1 2 Original Research Article 3 2 MICROBIAL ASSESSMENT OF SELECTED, LOCALLY 4 FERMENTED AND READY-TO-EAT CASSAVA PRODUCTS SOLD 5 IN LOKOJA, NIGERIA.

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8 ABSTRACT

9 This study was conducted to assess locally-fermented, ready-to-eat cassava products in Lokoja for microbial contamination. Sixty samples 10 comprising; twenty white garri, twenty yellow garri and twenty fufu were 11 subjected to microbial analysis. Samples were serially diluted to 10-4 and 12 appropriate dilutions inoculated by spread plate method unto Nutrient agar, 13 MacConkey agar and Potato Dextrose agar plates for Total aerobic plate 14 15 count (TAPC), Coliform count (CC) and Fungal count respectively. The 16 TAPC for white garri ranged from 1.0 x 101 to 7.0 x 103, the coliform count ranged from no growth (NG) to 7.1 x 103, while the mean fungal count 17 ranged from 1.0 × 102 to 3.0 × 103. The TAPC for yellow garri ranged from 18 1.1 x 102 to 9.0 x 103, the coliform count ranged from NG to 6.0 × 103 and 19 20 the fungal count ranged from 1.0 x 102 to 3.0 x 103. The TAPC of fufu ranged from 1.2 x 101 to 5.0 x 103, the coliform count ranged from NG to 21 22 3.0 x 103 and the fungal count ranged from 1.0 × 102 to 7.0 × 102. The bacteria isolated include Bacillus spp., Enterobacter spp., Pseudomonas 23 spp., Staphylococcus aureus, salmonella spp., Escherichia coli and 24 Klebsiella spp. The fungi isolated from the study samples include 25 Aspergillus niger, Cladosporium spp., Fusarium spp., Rhizopus spp., 26 Alternaria spp., Montospora spp., and Penicillium spp. The pH of the 27 samples ranged from 4.02 to 4.96 in white garri, 4.02 to 4.99 in yellow garri, 28 and 5.02 to 6.44 in fufu. Findings show that these widely consumed 29 fermented (ready-to-eat) cassava products presents (may represent) a 30 serious risk and route for transmission of food borne pathogens to 31 32 consumers and generally huge economic disadvantage to food handlers. 33 Improved manufacturing, packaging and storage practices in garri production and for public health purposes are strongly encouraged. 34

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40 1 Introduction

Food is the basic necessity for human survival and attainment of food security is the priority of any country. However it is important that food security should not be seen only in the perspective of availability either quantitatively or qualitatively. Therefore, food hygiene and safety should also be given important consideration in order to protect the health of the people as they could serve as vehicle for the transmission of food borne diseases [1] [2]. Food may be available but the source from which it is produced and / or processed may be unhygienic and even the chemicals that may be used to preserve it may cause serious health hazard [1].

Coliforms, particularly Escherichia coli are used as indicators of post 50 process contamination and also the presence of E. coli in foods serves as 51 an indicator of faecal contamination [3]. Coliforms are group of closely 52 53 related Gram negative, non-spore forming, rod-shaped aerobes and 54 facultative anaerobes that ferment lactose to produce acid and gas within 48 h at 35°C. They are mostly harmless and lives in soil, water and in the 55 gut of animals with few enteric pathogens including Salmonellae, Shigellae 56 57 and enteropathogenic E. coli [4].

58 Filamentous moulds and yeasts are common spoilage organisms of food 59 products and some species of *Penicillia* and *Aspergilli* have been reported 60 as spoilage organisms of a variety of foods on which they may produce a 61 quite number of mycotoxins [5].

Cassava ranks fourth in the list of major crops in developing countries after rice, wheat and maize and it is used for the production of a variety of West African foods [6]. In its natural state, it is toxic to man as it may contain high levels of linamarin, a cyanogenic glucoside. Hence, processing through fermentation, enhance its detoxification, improving the quality and hygienic safety of the food.

Garri and fufu happen to be one of the finished products of fermented 68 cassava and if not properly and carefully handled during processing and / 69 70 or storage, it could serve as vehicle for transmission of food borne pathogens. Moreover, there could be economic losses and widespread of 71 food borne illnesses as a result of contamination by these microorganisms. 72 73 Therefore, this study is aimed at evaluating locally fermented ready-to-eat cassava products (garri and fufu) for any microbial contamination and 74 microbiological safety with attempt at awareness creation on food safety to 75 76 consumers and relevant additions to the body of knowledge on this all important staple foods in order to inform right agricultural and health 77 policies. Furthermore, the obtained result will aid policy makers in making 78 79 necessary quality hazard, storage techniques as well as processing line crucial for the production of fermented staple food from cassava in Nigeria 80 81 and West Africa at large.

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86 2 Materials and Methods

87 2.1 Sample collection

88 A total of 60 samples of locally-fermented, ready-to-eat cassava products,

89 comprising 20 white garri, 20 yellow garri and 20 fufu were collected

aseptically from the five major markets in Lokoja Kogi state which includes;
Ganaja, Adankolo, Lokongoma, Old and New International Markets. In
each of the markets, four samples (i.e. from 4 vendors) of each of the
ready-to-eat locally fermented cassava products were collected in sterile
nylon bags and transported to Salem University Advanced Microbiology
Laboratory for analyses.

96 **2.2 Microbiological Analysis.**

Microbiological analysis was carried out using conventional microbiological
procedures. The analysis involved total aerobic plate count, fungal count
and coliform count. This was determined by the spread plate method using
standard microbiological techniques.

101 **2.2.1 Isolation and Identification of Microbial Isolates**

102 Ten gram (10g) of each sample were homogenized in 90 ml sterile distilled water (10⁻¹ dilution). Serial dilution of sample homogenate to 10⁻⁴ was 103 carried out also in sterile distilled water for colony count. Approximate 0.1ml 104 105 aliquot of appropriate dilutions were spread plated on plates of Nutrient 106 agar and Potato Dextrose agar supplemented with 0.2µg of 107 chloramphenicol (all from Bio-laboratory, Hungary). All Nutrient agar and 108 MacConkey agar plates were incubated at 37^oC for 24-48 h while all potato dextrose agar plates where incubated at 25°C for 72-120 h. All plates were 109 110 prepared in duplicate. Culture plates were examined, while enumeration and identification of colonies was carried out at the end of the incubation 111 112 period. The total microbial population was expressed as colony forming unit 113 per gram of the sample (cfu/g).

114 2.2.2 Detection of Hygiene indicator organisms and specific food 115 borne pathogens

Samples were plated on MacConkey agar, Manitol Salt agar and
Salmonella-Shigella agar (Oxoid, England) after pre-enrichment in Selenite
F broth and incubated at 35°C for 24-48 h, for isolation of Escherichia coli,
Staphylococci and Salmonellae respectively.

120 2.2.3 Coliform Test

One gram samples were also inoculated into Lactose broth in screw 121 capped test tubes with inverted Durham tubes and incubated at 37°C for 122 123 24-48 h. Tubes showing gas production and/or color change of dye were 124 reported as presumptive coliform test positive. These positive tubes were 125 streaked out on duplicate plates of Eosin Methylene Blue (EMB) agar for confirmatory test and incubated at 37°C and 44°C respectively for 24 h. 126 127 Growth of characteristic colonies on EMB medium represent confirmatory positive test which were Gram stained and inoculated into lactose broth for 128 complete coliform test. Gas production and/or color change of dye plus 129 Gram negative non spore bearing rod represent presence of coliform. 130

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133 2.2.4 Isolation and Identification of bacteria and fungi

Pure cultures of suspected colonies were obtained by repeated subcultureon nutrient agar plates and potato dextrose agar plates for bacteria and

fungi isolates respectively and stored on slants at 4°C until characterized.
The isolation and identification of the bacteria were carried out using
standard microbiological techniques including; Gram stain, catalase test,
coagulase test, indole test, citrate test, oxidase test [7]. All fungi isolates
were identified following previously described methods [8].

141 2.3 Determination of pH of the locally fermented ready-to-eat cassava 142 products

The pH was determined using digital pH meter calibrated with standard buffer solutions.Ten grams of each sample were weighed and homogenized in 20ml of sterile distilled water in a beaker for 1 min. The solution decanted and the pH of the suspension measured.

147 **2.4 Statistical analysis**

The mean of the total viable microbial count was subjected to analysis using SPSS version 20. The mean microbial load cfug⁻¹ and pH of the samples were presented in tables.

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154 3 Results and Discussion

The mean TAPC, fungal count, and coliform count results are shown in Table 3, and it reveals the samples had TAPC that are within the range of

10¹ to 10³. The fungal and coliform counts are about the same order. The 157 result of this study was in line with the earlier reported data [9] [10] 158 159 however, it was slightly lower than another previously reported [11], with counts that ranged from 10³ to 10⁴. Moreover a study conducted in Ebonyi, 160 Ogun and Ovo states in Nigeria reported microbial burden as high as 10⁶ 161 162 to 10⁷ respectively [12] [13], while another related work reported a fungal count as high as 10⁴ to 10⁶ [14]. The disparity in the microbial count from 163 164 these studies could be as a result of the processing method, the quality of water used in the production process and the length of exposure during 165 sale. A researcher equally observed back slopping used by some 166 167 processors to reduce the length of time for fermentation to compromise the 168 quality of the product [13], suggesting it could be the lead cause of high counts observed in their study. Meanwhile, the total aerobic plate count 169 170 and fungi counts of this study samples were within the acceptable limit. Ready-to-eat foods with plate counts of $\leq 10^3$ are within the acceptable limit 171 while counts of 10^4 to 10^5 are tolerable and counts that are $>10^6$ are totally 172 unacceptable [15]. Coliform was detected in most of the samples at high 173 counts 10² to 10³, and the presence of *E. coli* calls for serious concern. It 174 signifies poor sanitary condition and indicative of faecal contamination 175 176 during the production process and / or storage of the fermented ready-to-177 eat food under study. It is also indicative of the potential presence of 178 enteric pathogens and therefore makes the study samples of poor quality 179 for human consumption.

Bacterial isolates from the study samples were *Bacillus* sp., *Enterobacter*sp., *Pseudomonas* sp., *Staphylococcus aureus, salmonella* sp.,

Escherichia coli and Klebsiella sp. (Table 1). Most of the isolates were 182 glucose positive, indole negative, catalase positive and a reasonable 183 184 number of both Gram positives and Gram negatives (Table 4). The isolation of diverse microbial species from this ready-to-eat fermented 185 186 foods did not completely agree with the earlier findings [9] [10] [13] as each 187 author had dissimilarities in bacterial presence in their study samples. Most 188 of these studies reported the presence of Staphylococcus aureus, Bacillus 189 sp., Pseudomonas sp., E. coli and Klebsiella sp. The observation of 190 diverse bacteria isolates could be attributed to the fact that these studies 191 was carried out at different regions and from different sample markets of 192 which environmental conditions of the study areas could affect the 193 distribution of organisms. Buyer's attitude towards the exposed food products in the market could also contribute to the microbial load and 194 diversity as they touch the products with bare hands and taste it before 195 196 they buy. The presence of Salmonella in this study calls for concern as this organism is the common cause of human food poisoning, and 197 198 salmonellosis can affect all species of domestic animals and man. It is 199 important to draw to our attention that the young, aged, stressed, debilitated and pregnant individuals are more susceptible while the 200 201 immunosuppressed and those suffering from malnutrition are at risk for 202 salmonella infection [16]. The presence of Bacillus and Staphylococcus 203 aureus also calls for concern because some strains of these organisms are 204 known to be toxigenic and often implicated in food borne intoxication [17] 205 [18]. Bacillus a common environmental contaminant and a spore former 206 can withstand environmental stress and this may account for its presence in the samples. Meanwhile, *Staphylococcus aureus* is of human origin and their presence could therefore be from the food handlers, utensils and the environment. Moreover, garri and fufu is a common food widely consumed by all in Nigeria and increasing intake of it, especially dry garri as snacks or with cold water is an added practice that exposes the populace to serious health risk due to the microbial status of the product.

Fungal isolates from the samples collected were detailed in Table 2. More 213 214 so, among all fungal isolates; most authors reported the presence of Aspergillus sp., Fusarium sp. and Penicillium sp. in their study [14] [9] [10], 215 216 which agrees with the result of this study. Filamentous fungi are common environmental contaminants usually implicated in ready-to-eat foods 217 because they produce spores and this could explain their presence in the 218 219 study samples. More so, species of Aspergillus, Penicillium and Fusarium are known to produce mycotoxins [19] [20] [21] and their presence in the 220 221 study samples calls for serious concern.

222 The pH value of white garri and yellow garri ranged from 4.02 to 4.96 and 4.02 to 4.99 respectively while that of fufu ranged from 5.02 to 6.44 (Table 223 224 3). It was observed from the study that the pH values of fufu were higher 225 than that of the garri samples. The pH values recorded in this study was within the range of those reported in related studies [9] [10] [13] for the 226 227 garri samples. Moreover, a study conducted in Ebonyi state, Nigeria 228 reported higher pH values of 5.47 to 6.61 [12] which is in disagreement 229 with this study. This disparity observed could be attributed to the length of fermentation and storage time. A study reported a reduction in pH of 230

cassava mash from 6.2 to 3.2 over a period of 10 fermentation days under
ambient temperature of 28 – 32°C [22]. [14] It was also revealed from a
study that the longer the storage time, the higher the pH. Another study
attributed the increase in pH to be as a result of production of acidic
metabolites by microorganisms during their growth and proliferation [23].

236 Conclusion

237 This study has shown that the fermented staple food under study were contaminated with both bacterial and fungal species, with presence of 238 239 coliforms. Some of the isolated organisms are well-known causes of food 240 borne diseases and food intoxications. With these findings, the important of hygiene during processing and/or storage of these food products cannot be 241 242 overemphasized. It is also important that the food handlers properly covers the food product during storage and also reduce the length of its exposure 243 in the market place. The use of specific starter culture, effective HACCP 244 245 application and good manufacturing practice from farm to fork is also 246 suggested to help reduce the level of contamination and possibly ward off food borne pathogens. 247

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Market	Sample	Salm	Kleb	Staph	Bacillus	E.coli	Strept	Pseudo	Entero
Adankolo	White	+	+	+	-	-	-	-	-
	Yellow	-	-	+	+	-	-	-	-
	Fufu	-	+	+	-	-	-	+	-
Lokongoma	White	+	-	-	-	+	-	-	-
	Yellow	+	-	+	+	-	-	-	-
	Fufu	-	-	+	+	-	-	-	-
New	White	-	-	+	-	+	+	-	-
	Yellow	+	-	+	-	+	-	-	-
	Fufu	-	+	+	-	-	+	-	-
Old	White	+	-	+	+	+	-	-	-
	Yellow	-	-	+	-	+		-	+
	Fufu	+	-	+	-		+	-	-
Ganaja	White	-	-	+	-	+	-	-	-
	Yellow	-	-	+	- 0	+	-	-	-
	Fufu	-	-	+	7 7	-	-	+	-

Table 1: Distribution of bacteria in the food samples across the sampled markets

333 Key: Salm = Salmonella sp., Kleb = Klebsiella sp., Staph = Staphylococcus aureus,

334 **Pseudo = Pseudomonas sp., Entero = Enterococcus sp., + = present, - = absent**

Table 2: Distribution of fungi in the food samples across the sampled markets

Market	Sample	Asper	Peni	Mold	Mucor	Rhi	Fus	Clado	Alter	Monto
Adankolo	White	+	-	-	-	-	-	-	-	-
	yellow	+	+	+	-	-	-	-	-	-
	Fufu	-	+	-	-	-	-	-	-	-
Lokongoma	White	-	+	-	+	-	-	-	-	-
	yellow	-	-	-	-	+	-	-	-	-
	Fufu	-	-	-	-	-	+	-	-	-
New	White	-	+	+	+	-	-	-	-	-
	yellow	-	-	+	+	-	+	-	-	-
	Fufu	-	+	-	-	+	-	-	-	-
Old	White	-	-	-	+	+	-	-	-	-
	yellow	-	-	-	-	-	-	+	+	-
	Fufu	-	-	-	-	-	+	-	-	-
Ganaja	White	+	-	-	-	-	+	-	-	-

		yellow	-	-	-	+	-	-	-	-	+
		Fufu	-	-	-	+	-	-	-	-	-
337	Key: Aspe	r = Asper	gillus sp	o., Peni	= Penic	illium sp	., Rhi	= Rhi	zopus s	p., Fu	s =
338	Fusarium s	sp., Clado =	Clados	porium	sp., = Al	ter = Alter	maria s	sp., Mo	nto = Mo	ontosp	ora
339	sp., + = pre	esent, - = al	osent.								
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348 Table 3: Mean microbial load in cfu/g and pH

Sample		White garri				Yellow				Fufu			
Outlet		TAPC				Garri							
			CC	FC	рН	TAPC	CC	FC	рН	TAPC	CC	FC	рН
Adankolo	1	3.2 x 10 ²	2.0 x 10 ³	4.0 x 10 ²	4.20	2.0 x 10 ³	1.1 x 10 ³	3.0 x 10 ²	4.34	1.3 x 10 ²	2.0 x 10 ³	4.0 x 10 ²	6.23
	2	1.0 x 10 ¹	3.0 x 10 ³	3.0 x 10 ²	4.66	4.2 x 10 ²	1.0 x 10 ³	8.0 x 10 ²	4.83	2.3 x 10 ²	4.0 x 10 ²	7.0 x 10 ²	6.44
	3	3.0 x 10 ²	2.7 x 10 ³	3.0 x 10 ²	4.64	3.8 x 10 ²	2.0 x 10 ³	3.0 x 10 ²	4.02	3.0 x 10 ²	4.0 x 10 ²	2.0 x 10 ²	6.02
	4	1.8 x 10 ¹	3.0 x 10 ³	4.0 x 10 ²	4.91	2.0 x 10 ³	4.0 x 10 ²	4.0 x 10 ²	4.33	3.0 x 10 ²	NG	5.0 x 10 ²	5.93
Lokongoma	1	1.8 x 10 ²	NG	4.0 x 10 ²	4.36	3.2 x 10 ²	4.0 x 10 ²	7.0 x 10 ²	4.54	2.0 x 10 ²	1.4 x 10 ³	1.0 x 10 ²	5.64
	2	2.5 x 10 ²	2.7 x 10 ³	3.0 x 10 ²	4.43	3.1 x 10 ²	NG	2.0 x 10 ²	4.30	4.0 x 10 ²	3.4 x 10 ²	4.0 x 10 ²	5.52
	3	9.6 x 10 ²	2.0 x 10 ³	3.0 x 10 ²	4.22	4.7 x 10 ²	1.4 x 10 ³	5.0 x 10 ²	4.49	1.6 x 10 ¹	2.0 x 10 ³	4.0 x 10 ²	5.75
	4	1.5 x 10 ²	2.0 x 10 ³	6.0 x 10 ²	4.11	3.1 x 10 ²	3.4 x 10 ²	1.0 x 10 ³	4.67	1.2 x 10 ¹	3.0 x 10 ³	4.0 x 10 ²	5.46
New	1	7.0 x 10 ³	NG	3.0 x 10 ³	4.40	1.3 x 10 ²	6.0 x 10 ³	4.0 x 10 ²	4.52	3.2 x 10 ²	2.7 x 10 ³	3.0 x 10 ²	5.62
	2	1.0 x 10 ¹	7.0 x 10 ³	4.0 x 10 ²	4.43	1.6 x 10 ²	1.0 x 10 ³	6.0 x 10 ²	4.23	1.0 x 10 ³	3.0 x 10 ³	3.0 x 10 ²	5.41
	3	3.8 x 10 ²	2.0 x 10 ³	3.0 x 10 ²	4.59	9.0 x 10 ³	8.0 x 10 ²	9.0 x 10 ²	4.12	8.8 x 10 ²	NG	6.0 x 10 ²	5.03
	4	1.2 x 10 ²	3.0 x 10 ³	4.0 x 10 ²	4.17	1.2 x 10 ²	2.0 x 10 ²	4.0 x 10 ²	4.04	3.0 x 10 ³	2.7 x 10 ³	3.0 x 10 ²	5.14
Old	1	1.0 x 10 ³	8.0 x 10 ²	4.0 x 10 ²	4.08	1.1 x 10 ²	2.0 x 10 ²	5.0 x 10 ²	4.50	1.0 x 10 ³	2.0 x 10 ³	4.0 x 10 ²	6.35
	2	1.2 x 10 ²	7.1 x 10 ³	3.0 x 10 ²	4.87	1.5 x 10 ²	3.0 x 10 ²	9.0 x 10 ²	4.12	1.2 x 10 ²	2.0 x 10 ³	3.0 x 10 ²	6.23
	3	1.3 x 10 ²	3.0 x 10 ²	4.0 x 10 ²	4.96	1.6 x 10 ²	NG	5.0 x 10 ²	4.73	1.5 x 10 ²	3.0 x 10 ²	1.0 x 10 ²	6.10
	4	6.0 x 10 ³	NG	4.0 x 10 ²	4.15	5.0 x 10 ³	1.0 x 10 ²	1.0 x 10 ²	4.94	2.0 x 10 ³	NG	6.0 x 10 ²	6.06
Ganaja	1	1.2 x 10 ²	2.0 x 10 ²	3.0 x 10 ²	4.96	1.8 x 10 ²	4.2 x 10 ³	4.0 x 10 ²	4.56	1.1 x 10 ²	2.0 x 10 ²	1.0 x 10 ²	5.64
	2	1.9 x 10 ²	2.0 x 10 ²	1.0 x 10 ²	4.02	1.3 x 10 ²	3.1 x 10 ³	3.0 x 10 ³	4.15	1.5 x 10 ²	2.0 x 10 ³	3.0 x 10 ²	5.43
	3	1.5 x 10 ²	NG	6.0 x 10 ²	4.14	1.2 x 10 ²	1.2 x 10 ²	5.0 x 10 ²	4.77	1.6 x 10 ²	8.0 x 10 ²	1.0 x 10 ²	5.02
	4	2.0 x 10 ²	NG	1.0 x 10 ²	4.25	5.0 x 10 ³	NG	1.2 x 10 ²	4.99	5.0 x 10 ³	2.0 x 10 ²	6.0 x 10 ²	5.13

350 Key: TAPC = Total Aerobic Plate Count, CC = Coliform Count, FC = Fungal Count, NG = No Growth.

Table 4: Biochemical characteristics of bacterial isolates

Bacteria found	Gram stain	H ₂ O ₂	Glucose	Citrate	Indole	Endospore	Oxidase	Coagulase	Catalase
Salmonella sp.	-		+	-	-	-	-	-	+
Klebsiella sp.	-		+	+	-	-	197-	-	+
S. aureus	+		+	+	-	-		+	+
Bacillus sp.	+		+	+	-	+	\mathbf{X}		+
E. coli	-		+	-	+	-		-	+
Streptococcus sp.	+		+					-	-
Pseudomonas sp.	-	-	-	+	-	VV	+	-	+
Enterococcus sp.	+	-	+	-	-	-	-	-	-

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- 378