

Original Research Article

MICROORGANISMS ASSOCIATED WITH THE PRODUCTION OF A NIGERIAN FERMENTED BEVERAGE, 'AGADAGIDI'

ABSTRACT

Aim: The microbial types, occurrence, loads and interactions were studied during the production of a Nigerian fermented beverage, 'Agadagidi', from overripe plantains.

Place and Duration of Study: Federal University of Technology Akure, Ondo State, Nigeria. March- July, 2012.

Methodology: Isolation, enumeration and identification of bacteria and fungi were carried out by using standard pour plate, morphological, biochemical and physiological characterization methods. Antagonistic and mutualistic interactions among the microorganisms were investigated using agar well diffusion method.

Results: *Bacillus subtilis*, *B. megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* species, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *L. fermentum*, *Leuconostoc mesenteroides*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium notatum*, *Trichoderma viridea* *Saccharomyces cerevisiae*, *Candida utilis* and *Geotrichum* species were identified during the production of the beverage. The loads of bacteria (TB), Lactic Acid Bacteria (LAB), enterobacteriaceae and fungi of the uncrushed plantain pulp were $2.6 \times 10^7 \pm 0.05$ cfu/ml, $6.7 \times 10^6 \pm 0.05$ cfu/ml, $3.8 \times 10^5 \pm 0.05$ cfu/ml and $2.0 \times 10^7 \pm 0.05$ sfu/ml. At 0 hour of fermentation, the loads of TB, fungi and enterobacteriaceae increased. Then after, the TB, enterobacteriaceae and fungi counts decreased to $2.0 \times 10^5 \pm 0.11$ cfu/ml, $1.3 \times 10^5 \pm 0.11$ cfu/ml and $1.03 \times 10^5 \pm 0.05$ sfu/ml respectively. In contrast, the LAB cell number increased to $8.6 \times 10^7 \pm 0.1$ cfu/ml at the end (48 hours) of fermentation. The level of the microbial occurrence was 25 to 100% with *B. subtilis*, *L. plantarum*, *L. mesenteroides*, *S. cerevisiae* and *C. utilis* occurring as the highest. *B. megaterium*, *E. spp.*, *A. niger* and *T. viridea* occurred least. There was positive co-existence between Yeast and LAB. The yeasts and LAB exhibited antagonism against other bacteria.

Conclusion: The data obtained in this work has shown some functional microflora and their relationship during the production of "Agadagidi". This information can contribute to a better understanding of the "Agadagidi" production process for a consistent quality beverage.

Keywords: Microbial community; “Agadagidi” a Nigerian Fermented beverage; Microbial loads; Antagonism.

1. INTRODUCTION

Plantain (*Musa sapientum* var. *Paradisiacal* Linn) is one of the most important staple food crops for millions of people both in developed and developing countries. It is one of the foods commonly consumed in the West Africa sub-region, Northern America, Mexico and the Caribbean. In Nigeria, its consumption cuts across the indigenous groups and the numerous socio-economic classes because of the ease of preparation and consumption [1]. In West and central Africa, more than 10 million tons are produced annually and are traded locally [2, 3].

“Agadagidi,” a cloudy effervescent sweet-sour taste typical African traditional alcoholic beverage is made from overripe bananas and plantains through fermentation. It is common in south-western part of Nigeria [4].

The techniques used in the production of wines from tropical fruits are similar to those of grape wine production which include pressing out the juice, fermenting, maturing and bottling [5]. Fermentation leads to changes in appearance of food which is characterized by different reactions of microorganisms [6]. Food fermentation represents one of the oldest known uses of biotechnology. Fermented foods and beverages forms a significant proportion of all diets worldwide; they are typically about one-third of the foods consumed globally. Fermentation of foods covering a wide range of microbial and enzymatic processing of foods and ingredients is used to achieve desirable characteristics such as prolonged shelf-life, improved safety, attractive flavour, nutritional enrichment and promotion of health [7, 8]. The fermentation of overripe plantain to produce “Agadagidi” is a waste prevention processing of plantain. Plantain is a perishable crop which has much less value when it is overripe; hence it is used for wine production [9-11].

At present, there is no adequate information on the spectrum of microorganisms and microbial interaction associated with the production of overripe plantain pulp to yield ‘Agadagidi’. Microbial information on the production of “Agadagidi” will contribute to the development of starter cultures with predictable characteristics, for use in small-scale and commercial production of stable and consistent quality ‘Agadagidi’. Therefore, this research was proposed to reveal the microbial community and confirmation of mutualism or commensalism and antagonistic interaction during the production of “Agadagidi”.

2.0 MATERIALS AND METHODS

2.1 Traditional Preparation of “Agadagidi” sample

The production of “Agadagidi” was done in the laboratory based on the local or indigenous method. The plantains used were bought from the king’s market, Akure, Ondo State. The Overripe plantain pulps peels were washed in tap water to remove debris and dirt. The plantain was peeled and the pulps were crushed in portable tap water at a ratio of 1:5 (w/v) in a clean container, covered and left to ferment for 2 days at ambient temperature of $27\pm 2^{\circ}\text{C}$. Samples were withdrawn from peeled, uncrushed pulps and at 0 h, 24 h and 48 h of fermentation. The fermented liquid was filtered with a clean muslin cloth to remove the plantain mashes. The liquid then served as “Agadagidi”.

2.2 Enumeration and Isolation of Microorganisms

Serial dilutions (1:10 v/v) were made with the samples collected and pour plated on nutrient agar for mesophilic bacteria, Man Rogosa Sharpe agar at pH 5.5 for lactic acid bacteria (LAB), Malt Extract Agar (supplemented with streptomycin sulphate) for yeasts, Eosin Methylene Blue for the isolation of enterobacteriaceae. Incubation was carried out at 37°C for total bacteria and LAB and 27°C for fungi. Colonies and spore forming units formed on the media were counted and subcultured. Cultural, microscopic and biochemical characteristics of the isolates were determined according to the methods of [12-15].

2.3 Determination of Positive and Negative Microbial Interactions between the Isolates

Mutualism/commensalism and antagonism between the microbial isolates were determined using Muller Hilton agar and Agar Well Assay method with slight modification as described by [16].

2.4 Statistical analysis

Data obtained were subjected to Analysis of Variance (ANOVA) and separation of means was done with Duncan’s New Multiple Range Test at 95% confidence level using SPSS 16.0 version.

3.0 RESULTS

The microbial composition of ‘Agadagidi’ is made up of nine bacteria and eight fungi. The bacteria consist of two species of *Bacillus* and *Lactobacillus*, one species of *Staphylococcus*, *Enterococcus*, *Escherichia coli*, and *Leuconostoc*. *Pediococcus acidilactici* was also isolated. Among the fungi, three species of *Aspergillus*, one species of *Trichoderma* and *Penicillium* were the moulds isolated. *Geotrichum*, *Saccharomyces* and *Candida utilis* were the yeasts obtained (Table 1).

Table 1 also revealed the types of microbes associated with the “Agadagidi” production. *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Candida utilis* had the highest (100%) occurrence throughout the stages of production. *Bacillus megaterium*, *Enterococcus* spp., *A. niger* and *T. viridea* occurred lesser (25%) than other microorganisms.

Table 1: The occurrence of bacteria and fungi during the production of “Agadagidi”

Microorganism	Plantain Pulp (Uncrushed)	Fermentation: (hr)			Level of Occurrence (%)
		0	24	48	
<i>Bacillus subtilis</i>	+	+	+	+	100
<i>Bacillus megaterium</i>	+	-	-	-	25
<i>Staphylococcus aureus</i>	+	+	-	-	50
<i>Escherichia coli</i>	+	+	-	-	50
<i>Enterococcus</i> species	-	+	-	-	25
<i>Lactobacillus plantarum</i>	+	+	+	+	100
<i>Lactobacillus fermentum</i>	+	+	+	-	75
<i>Leuconostoc mesenteroides</i>	+	+	+	+	100
<i>Pediococcus acidilactici</i>	-	+	+	+	75
<i>Aspergillus flavus</i>	+	+	-	-	50
<i>Aspergillus niger</i>	+	+	-	-	50
<i>Aspergillus fumigatus</i>	+	+	-	-	50
<i>Penicillium notatum</i>	+	+	-	-	50
<i>Trichoderma viridea</i>	+	-	-	-	25
<i>Sacharomyces cerevisiae</i>	+	+	+	+	100
<i>Candida utilis</i>	+	+	+	+	100
<i>Geotrichum</i> species	+	+	+	-	75

Legend:

+ Present

- Absent

During the production of “Agadagidi”, the populations of bacteria, fungi and enterobacteriaceae followed the same trend. Their populations increased at 24 hr and decreased at 48 hours of fermentation respectively (Fig. 1, 2 and 3). The pattern of lactic acid

bacteria load differs with decrease population at 24 hours and increase at 48 hours (Fig. 4). The total bacterial population of the overripe plantain pulp was $2.6 \times 10^7 \pm 0.05$ cfu/ml which increased before fermentation (0 hr) to $5.2 \times 10^7 \pm 0.05$ cfu/ml (Figure 1). This load decreased significantly to $2.0 \times 10^5 \pm 0.11$ cfu/ml at 48 hours of fermentation. Fungal load of $2.0 \times 10^7 \pm 0.05$ cfu/ml was obtained on the ripe plantain pulp which increased and decreased at 0 hr and 24 hr of fermentation to $2.2 \times 10^7 \pm 0.05$ cfu/ml and $4.3 \times 10^5 \pm 0.05$ cfu/ml respectively (Fig. 2). At the end (48 hr) of fermentation, the population of fungi was $1.03 \times 10^5 \pm 0.05$ cfu/ml. Figure 3 shows enterobacteriaceae load during the preparation of “Agadagidi.” The population was 2.8×10^7 cfu/ml at 0 hr of fermentation. There was a reduction in the enterobacteriaceae load to $1.3 \times 10^5 \pm 0.11$ cfu/ml at 48 hr of fermentation.

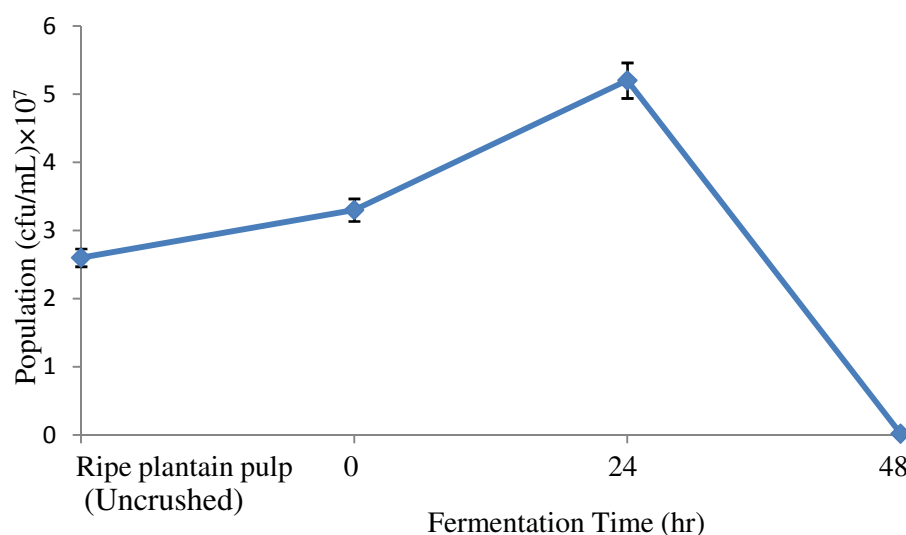


Fig. 1: Total bacterial load during production of “Agadagidi”

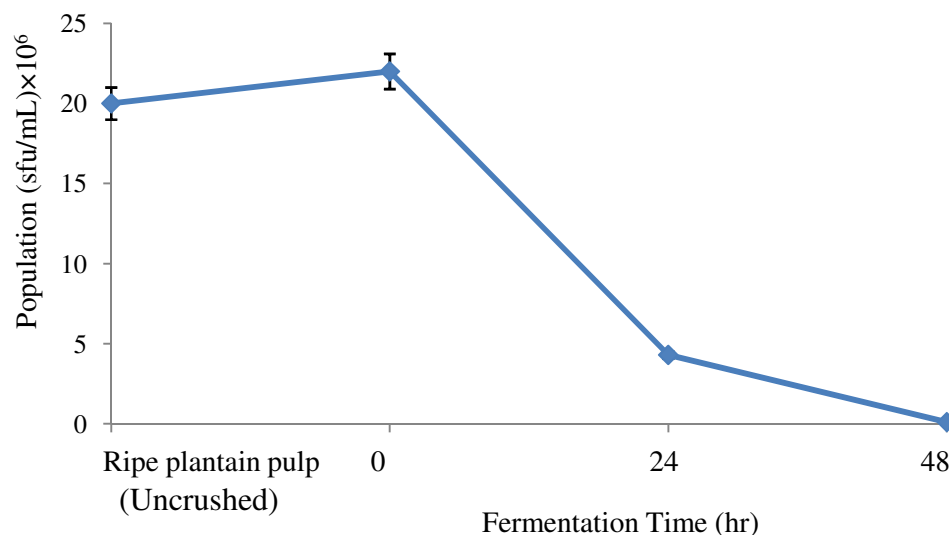


Fig. 2: Fungal load during the production of "Agadagidi"

Lactic acid bacteria had a load of $6.7 \times 10^6 \pm 0.05$ cfu/ml on the overripe plantain pulp (Fig. 4). The load reduced after 24 hr of fermentation to $4.2 \times 10^5 \pm 0.05$ cfu/ml and thereafter (48 hr) increased to $8.6 \times 10^7 \pm 0.1$ cfu/ml.

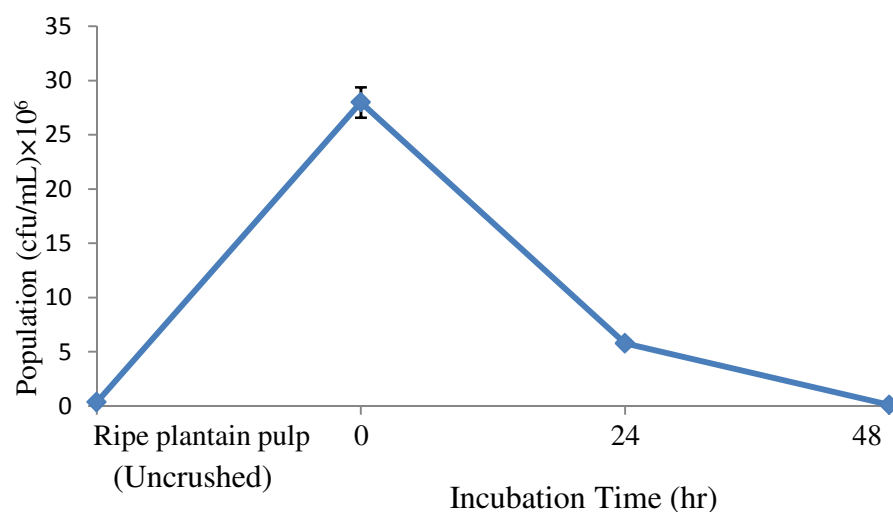


Fig. 3: Enterobacteriaceae load during the production of "Agadagidi".

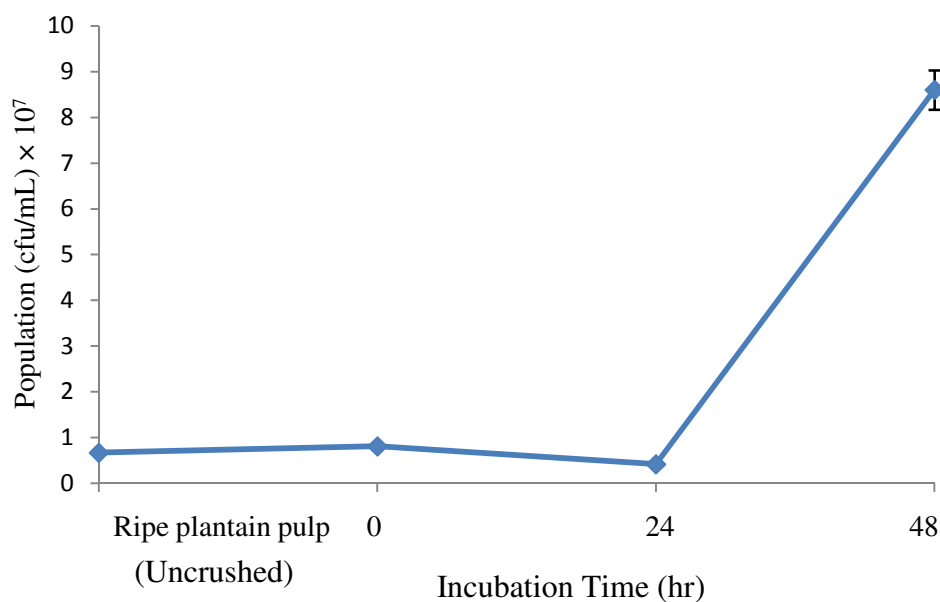


Fig. 4: Lactic acid bacteria load during the production of "Agadagidi"

Table 2: Interaction between Lactic acid bacteria, yeasts and some of the bacteria isolated during the production of "Agadagidi"

Microorganism (LAB and yeast)	<i>Enterococcus</i> spp.	<i>Lactobacillus</i> <i>plantarum</i>	<i>Bacillus</i> <i>subtilis</i>	<i>Lactobacillus</i> <i>fermentum</i>	<i>Escherichia</i> <i>coli</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Bacillus</i> <i>megaterium</i>	<i>Leuconostoc</i> <i>mesenteroides</i>	<i>Saccharomyces</i> <i>cerevisiae</i>	<i>Candida</i> <i>utilis</i>
<i>Leuconostoc</i> <i>Mesenteroides</i>	+	-	+	-	+	+	+	-	-	-
<i>Lactobacillus</i> <i>fermentum</i>	+	-	+	-	+	+	+	-	-	-
<i>Lactobacillus</i> <i>plantarum</i>	+	-	+	-	+	+	+	-	-	-
<i>Lactococcus</i> <i>lactis</i>	+	-	+	-	+	+	+	-	-	-
<i>Saccharomyces</i> <i>cerevisiae</i>	+	-	+	-	+	+	+	-	-	-
<i>Candida utilis</i>	+	-	+	-	+	+	+	-	-	-

Table 2 shows the interactions which occurred among the microorganisms. Lactic acid bacteria (LAB) and yeasts inhibited the growth of other bacteria but not LAB members. The

interaction between the yeasts and the lactic acid bacteria were positive as zone of inhibition was not created when they were co-cultured.

DISCUSSION

Many species of microorganisms were isolated during the production of “Agadagidi.” The cultural and biochemical properties exhibited by the microorganisms were similar to those described by [12-14] which in turn gave the names of the microorganisms in table 1. The 100% occurrence of *Leuconostoc mesenteroides* *L. plantarum* and *Bacillus subtilis* isolated could be as result of their dominancy and the ability to withstand acidic condition associated with the fermentation stages. *Leuconostoc mesenteroides* and *L. plantarum* are heterofermentative, hence they were highly resistant to acid. [17] confirmed that their dominancy is determined by the sensitivities of microorganisms to the acidic conditions that develop during the fermentation. The presence of *B. subtilis* during the production of “Agadagidi” may be due to contamination through their endospores from dust, air and peels. Bacilli are spore forming bacteria, able to withstand harsh conditions which are widely distributed in nature and in many cases with a pH as low as 3.9 [18, 19]

The appearance of the two yeasts, *Saccharomyces cerevisiae* and *Candida utilis* throughout the stages of the production of ‘Agadagidi’ can be attributed to the environment and the fruits itself. These yeasts are naturally associated with ripened fruits and they are known to be responsible for alcoholic fermentation [20-21]. This data is similar to the report of [22] who identified yeasts species in “Tchapalo” production and observed highest frequency with *S. cerevisiae* (87.36%), followed by *Candida tropicalis* (5.45%) and *Meyerozyma caribbica* (2.71%). The presence of moulds on the uncrushed ripened plantain pulp, after adding water may be as a result of improper handling of the ripe plantain. The peel possibly contained moulds which might have been transferred the plantain pulp. The elimination of moulds stated during fermentation may be as result of high loads and activities of LAB during fermentation. Bacteria have been shown to suppress the growth of mould during fermentation [23]. [24] who worked on yeasts and moulds associated with “Ogi” similarly observed moulds on the surface of raw maize grains for “Ogi” production. The increase in the load of bacteria, fungi and enterobacteriaceae in mashed overripe plantain pulp (0 hr fermentation) may be as a result of the potable tap water added. Portable water passes through pipes that may not have been treated or sanitized for a long period of time and as a result microorganisms present in the pipes might have increased the microbial loads. [25, 26] confirmed the presence of coliform bacteria in “Kunun zaki” where tap water was the source of water used. The decrease in total bacterial and

186 fungal loads after 24 hr of fermentation may be as a result of the increase in population of the
187 LAB that must have formed acid thereby reducing pH (acidity) which seems to be detrimental to
188 the other bacteria. This data agrees with that of [27, 28] who explained that LAB produce many
189 organic acids such as lactic, acetic and propionic acids as end products during fermentation
190 which provide an acidic environment unfavourable for the growth of many pathogenic and
191 spoilage microorganisms.

192 The increment LAB population after 24 hr may be due to their ability to proliferate in the
193 presence of the metabolic product synthesized by the yeasts. This observation is similar to that
194 of [29] who worked on “Akamu”. They observed that LAB occurred early in the fermentation,
195 increasing rapidly from 1.6×10^7 cfu/g to 7.1×10^8 cfu/g after 72 hr. The drop in population of
196 enterobacteriaceae during fermentation was as a result of the low pH and acid production by
197 some of the bacteria which could destroy the cells of these microbes. Similar observation was
198 obtained in the [30] work, who reported decrease in the population of enterobacteriaceae as the
199 pH dropped was due to the effect of lactic acid bacteria present in ‘bushera’ and ‘kirario’

200 LAB and yeasts evaluated in this study showed antagonistic interaction against other
201 isolated bacteria, which mean that these microbes might have produced inhibitory metabolites.
202 This conforms with the report of [31, 32] who documented that the ability of antimicrobial
203 compounds produced by LAB enabled them to exert strong antagonistic activity against food
204 contaminating microorganisms. None of the LAB isolates inhibited any of the yeast cultures. The
205 association between the yeasts and LAB could therefore be mutualism. Several authors have
206 reported similar co-existence and positive interactions between yeasts and lactic acid bacteria in
207 different African fermented foods [24, 33].

208 The stimulatory effect of yeasts on lactic acid bacteria during fermentation has been
209 attributed to the provision of some compounds such as soluble nitrogenous compounds, B-
210 vitamins, CO₂, pyruvate, propionate, acetate and succinate [34]. It has also been shown that
211 yeasts multiplication is associated with an increase in acid formation in fermented products.

212 CONCLUSION

213 This study has provided valuable information on the types of microbial communities and
214 their interactions during the production of “Agadagidi”. *Lactobacillus plantarum*, *Leuconostoc*
215 *mesenteroides*, *B. subtilis*, *S. cerevisiae* and *C. utilis* were found to be predominant in the
216 production of the beverage. This research also established positive and negative interactions
217 between the isolated microorganisms. This will contribute to the development of strains with
218 predictable characteristics for use in small and large scale production.

RECOMMENDATION

It is therefore recommended that there should be proper handling of the overripe plantain pulp and tap water should also be boiled before use so as to reduce or eliminate undesirable microorganisms.

REFERENCES

1. Solanke AO and Falade KO. A review of the uses and methods of processing banana and plantain (*Musa* spp.) into storable food products. *Journal of Agricultural Research and Development*. 2010; **9**(2): 87-96.
2. Latham, MC. La nutrition dans les pays en development, In Info Musa Africa :Abstract from International Conference on plantain and banana for Africa. *International Journal on Banana and Plantain*. 2001; **16**(1):5 -12.
3. Nse-Nelson FA, Oke UR, Adindu JU. Analysis of plantain marketing in Ikwuano local government area of Abia State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*. 2016; **12**(1): 85-89.
4. Omojasola PF, Davies OF, Kayode RM. The effect of chemical preservatives, pasteurization and refrigeration on the shelf life of “Agadagidi” a fermented plantain drink. *Research Journal of Microbiology*. 2012; **7**(3): 145-157.
5. Okafor N. Modern Industrial Microbiology and Biotechnology. Science Publishers, Enfield, N. Y, U. S. A. 2007; pp. 530.
6. Ray M, Kuntal Ghosh S, Singh K, Chandra M. Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal Ethnic Foods*. 2016; **3**: 5-18.
7. Holzapfel WH. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*. 2002; **75**: 197–212.
8. Tamang JP. Health Benefits of Fermented Foods and Beverages. CRC Press, Taylor and Francis Group, New York. 2015; pp. 1-110.
9. Sanni AI, Oso BA. The production of “Agadagidi”, a Nigerian fermented beverage. *Die Nahrung*, 1988; **32**: 319-326.
10. Chia CL, Huggins CA. Bananas. In: Community Fact Sheet fruit. Hawaii. CTAHR edition. 2003; pp. 23-29.
11. Areola JK, Yussuf IO. The processing and nutritional analysis of plantain wine. *International Journal of Business and Educational Policies*. 2011; **7**(2): 238-245.
12. Deak T Beuchat LR. Yeasts associated with fruit juice concentrate. *Journal of Food Protection*. 1993; **56**: 777-782.
13. Sanni AI, Lonner C, Marklinder L, Johanssan ML, Molin G. Starter cultures for the production of “Ogi”, a fermented infant food from maize and sorghum. *International Journal of Food Chemistry, Microbiology and Technology*. 1994; **16**: 29-33.
14. Brenner J, Kreig V, Stanley T. Bergey’s Manual of Systematic Bacteriology. The Proteobacteria. 2nd Edition. Published by Springer, New York. 2005; pp. 587-848.
15. Fawole MO, Oso, BA. Characterization of Bacteria: Laboratory Manual in Microbiology. 5th Edition. New Spectrum Books Publisher, Ibadan, Nigeria. 2007; pp. 24-33.

16. Oriola O, Boboye B, Adetuyi F. Bacterial and fungal communities associated with the production of a Nigerian fermented beverage, 'Otika'. 2017. *Journal in Advances in Microbiology*, Under Review.
17. Ampe F, Sirvent A, Zakhia, N. Dynamics of the microbial community responsible for traditional sour cassava starch fermentation studied by denaturing gradient gel electrophoresis and quantitative rRNA hybridization. *International Journal of Food Microbiology*. 2001; **65**: 45– 54.
18. Hanlin JH Spoilage of acidic products by *Bacillus* species. *Dairy Foods Environment*. 1998, **18**: 655-659.
19. Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS. Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta*. *Advances in Applied Science Research*. 2012; **3**(2): 844-848.
20. Sanni AI, Onilude AA, Ibidapo OT. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. *Food Chemistry*. 1999; **65**:35-39.
21. Jay, J. M., Loessner, M. J. and Golden, D. A. (2005). *Modern Food Microbiology*. 7th edition. Springer Science and Business Media, New York, U. S. A. pp. 101- 125.
22. N"guessan FK, Brou K, Jacques N, Casaregola S and Dje, KM. Identification of yeasts during alcoholic fermentation of "Tchapalo", a traditional sorghum beer from Côte d'Ivoire. *Anton Leeuwenhook International Journal of General and Molecular Microbiology*. 2011; **99**: 855-864.
23. Dalié DK, Deschamps AM, Richard FF. Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control*. 2010; **21**: 370–380.
24. Omemu AM, Oyewole OB, Bankole MO, Akintokun AK. Yeasts and moulds associated with "Ogi" - a cereal based weaning food during storage. *Research Journal of Microbiology*. 2007; **2**(2) 141-148.
25. Adegoke GA, Asaye O, Shridhar MK. Microbiological and physico-chemical parameters of water used by some brewery, bakery and soft drink plant in Oyo state, Nigeria. *Journal of Science and Technology*. 1993; **3**: 92-95.
26. Amusa NA. Microbiological and nutritional quality of hawked "Kunun" (a sorghum based non-alcoholic beverage) widely consumed in Nigeria. *Pakistan Journal of Nutrition*, 2007; **8**: 20-25.
27. Babatunde DA, Oladejo PO. Identification of Lactic Acid Bacteria isolated from Nigerian Foods: Medical importance and comparison of their bacteriocin activities. *Journal of Natural Sciences Research*, 2014; **4**(23): 246-252.
28. Teshome G. Review on lactic acid bacteria function in milk fermentation and preservation. *African Journal of Food Science*. 2015; **9**(4): 170-175.
29. Ogbonnaya N, Chukwu, BC. (2012). Studies on "Akamu", a traditional fermented maize food. *Review on Children Nutrition*, **39**: 180-184.
30. Muyanja, CM, Narvhus JA, Treimo, J, Langsrud T. Isolation, characterization and identification of lactic acid bacteria from "Bushera": A Ugandan traditional fermented beverage. *International Journal of Food Microbiology*. 2003; **80**: 201-210.
31. De Martinis ECP, Publio MRP, Santarosa PR, Freitas FZ. Antilisterial activity of lactic acid bacteria isolated from vacuum packaged Brazilian meat and meat products. *Brazil Journal of Microbiology*, 2001; **32**: 32-37.
32. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Journal of Applied Microbiology*. 2003; **99**: 77-84.

- 304 33. Gulitz A, Stadie J, Ehrmann M, Vogel R. Comparative phylobiomic analysis of bacteria
305 community of water kefir by 16S rRNA gene amplicon sequencing and ARDRA analysis.
306 *Journal of Applied Microbiology*. 2013; **114**: 1082-1091.
- 307 34. Stadie J, Gulitz A, Ehrmann M, Vogel R. Metabolic activity and symbiotic interaction of lactic
308 acid bacteria and yeasts isolated from water kefir. *Food Microbiology*. 2013; **35**: 92-98.
309