based fermented foods, *obushera* and *kwete*, have been produced using probiotic strain
207 *Lactobacillus rhamnosus* yoba (Mukisa et al., 2019; Wacoo et al., 2019). Although the probiotic strain
208 is not of African origin, the inherent presence of LAB species in IFFs from Africa suggests that IFFs
209 may be sources of novel probiotic strains and warrants further studies.

210 The presence of *Enterococcus* spp., notably *Enterococcus faecium* and *Enterococcus faecalis*, in food
211 systems is complex due to their increasing medical relevance as aetiological agents of nosocomial
212 infections and their ability to disseminate antibiotic resistance determinants, particularly
213 vancomycin (Oladipo et al., 2013; Oguntoyinbo and Okesuo, 2013). However, *Enterococcus* spp. are
214 usually observed in IFFs, particularly in fermented dairy products where they are considered as
215 contaminants (Jans et al., 2017). Although not recognised on the Qualified Presumption of Safety list
216 (EFSA Panel on Biological Hazards, 2021), the positive technological contributions of enterococci
217 include the production of extracellular polysaccharides, which influence the texture of fermented
218 dairy products, and flavour and aroma development (Jans et al., 2017; Obioha et al., 2021). Some
219 strains also produce bacteriocins, and their consideration for probiotics is receiving more research
220 attention (Hanchi et al., 2018).

221 *Saccharomyces cerevisiae* is the most commonly isolated fungal species from African IFFs and has an
222 essential role in the alcoholic fermentation of cereals and palm sap. Other dominant genera include
223 *Candida*, *Pichia* and *Kluveromyces* (Table 1). In addition to fermenting the sugars available in the
224 natural substrates to alcohol and carbon dioxide, fungi produce flavour and aroma compounds such
225 as organic acids, esters and carbonyls and contribute to textural changes via pectinases and
226 cellulases (Cason et al., 2020; Johansen, 2019). Yeasts may also contribute to organic acid production
227 by LAB species present during the fermentation (Ferreira and Mendes-Faia, 2020). Fungi, therefore,
228 play a significant role in the final organoleptic characteristics of alcoholic IFFs over and above alcohol
229 production.

230 *Bacillus* spp. dominate the fermentation of protein-rich legumes and seeds. An important aspect of
231 the production process of alkaline fermented condiments such as *iru*, *soumbala* and *bikalga*
232 produced from these protein-rich substrates is the long cooking time, and this heating process may
233 select for spore formers that are more heat resistant (Ouoba et al., 2007; Parkouda et al., 2009). In
234 addition, the degradation of proteins during fermentation by *Bacillus* spp., most significantly, *B.*
235 *subtilis*, *B. pumilus*, and *B. licheniformis*, leads to the accumulation of peptides and ammonia. This

236 leads to an increase in pH, which also favours the proliferation of *Bacillus* spp. (Ouoba et al., 2008).
237 *Bacillus* spp. are also frequently isolated from fermented cassava products (Anyogu et al., 2014;
238 Assanvo et al., 2017; Padonou et al., 2009). Their ability to produce enzymes that hydrolyse cassava
239 tissue has been reported to be responsible for the textural changes that occur during fermentation
240 (Amoa-Awua & Jakobsen, 1995).

241 Molecular based techniques including fingerprinting-based methods, e.g., repetitive element
242 palindromic (rep)-, intergenic transcribed spacing (ITS)-PCR in combination with the sequencing of
243 ribosomal RNA (16S and 26S) or other housekeeping genes, e.g. *rpoA*, *rpoB*, *gyrA* and *pheS* are now
244 routinely used to identify and characterise microorganisms in IFFs (Oguntoyinbo and Okesuo, 2012;
245 Owusu-Kwarteng et al., 2012; Tadesse et al., 2019). These have provided new insights compared to
246 earlier studies, which relied only on phenotypic identification (Abegaz, 2007; Nyambane et al.,
247 2014), which do not always provide sufficient information. For example, molecular studies on
248 submerged cassava fermentation have highlighted the dominance of *Weissella confusa* (Anyogu et
249 al., 2014; Padonou et al., 2009), a microorganism that had not been previously reported in studies
250 relying on phenotypic methods (Omafuvbe et al., 2007; Oyewole & Odunfa, 1988). *Weissella* spp. are
251 also now frequently identified in other IFFs, e.g. cereals (Angelov et al., 2017; Oguntoyinbo et al.,
252 2011) and dairy products (Akabanda et al., 2013). The extensive species diversity revealed by the use
253 of genotyping techniques has been reported for other IFFs (Achi, 2005; Aderigbigbe et al., 2011;
254 Nwaiwu and Itumoh, 2017; Ouoba et al., 2012) and has also uncovered new microbial species
255 (Ouoba et al., 2015, a, b).

256 More recently, culture-independent methods such as PCR-DGGE and next-generation sequencing
257 techniques are used to investigate the metagenomics associated with IFF production (Cason et al.,
258 2020; Djeni et al., 2020; Walsh et al., 2017). An advantage of this approach is that it provides
259 detailed information about the microbial community associated with different fermentation stages
260 without the need for isolation. This results in a less biased microbial profile compared to culture-
261 dependent methods as IFFs may contain uncultivable species (Bigot et al., 2015). Accurate data of

262 the microbial community involved during IFF production eliminates chance isolates that can be
263 recovered on agar from the screening process for potential starter cultures.
264 These more recent reports have confirmed that the microbes present in IFFs are more diverse than
265 earlier reported. While investigating the microbial community of *soy-daddawa*, Ezeokoli et al. (2018)
266 identified *Exiguobacterium* spp. for the first time. Diaz et al. (2019) observed *Zymomonas* spp. for
267 the first time in fermented cereal, dairy, cassava and locust bean products. Although the
268 contribution of these organisms to the fermentation process requires further study, their
269 identification reveals new insights into IFFs. The factors that influence the composition of the
270 microbial community has been noted to include fermentation conditions, pH changes, and
271 geographical location (Houngbedji et al., 2018; Moonga et al., 2020). However, metagenomic studies
272 have revealed that geographical location is not a consistent factor (Cason et al., 2020; Parker et al.,
273 2018). Understanding the microbes involved in African IFF production will help in the design of
274 starter cultures. However, culture-independent methods rely solely on DNA, so do not allow for the
275 direct selection of microbial starters. Depending on the method used, DNA may be extracted from
276 live and dead cells (Mukisa et al., 2012; Diaz et al., 2019). These limitations may be overcome by
277 using both culture-dependent and independent methods (Adewunmi et al., 2012; Schoustra et al.,
278 2013).

279 Despite these recent developments, there remain significant knowledge gaps concerning the
280 microbiota of IFFs from Africa. Our review of the published research in this area highlights that
281 further studies are required, particularly for alcoholic beverages, fermented meat and fish products
282 (Gagaoua & Boudechicha, 2018; Djeni et al., 2020). These gaps limit the transition from household to
283 large-scale controlled production of IFFs with consistent quality and safety attributes.

284 **4. Microbial hazards in indigenous fermented foods**

285 Globally, food safety remains a significant challenge. The World Health Organisation (WHO)
286 estimates that as many as 1 in 10 people fall ill, and more than 120,000 children under 5 die each
287 year after consuming unsafe food. Africa bears a high burden of the global incidence of foodborne

288 illness with an estimated annual morbidity of 90 million (WHO, 2015; WHO 2017b). Microbial
289 hazards, including foodborne pathogens and their toxins, are primary aetiological agents of
290 foodborne disease (FBD) and a growing public health issue.

291 Fermented foods are generally considered safe. Fermenting organisms, especially LAB, produce a
292 range of antimicrobial compounds, e.g., organic acids, ethanol, bacteriocins and hydrogen peroxide,
293 which are antagonistic to the growth and survival of foodborne pathogens (Adinsi et al., 2017;
294 Devuyst and Vandamme, 1996; Mpofu et al., 2016). Cason et al. (2020) reported the decline of
295 pathogenic and spoilage organisms during cereal fermentation for *sesotho* production. Similar
296 observations were made by Karamoko et al. (2012), who noted a 4 – 7 log reduction in faecal
297 coliform counts within a 24 h period in fermenting palm wine. When investigating the
298 microbiological quality of milk products in Tanzania, Schoder et al. (2013) detected *Salmonella* and
299 *Escherichia coli* in raw milk but not in fermented milk. In addition, Oguntoyinbo and Narbad (2015)
300 isolated bacteriocin producing *Lactobacillus plantarum* strains from *kunu* and *ogi* that showed
301 antimicrobial activity against *Salmonella enterica*.

302 However, indigenous practices for food production are often based on spontaneous fermentation,
303 i.e., chance inoculation or the use of backslopping where utensils from a previous fermentation are
304 reused (Caplice & Fitzgerald, 1999). Limited knowledge and utilisation of Hazard Analysis and Critical
305 Control Points (HACCP) and good manufacturing processes (GMP) by farmers, food producers and
306 handlers can lead to production and processing occurring under unhygienic conditions. These factors
307 lead to variation in the microbial profile of IFFs, and consequently, the presence of spoilage and
308 pathogenic bacteria in these foods cannot be ruled out (Oguntoyinbo, 2014; Olasupo et al., 2016).

309 Despite the lack of surveillance of foodborne infections in many African countries, several studies
310 have investigated the prevalence of major foodborne pathogens in IFFs from Africa. Reports on
311 microbiological hazards associated with IFFs from Africa are presented in Table 2. Microbes of public
312 health significance, including *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*,

313 have been reported in IFFs from Africa. The introduction of pathogenic organisms can occur at
314 different stages of the value chain via raw materials, the processing environment, and food handlers.
315 An evaluation of the microbiological quality of water used for processing, fermenting broth, and the
316 fermented cassava product, *lafun*, identified microbial hazards such as coliforms, including
317 *Salmonella* spp. and *Staphylococcus* spp. (Lateef & Ojo, 2015). Potentially pathogenic bacteria have
318 been found in utensils used for the fermentation process (Gran et al., 2002; Jans et al., 2017);
319 however, only a few studies investigate the complete production chain to identify the source of
320 contamination (Ademola et al., 2018; Thorsen et al., 2015). Adedeji et al. (2017) reported
321 similarities in the microbial profiles of potentially pathogenic bacteria isolated from two fermented
322 condiments from the same producer at the retail level. It is important to note that these bacteria
323 were not present in the raw materials, suggesting unhygienic processing and handling. This
324 underscores the need for further research to identify contamination sources to support the
325 management of food safety hazards.

326 The microbiological safety of fermented vegetable proteins usually dominated by *Bacillus* spp. and
327 *Staphylococcus* spp. requires consideration, as acid production, a potent antimicrobial attribute in
328 lactic fermented IFFs, is not present (Ahaotu et al., 2013; Ouoba et al., 2019). *Bacillus cereus* and
329 *Staphylococcus aureus* can produce toxins in food during their growth and have been identified in
330 these products (Oranusi et al., 2015). *Bacillus* spp. are the dominant organisms involved in the
331 fermentation of oil bean seeds; however, the presence of *Bacillus cereus* is routinely reported
332 (Ahaotu et al., 2013; Ouoba et al., 2008b; Parkouda et al., 2009; Thorsen et al., 2015). Ahaotu et al.
333 (2013) isolated *Bacillus cereus* capable of producing enterotoxins in *ugba* under fermentation
334 conditions. A similar observation was made by Ouoba et al. (2008b) when investigating *B. cereus*
335 involved in locust bean fermentation for soumbala production. However, these are heat-labile toxins
336 that should be denatured with adequate cooking. Thorsen et al. (2015) detected the heat-stable,
337 emetic type toxin-producing *B. cereus* strains in fermented baobab seeds, which is of concern.

338 The metabolic activities of some microorganisms involved in the fermentation process provide
339 antagonistic conditions to the growth and survival of foodborne pathogens. However, inappropriate
340 handling and the use of unsanitary packaging material can introduce microbial hazards post-
341 processing (Adinsi et al., 2017; Mpofu et al., 2016; Schoder et al., 2013). The occurrence of
342 potentially pathogenic bacteria at the retail level across all food categories highlights the potential
343 risks to public health associated with IFFS (Odom et al., 2012; Owusu-Kwarteng et al., 2018). Of
344 particular concern is the isolation of pathogenic bacteria from cereal-based and dairy fermented
345 products, some of which are used as weaning or complementary foods (Adekoya et al., 2019; Samet-
346 Bali et al., 2016). The presence of these organisms in ready-to-eat products suggests that IFFs may
347 serve as vehicles of pathogenic bacteria. Therefore, the safety of these foods should not be taken for
348 granted or assumed. The use of next-generation sequencing methods has provided more insight in
349 identifying microbial hazards in IFFs, highlighting a role for metagenomic approaches as food safety
350 tools (Walsh et al., (2017).

351 **5. Antibiotic resistance of microbes from indigenous fermented foods from Africa**

352 The availability of antibiotics represented a landmark achievement in medicine and led to a
353 significant decrease in mortality and morbidity from infectious disease agents (Spellberg, 2014).
354 However, in recent times, an increasing number of pathogenic bacteria, including those that cause
355 foodborne diseases, have become resistant to treatment with the antibiotic drugs currently available
356 (WHO, 2017b). This scenario, termed antibiotic resistance (AR), has become one of the greatest
357 threats to global public health and food security (McEwen & Collingon, 2018). It has been estimated
358 that if left unchecked, the number of deaths attributed to AR each year could rise to 10 million by
359 2050 (O'Neill, 2016).

360 Antibiotic resistance is increasingly recognised as a food safety issue. The consumption of food
361 contaminated with AR foodborne pathogens such as *Salmonella* could lead to treatment failure.
362 Commensal bacteria such as *Escherichia coli* and *Enterococcus* spp. in food may transfer AR genes to
363 human pathogens (WHO, 2017b). Drivers of AR include the overuse of antimicrobial drugs in human

364 and veterinary medicine. In addition to treatment, antimicrobials are used in agriculture and
365 aquaculture as prophylaxis and in some parts of the world as growth promoters (Nhung et al., 2017;
366 Van Boeckel et al., 2014). These factors lead to the spread of AR bacteria and AR genes (ARG) in the
367 environment.

368 A comprehensive evaluation of the safety of IFFs in Africa should consider their role as vehicles of
369 both pathogenic and AR bacteria. However, in Africa, the contribution of the food chain to the
370 overall burden of AR is mostly unknown as there are little to no surveillance systems for monitoring
371 antibiotic use in agriculture and food products (Founou et al., 2016; Oloso et al., 2018). For the most
372 part, antibiotic use remains unregulated in Africa (Oguntoyinbo & Okesuo, 2012; Van et al., 2020).

373 Reports of AR bacteria associated with some African IFFs are presented in Table 3. Some of these
374 studies have documented multiple drug-resistant (MDR)- bacteria in technologically relevant,
375 indicator and potentially pathogenic organisms. Phenotypic resistance to more than three classes of
376 antibiotics was observed in *Escherichia coli* O157:H7 and *Shigella* spp. isolated from cheese products
377 available for retail sale in Egypt (Ahmed and Shimamoto, 2015 a,b). Fowoyo and Ogunbawo (2017)
378 isolated 255 coagulase-negative staphylococci (CoNS) from fermented dairy, cereal and oilseed
379 products in Nigeria. Of the total isolates, 27% exhibited MDR-phenotypes. Oguntoyinbo and Okesuo
380 (2012) observed MDR *Enterococcus* spp. in *wara*, a fermented cheese. Ouoba et al., (2019) also
381 identified MDR-*Staphylococcus* spp. in fermented vegetable products. However, other reports
382 showed AR to one or two classes or overall sensitivity to the antibiotics tested. Owusu-Kwarteng et
383 al., (2017) noted that *Bacillus cereus* isolated from fermented dairy products in Ghana only showed
384 resistance to beta-lactams. *Enterococcus* spp. in IF products in Tunisia were shown to be sensitive to
385 beta-lactams, aminoglycosides and macrolides (Rehaim et al., 2016).

386 Vancomycin is a critically important antibiotic, often used as a last resort treatment (WHO, 2019).
387 Vancomycin resistance has been noted in isolates from African IFFs (Awopetu et al., 2016;
388 Oguntoyinbo & Okesuo, 2012; Ouoba et al., 2008a). However, Rehaim et al., (2016) reported that
389 *Enterococcus* spp. isolated from Tunisian fermented food products were susceptible to vancomycin.

390 A similar observation was also noted by Owusu-Kwarteng et al., (2017, 2018) when investigating *B.*
391 *cereus* and *L. monocytogenes* in fermented dairy products.

392 It is important to note that most bacterial species have intrinsic and induced resistance mechanisms
393 to specific antibiotic drugs (Reygaert, 2018). From a food safety perspective, the ability of foodborne
394 bacteria to transfer resistance traits via mobile genetic elements to other bacteria is a primary
395 concern. There are some reports of AR genetic determinants associated with African IFFs. Ahmed
396 and Shimamoto (2015a) screened shiga toxin-producing *Escherichia coli* for antibiotic-resistant genes
397 (ARG) and identified beta-lactamase encoding genes in all (n=5) isolates. Two of these isolates
398 possessed plasmid-mediated quinolone resistance genes. Conjugation experiments undertaken by
399 Ouoba et al., (2019) showed that CoNS from fermented condiments produced transconjugants with
400 increased resistance to erythromycin and tetracycline. Unfortunately, most investigations of AR in
401 African IFFs only consider phenotypic resistance (Adimpong et al., 2012; Awopetu et al., 2016;
402 Eruteya and Eze, 2017). For this review, we found only one study (Aka et al., 2020) where ARGs in
403 IFFs from Africa were investigated using whole-genome sequencing.

404 The prevalence of AR phenotypes in bacteria isolates from retail level IFFs in Africa and the high
405 levels of resistance observed in some strains provide some evidence that these foods may serve as a
406 reservoir for AR and is of public health significance. There remain considerable research and data
407 gaps in this area, underscoring the need for large scale and long-term surveillance studies
408 coordinated at national and international levels.

409 **6. Discussion & Recommendations**

410 According to the United Nations, 50% of global population growth between now and 2050 is
411 anticipated to occur in Africa (UN, 2020). However, Africa currently has the 2nd highest number of
412 undernourished people globally, and about 20% of the population on the continent is already
413 considered to be food insecure (FAO et al., 2020). The contribution of IFFs to combating food
414 insecurity via increased food availability, improved nutrition and income generation is well
415 established (Franz et al., 2014; Okafor, 1992; Rolle and Satin, 2002; Setta et al., 2020).

416 The global market for fermented products is predicted to surpass \$20 billion by 2022 (Sivamaruthi et
417 al., 2019). This demand is fuelled in part by the increasing popularity of some IFFs, such as
418 *kombucha* and *kefir*, in the international market as consumers become more aware of their
419 beneficial effects (Soni et al., 2014). Besides meeting domestic demand from an increasingly urban
420 populace, African IFFs could also become a source of foreign revenue via exports for a growing
421 diaspora community and beyond. This requires improving the value chains that deliver IFFs with
422 consistent quality and safety attributes to local and international markets using modern, industrial
423 processes, including well-characterised starter cultures.

424 More work remains to be done towards achieving large scale production of IFFs from Africa.
425 However, there are some success stories. For example, the production of the widely consumed
426 sorghum-based fermented beer *umqombothi* has been industrialised in South Africa. Cereal-based
427 porridges *ogi* and *uji*, and the alcoholic beverage palm wine are also now produced commercially
428 (Adebo, 2020; Nwaiwu & Itumoh, 2017). Significant advances have been made in identifying
429 dominant strains in IFFs and characterising technological aspects which make them suitable for use
430 in controlled fermentations (Aderigbigbe et al., 2011; Ahaotu et al., 2013; Aka et al., 2020;
431 Houngbédji et al., 2018; Moodley et al., 2019; Oguntoyinbo & Narbad, 2015; Sawadogo-Lingani et
432 al., 2008). However, transferring these technologies to producers presents some challenges,
433 including the stability, activity, and viability of these cultures (Benkerroum & Tamime, 2004;
434 Benkerroum, 2013; Rolle and Satin, 2002). Although several studies have identified and
435 characterised potential starters for use in soubala fermentation, most processors still utilise
436 spontaneous fermentation processes (Ouoba et al., 2004; Compaore et al., 2020; Parkouda et al.,
437 2009). To overcome some of these obstacles, an alternative approach used by some researchers is
438 the utilisation of commercially available starter cultures, as has been reported for the production of
439 *obushera* using *Lact. rhamnosus* yoba (Mukisa et al., 2019) and *pito* using *Lact. delbrueckii* and
440 *Sacch. cerevisiae* (Djameh et al., 2019). However, this may not be a sustainable solution due to cost
441 implications.

442 Selecting starter cultures from the autochthonous community is often recommended as these
443 cultures are considered more adapted to the fermentation parameters of the IFF and may show
444 wider metabolic diversity required for achieving desirable properties and functional characteristics
445 of the product (Ashaolu, 2019; Casquete et al., 2011). However, there is a lack of data showing if
446 autochthonous fermenting organisms possess unique biochemical properties compared to similar
447 strains obtained from other sources, which could be further explored. There is also a need for more
448 studies that evaluate the application of starter cultures as monocultures or in combination for IFF
449 production outside the laboratory environment (Compaore et al., 2020; Kimaryo et al., 2000; Mukisa
450 et al., 2016). Beyond technology transfer, more progress is required to improve raw materials,
451 conduct laboratory, and pilot plant production before scaling up to industrial production (Okafor et
452 al., 1992; Benkerroum, 2013).

453 Food fermentations are complex microbial ecosystems (De Filippis et al., 2017). The use of
454 metagenomics in studying African IFFs has provided more insight into the microbiota and succession
455 dynamics of fermenting microorganisms (Cason et al., 2020; Diaz et al., 2019). However, only a
456 limited number of IFFs have been investigated using this approach, and future research efforts
457 should be directed here. Metagenomic data can be combined with outputs from other 'omics'
458 technologies such as metabolomics and transcriptomics to develop a more comprehensive
459 understanding of the relationships between the microorganisms present in the food, their metabolic
460 interactions, and what these contribute to the fermentation process (De Filippis et al., 2017;
461 Kergourlay et al., 2015). This will allow for a more informed starter selection process for improved
462 fermentation processes.

463 The prevalence of foodborne disease remains severe in many African countries. Factors contributing
464 to this include food preparation with contaminated water, poor hygiene, inadequate storage
465 facilities, food safety knowledge, and insufficient food safety legislation and implementation (Belli et
466 al., 2013). Currently, IFFs are mainly marketed via the informal economy in open markets, street
467 vending and household producer/seller, therefore outside the scope of official health regulatory

468 standards where these exist. The reviewed studies suggest that contamination of IFFs mainly occurs
469 post-processing. Similar to our observations, a meta-analysis of the prevalence of foodborne
470 pathogens in ready-to-eat food from seven African countries showed that *Enterobacteriaceae*,
471 *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* were the most
472 frequently reported organisms (Paudyal et al. 2017).

473 Given the potential for the introduction of food safety hazards at each production stage, the design
474 and implementation of quality assurance and management systems, including GMP and HACCP in
475 commercial food production, is recommended or incorporated into legislation as an effective
476 strategy for improving food safety (Kafetzopoulous, 2013; Ropkins & Beck, 2000). There are now
477 several reports on the development of HACCP systems for IFF production (Asagbra et al., 1998;
478 Fasoyiro et al., 2010; Lateef & Ojo, 2015; Oguntoyinbo, 2012;). Some studies have demonstrated the
479 effective use of these approaches in improving the microbiological quality and safety attributes of
480 some products such as *lafun* (Obadina et al., 2008) and *kenkey* (Amoa-Awua et al., 2007). However,
481 there is a need for more data evaluating the application of HACCP systems in the commercial
482 production of IFFs and on studies that report on producers and handlers' food safety knowledge,
483 attitudes, and practices. Byakiya et al., (2019) noted that processors of *Obushera*, a fermented
484 beverage widely consumed in Uganda, showed good food safety knowledge but observed poor
485 hygienic practices. Amoa-Awua et al., (2007) reported significant improvements in the safety
486 characteristics of *kenkey* after the implementation of a HACCP and GMP system. However, they
487 noted that the level of formal education of processors was a significant hurdle when applying quality
488 assurance systems in IFF production. After observing poor manufacturing and hygienic processes
489 during the production of *ice kenkey* (Atter et al., 2015), researchers developed a simplified manual
490 to train *ice kenkey* processors in GMP & HACCP principles. These studies highlight the need for more
491 extension work, including training on basic food safety and hygiene, GMP, and HACCP, for food
492 producers and handlers.

493 Assessing health risks to consumers from IAFFs requires more and better-quality data to underpin
494 quantitative exposure assessments. There is a scarcity of research studies that focus on the
495 microbiological safety of African IFFs. In some cases, the observation of pathogenic or indicator
496 bacteria are incidental, occurring where investigation of the fermented product's microbial
497 community is the main objective (Anyogu et al., 2014; Oranusi et al., 2015; Parkouda et al., 2010).
498 This means that beyond identification, important information such as microbial load, virulence
499 factors, or antimicrobial resistance determinants are not investigated and recorded.
500 Another constraint in the quality of data collected is study design. Some reports do not include the
501 number of samples collected to support estimating prevalence or utilised convenience sampling,
502 which may not be representative. Many reports rely on conventional techniques for identifying
503 foodborne pathogens, which may be misleading or do not provide sufficient information, e.g.,
504 species identification. While the presence of potential pathogens in a food product is a cause for
505 concern, health risks associated with consumption must consider any national, regional, or
506 international safety standards, such as the Codex Alimentarius. Except for a few exceptions (Byakika
507 et al., 2019; Kouame et al., 2013; Gran et al., 2002), these standards are often not referred to
508 indicate the microbiological quality of the food being studied.
509 Antibiotic resistance of microorganisms has not been extensively studied in African IFFs. A significant
510 AR data gap also exists in African clinical settings. In a systematic analysis, Tadesse et al., (2017)
511 reported that AR data was not available for as many as 40% of African countries. The analysis
512 concluded that resistance to commonly prescribed antibiotics was prevalent, and the quality of
513 microbiological data is of serious concern. In the absence of rigorous government surveillance and
514 regulation for IFF production in many parts of Africa, more studies will need to be carried out by the
515 scientific community to raise the necessary awareness required and monitor prevalence trends,
516 especially of acquired AR.
517 Many of the technologies required to investigate IFFs and produce the innovation needed in the
518 sector remain inaccessible to many researchers in low and middle-income countries (LMICs) due to

519 resource constraints in technical know-how and infrastructure. This limits opportunities to publish in
520 more impactful journals, reducing the visibility of the outputs obtained, despite their importance to
521 the scientific community. There is a need for more initiatives that support sustainable international
522 collaborative efforts that bring together scientists in LMICs and High-income countries to share
523 expertise and develop equitable capacity building research activities. An example of this is the
524 ENRECA/DANIDA project, “Capability Building for Research and Quality Assurance in Traditional Food
525 Processing in West Africa”, which has supported several successful collaborative research efforts in
526 value-added processing of IFFs in some West African countries.

527 Research innovation must translate into improvements in IFF processing technology. This requires
528 significant investment in stakeholder management between policymakers, scientists, and producers.
529 Public sector funding could target knowledge transfer partnerships between research institutions
530 and small-medium- enterprise (SME) producers, which must benefit both partners. Research
531 projects can be designed in collaboration with SMEs, focusing on the real-world problems
532 encountered by producers (Moodley et al., 2019). This model can contribute towards the buy-in of
533 producers, required to drive change along the food processing value chain.

534 Weak enforcement of food safety regulations poses risks to public safety. Additionally, surveillance
535 systems that should provide accurate and reliable data on the burden of foodborne illness in IFFs are
536 often inadequate. This means that the number of illnesses or outbreaks associated with IFFs may go
537 unreported. In 2012, an outbreak of botulism in Canada was linked to an African fermented fish
538 product, fesikh, which led to a voluntary withdrawal from sale by the manufacturer (Walton et al.,
539 2014). Many public health agencies across Africa have limited access to the required infrastructure
540 to gather epidemiological evidence to support this type of timely action.

541 ‘One Health’ is a transdisciplinary approach introduced by the WHO as a framework to be used by
542 relevant stakeholders in developing and implementing strategies to safeguard public health (WHO,
543 2017c). The African Center for Disease Control (Africa CDC) has recently published a ‘One Health’
544 framework for managing zoonotic infections (Africa CDC, 2020). However, food safety and

545 antimicrobial resistance are also important priority areas for public health, requiring urgent
546 attention in the African region. At a national level, policymakers also need to empower regulatory
547 agencies with the required legal frameworks and infrastructure to develop and enforce food safety
548 standards.

549 **7. Conclusion**

550 Indigenous fermented foods have great potential in combating food insecurity in Africa and harbour
551 a vast genetic potential of valuable undiscovered strains. To achieve the goal of improving and
552 scaling up fermentation technology, the use of advanced molecular biology tools, including whole-
553 genome sequencing, is required to accurately identify the microbial community in IFFs, both
554 beneficial and harmful. A comprehensive understanding of the microbial community of IFFs could
555 identify biomarkers for assessing the quality and safety attributes of these foods, including
556 technological characteristics, virulence factors and antibiotic resistance.

557 The presence of pathogenic & AR bacteria in ready to eat IFFs constitute a risk to public health.
558 Harmful bacteria may enter the food chain via the raw material, inadequate fermentation to lower
559 the pH sufficiently in lactic fermented foods or post-processing contamination. The production of
560 antimicrobial compounds by fermenting organisms may be insufficient to eradicate pathogenic
561 organisms in the final product, and they are not substitute for GMP. Hygiene improvement in
562 handling raw food, increased surveillance, and uniform protocols for sampling and identification is
563 suggested to help evolve a common approach to studies of indigenous foods.

564 Any strategies to guarantee IFFs free of microbial hazards require considerable investment and
565 collaboration from relevant stakeholders – consumers, producers, industry, policymakers, and
566 scientists.

567 **Acknowledgements**

568 The authors are grateful for the support of the Nigerian Applied Microbiologists network in
569 developing this collaborative effort.

570 **Funding**

571 This research did not receive any specific grant from funding agencies in the public, commercial, or
572 not-for-profit sectors.

573 **Author's contributions**

574 Amarachukwu Anyogu: Conceptualisation, Data Collection, Formal Analysis, Writing - Original draft
575 preparation, Project Administration. Ogueri Nwaiwu: Conceptualisation, Data Collection, Writing –
576 Original draft preparation, Writing – Review and Editing, Supervision. Ayomide Olukorede: Data
577 Collection, Writing – Original draft preparation. Writing – Review and Editing. Christian Anumudu:
578 Data Collection, Writing – Original draft preparation. Helen Onyeaka: Data Collection, Writing –
579 Review and editing. Esther Areo: Data Collection, Writing – Original draft preparation. Obadina
580 Adewale: Writing – Review and editing, Supervision. All authors read and approved the final
581 manuscript.

582 **Declarations of interest:** None

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