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Full Length Research Paper

Comparative study on the microbial load of *Gari, Elubo-isu* and *Iru* in Nigeria

Osho A.[1*], Mabekoje O. O. and Bello O. O.

Department of Microbiology, Olabisi Onabanjo University, Nigeria.

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Microbiological investigation was carried out on the safety of three locally fermented foods (*gari, elubo-isu* and *iru*). Twelve different samples each of three locally fermented food products- namely- *gari*, *elubi-isu* and *iru* were collected from the central markets at three different states in Nigeria (Ijebu-Igbo Central Market, Ogun State; Bodija Market, Ibadan, Oyo State and Garki Central Market, Abuja) and evaluated microbiologically. Various types of microorganisms were isolated from the three locally fermented food products (*gari*, elubo-isu and *iru*), and characterized. Both bacteria and fungi were isolated from these evaluated fermented food products. The mean total bacterial count in the samples of *gari*, *elubo-isu* and *iru* were 13.1 x 10^4, 11.4 x 10^5 and 9.5 x 10^6 cfu/ g, respectively. Also the mean total viable count of mould was 1.4 x10^4, 3.4 x 10^5 and 7.4 x 10^6 cfs/g, respectively. Bacteria isolated from *gari* include *Actinomyces* sp., *Bacteriodes* sp.*Corynebacterium* sp.,*Pseudomonas* sp., *Lactobacillus* and *Le uconostoc* sp., while the isolated moulds from *gari* include *Culicidospora gravida, Diplococcium spicatum, Geotrichum candidum, Passalora bacilligera,Scolecotrichum graminis, Tallospora aspera,* and *Varicosporium* sp. Bacteria isolated from elubo-isu include-;*Lactob* sp., *Streptococcus* sp. and *Listeria* sp., while the isolated moulds were *Articulospora inflate, Aspergillus niger, Aspergillus rapens*, *Aspergillus flavus* and *Lemonniera aquatica.* Also, the bacteria isolated from *iru* were *Bacillus* sp., *Pediococcus* sp., *Streptococcus* sp. and *Lactobacillus* sp., while the mould isolated from *iru* were *Aspergillus fumigatus, Rhizopus stolonifer* and *Triscelophorus monosporus.* It was concluded that the low microbial counts recorded for the evaluated fermented food products are tolerable and, thus, still safe for human consumption.

Key words: Fermented foods, bacteria, fungi, safety.

INTRODUCTION

Gari is a product of cassava; *elubo-isu*, a product of yam and *iru* a product of locust bean seeds mainly used as condiments in soups, sauces and porridges among consuming populations in Nigeria. The microflora involved in the fermentation of these foods have been isolated and characterized (Oyewole and Odunfa, 1990). The method of preparation of *gari* and *elubo-isu* has been explained by Latunde-Dada, (2000) while the method of preparation of *iru* was explained by Odunfa (1981). Fermented foods are of great significant because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet (Steinkraus, 1997). Nigeria is endowed with a wide range of fermentable indigenous staple foods that serve as raw materials for agro-allied cottage industries. These industries utilize small-scale equipment while adding value to such local produce (Latunde- Dada, 2000). Although fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumers' tastes and preferences for such foods (Achi, 1992). The introduction of foreign high technology products, especially processed ones because of globalization and liberation of the economy, radically changed the Nigerian food culture into a mixed grill of both foreign and local dishes (Ojo, 1991). Fermented products remain of interest since they do not require refrigeration during distribution and storage. The traditional condiments have not attained commercial status due to the very short life, objectionable packaging materials, stickiness and the characteristic putrid odour

*Corresponding author. E-mail: adelekeosho@yahoo.om. Tel: +234 8034714411.

Table 1. The total viable counts (TVC) of bacteria in *gari* (G), elubo-isu (E) and *iru* (I) in Nigeria.

Isolate	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	Mean
TVC/cfu/g X 10^4	8	20	16	20	13	21	5	20	11	3	6	13	13.1 cfu/g x 10^4
Isolate	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
TVC/cfu/g X 10^5	3	12	13	2	11	16	24	2	16	20	3	15	11.4 cfu/ g x 10^5
Isolate	11	12	13	14	15	16	17	18	19	110	111	112	Mean
TVC/cfu/g X 10^6	11	9	16	9	10	8	8	9	15	7	7	5	9.5 cfu/ g x 10^6

(Arogba et al., 1995). According to Steinkraus (1997), fermented foods have a very good safety record even in the developing world where foods are manufactured by people without training in microbiology or chemistry but fermented foods by themselves cannot solve the problems of contaminated drinking water and improper personal hygiene in food handlers. These problems make the safety of fermented foods debatable and questionable in developing countries. Some authors have shown that *Esherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Shigella* have high survival rate in fermented foods (Inatsu et al., 2004). The objective of this work is to determine the microorganisms associated with *gari*, elubo-isu and *iru* sold in Nigeria.

MATERIALS AND METHODS

Materials

Twelve samples each of the traditionally fermented food products of African locust bean (*iru*/dawadawa), yam (*elubo-isu*) and cassava (garri) were collected from local sellers in Ijebu-Igbo Central Market, Ogun State; Bodija Market, Ibadan, Oyo State and Garki Central Market, Abuja, Nigeria. Four samples each of the fermented food products were bought from the different markets. Aluminum foil, cotton wool, test tubes and pipettes used for this study were purchased from Eko-Idumota Market in Lagos. All chemicals and reagents used were of analytical standard.

Isolation of microorganisms

Fermented samples of *iru* were taken aseptically from traditionally fermented beans. One gram of the sample was weighed and thoroughly mashed with laboratory pestle and mortar and mixed with 9 ml of normal saline water as a diluent in a McCartney bottle and the content was thoroughly shaken. Subsequent serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of the diluent, using sterile pipette. The samples were screened for the presence of spoilage and pathogenic bacteria and fungi. Nutrient Agar was inoculated with 10^{-5} or more dilution of the sample(s) for bacteria and Potato Dextrose Agar was inoculated with 10^{-3} or more dilution of the sample (s) for fungi. The NA plates were incubated at 35°C for 24 h and PDA plates were incubated at 25°C for 48 - 72 h.

10 g from each sample of *gari* and *elubo-isu* were added to 90 ml of 0.1% (W/V) sterilized peptone water in a beaker and allowed to stand for 5 min with occasional stirring. Portions (0.1 ml) of different serial decimal dilutions were spread-plated on potato dextrose agar for total fungi count. The media used were prepared and incubated according to the labeled manufacturer instructions. The colonies were enumerated and expressed as colony forming unit per gram (cfu/g) (Vanderzannt and Splittstoesser, 1992).

Characterization and identification of isolates

Representative colonies obtained after incubation were sub-cultured on nutrient agar which was incubated for 24 h at 35°C. The cultural characteristics of isolates on the agar plates were observed. The motility of the isolates was examined using hanging drop technique. Gram staining reactions and cell morphology from heat fixed smears were done. The identification procedures for the micro-organisms were carried out using Cowan and Steel (1985) methods. Pure cultures of the different organisms isolated were sub-cultured and preserved on agar slants at the appropriate refrigeration temperature (4°C). For fungi, a smear of the mycelial growth on PDA was prepared on sterile slides, obtained with cotton blue in lactophenol and observed under the microscope for identify-cation. The fungal isolates were identified based on examination of the conidial heads, phialides, conidiophores and presence of foot cells or rhizoids (Bounds et al., 1993).

RESULTS AND DISCUSSION

Table 1 shows the total viable counts and average total viable counts of the bacteria in samples of *gari*, *elubo-isu* and *iru* collected from sellers in the three different markets. The average total viable bacterial counts were 13.1 x 10^4, 11.4 x 10^5 and 9.5 x 10^6 cfu/ g in *gari*, *elubo-isu* and *iru*, respectively. While the average total viable fungal counts were 1.4 x 10^4, 3.4 x 10^5 and 7.4 x 10^6 cfs/g in *gari*, *elubo-isu* and *iru*, respectively (Table 2). A total of ten bacteria species were isolated from the samples collected. Six bacteria; *Leuconostoc* sp. *Pseudomonas* sp., *Bacteriodes* sp., *Actinomyces* sp., *Corynebacterium* sp. and *Lactobacillus* sp. were isolated from *gari* samples. Four bacteria; *Streptococcus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Listeria* sp. were isolated from samples and four bacteria; *Lacto-bacillus* sp., *Streptococcus* sp., *Pediococcus* sp. and *Bacillus* sp. were also isolated from *iru* samples (Table 3). A total of fifteen mould species were isolated from samples (Table 3). Seven mould isolates; *Geotrichum candidum, Scolecotrichum graminis, Tallospora aspera, Passalora bacilligera, Varicosporium species, Culicidospora gravid* and *Diplococcium spicatum* were isolated from *gari* samples, five mould isolates; *Articulospora inflate, Aspergillus niger, Aspergillus rapens, Aspergillus flavus* and

Table 2. The total viable counts (TVC) of fungi in *gari* (G), elubo-isu (E) and *iru* (I) in Nigeria.

Isolate	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	Mean
TVC/cfu/g X 10^4	1	1	1	1	6	1	1	1	1	2	0	1	1.4 cfs/g x10^4
Isolate	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	G11	G12	Mean
TVC/cfu/g X 10^5	3	3	2	2	8	9	3	3	-	4	2	2	3.4 cfs/ g x10^5
Isolate	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	Mean
TVC/cfu/g X 10^6	8	9	10	6	10	8	7	9	4	11	3	4	7.4 cfs/ g x 10^6

Table 3. Microorganisms associated with some local fermented foods sold within Nigeria.

	Bacteria	Moulds
Gari	*Leuconostoc sp*	*Geotrichum candidum*
	Pseudomonas sp.	*Scolecotrichum graminis*
	Bacteriodes sp.	*Tallospora aspera*
	Actinomyces sp.	*Passalora bacilligera*
	Corynebacterium sp.	*Varicosporium species*
	Lactobacillus sp.	*Culicidospora gravida*
		Diplococcium spicatum
Elubo-isu	*Streptococcus* sp.	*Articulospora inflate*
	Lactobacillus sp.	*Aspergillus niger*
	Listeria sp.	*Aspergillus rapens*
		Aspergillus flavus
		Lemonniera aquatica
Iru	*Lactobacillus* sp.	*Rhizopus stolonifer*
	Streptococcus sp.	*Aspergillus fumigatus*
	Pediococcus sp.	*Triscelophorus monosporus*
	Bacillus sp.	

Lemonniera aquatica were isolated from elubo-isu samples, and three mould isolates; *Aspergillus fumigatus, Rhizopus stolonifer* and *Triscelophorus monosporus* were found in *iru* samples.

The bacterial and fungal loads were highest in *iru*, followed by *elubo-isu* and lastly by *gari*. The dynamics of fermentation in any food matrix is a complex microbiological process involving interactions between different microorganisms (Omafuvbe et al., 2003). The contribution of the accompanying flora of fermenting substrates is determined by the substrate composition and hygiene during production. During fermentation, the microorganisms use the nutritional components of the seeds, converting them into products that contribute to the chemical composition and taste of the condiment. Quite a number of *Bacillus species* have been isolated from various fermented food condiments. Although yeasts and other bacteria are also seen, only part of them can be considered to play a substantial role in fermentation processes. For example, non-fermenting species may just be ubiquitous contaminants although they may affect the flavour of the final product when occurring in high numbers. It can be deduced that *iru* was most susceptible to contamination with microorganisms probably due to mode of preparation and handling. Selection of starter cultures for large-scale industrial processes may require genetic modification to introduce a number of properties. These may offer nutritional benefit in the form of increased protein production or compatibility to mixed culture fermentations. Fermented condiments have a characteristic organoleptic quality, which probably are the most important factors for consumers. There are a few data on the flavour components of fermented condiments and how this can be improved by fermenting microorganisms (Dakwa et al., 2005). Further research is needed in these areas. Taking into account the increasing demand particularly by urban populations in Nigeria, there are certainly prospects for industrialization of traditionally fermented food products. Commercial availability of ready-to-use fermented products saves much labour and time in the household (Nout and Sarkar, 1999).

From the results, the bacterial load varies from one seller to another and it was clear that *gari* was

bacteriologically safer for consumption compared to *elubo-isu* and *iru*. Lactobacillus which is a probiotic was isolated from all the samples and it shows that the small-scale fermented foods are a good source of probiotics. The results of this study agreed with the findings of Ijabadeniyi (2007) but with new identification of some other microbes involved in the fermentation of *gari*. However, this study greatly differs from the work of Victor (2009) who reported that the organisms isolated from fermented *iru were Bacillus, Micrococcus and Staphylococcus species* which were all bacterial isolates.

In this study, most of the fungi isolated from the fermented foods, except for *A. flavus* and *Passalora bacilligera* isolated from elubo-isu and *gari* respectively, were non-pathogenic. More effort is needed to improve the present packaging and presentation status of traditional African fermented foods. Apart from the obvious effects of improving shelf life of product, this will help to increase product popularity and general help to increase product popularity and acceptability. Scientists have a lot to contribute in this as it will guarantee better income for more families and individuals that depend a lot on the fermented food for their survival in Africa.

In conclusion, fermented foods such as *gari*, *elubo-isu* and *iru* sold within Nigeria are considered safe and wholesome for consumption because food pathogens such as *Salmonella, Shigella, E. coli, Campylobacter jejuni* and *Clostridium perferinges* were not isolated from them; however their safety can be insured and improved upon greatly by using quality raw materials, using unique starter cultures that have the ability to detoxify, maintaining proper hygienic standards in the processing environment and using proper packaging in the scaling up process (Ijabadeniyi and Omoya, 2006b). Mention was made already of the choice of raw materials, fermentation conditions, and starter cultures used (Achi, 2005).

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