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EVALUATION OF MICRO-ORGANISMS ASSOCIATED WITH GARRI SOLD IN EKPOMA AND IT'S ENVIRON

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ABSTRACT

The microbiological assessment study on Garri sold at different markets in Ekpoma and its environ was carried out to evaluate the bacteria species associated with the type sold. White, yellow and Ijebu Garri types were considered in this study. Bacteria isolates were identified using Gram staining technique, microscopy, the characteristics of the bacteria on MacConkay and Nutrient Agar plate were determined using biochemical tests such as Catalase test, Oxidase test and Coagulase test, the microbes identified included *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*.. Ijebu garri recorded a 66.6% contamination, white garri had 40% contamination while the yellow garri was least contaminated at 33.3% contamination. The use of hygienic packaging by producers and retailers in Ekpoma, is highly recommended to ensure food safety and consumer protection.

Key words: Garri, Bacteria species, Market, Ekpoma, Contamination

INTRODUCTION

Garri is a creamy – white, granular flour with a slightly fermented flavor and slightly sour taste, made from fermented, gelatinized fresh cassava (*ManihotesculentaCrantz*) tubers. Garri is widely known in Nigeria and other West African countries, as a staple food eaten mostly by the mid-western part of Nigeria as Red-Garri and White Garri, while the western part eats it as *Ijebu*Garri. It is commonly consumed either dry or soaked in cold water with sugar, coconut, roasted groundnuts or dry fish as compliments, or as a paste made with hot water and called "Eba" which is eaten with varieties of African Soups. Garri is traditionally made at home in Africa, using mechanized means. It can also be made into commercial quantities. Before the advent of machines, the cassava was hand grated. The tubers are harvested, peeled, removing the covering, and the white pulp is grated in a Garrigrinding machine. The grated produce is then put into a jute sack and the sack tied. Traditionally, this is left to ferment for three to seven days depending on the type of garri being made. This step is very important, as the fermentation process helps to reduce and detoxify the high cyanide content of cassava (Oke, 1969). There are several factors which influence the quality of Garri; processing conditions and storage conditions (Obadina*et al*., 2009).

It is a very popular cassava product in Edo state and across Nigeria, due to its wide economic, social and geographical preference, but some known health hazards such as food poisoning that could result from mishandling, unsatisfactory sterilization of products and packaging, storage and distribution of garri. Much work has not been carried out locally to isolate pathogenic organisms that could damage consumers' health. Therefore effort was put into analyzing smaller-scale local manufactured products, where it is considered that hygienic conditions may not be of highest priority.

The local handling and processing method of Garri has been found to be generally unhygienic, and may cause serious health/environmental hazards to the final consumers, Obadina *et al*., (2009)**,** observed that after fermentation of the cassava product (Garri) a change in odour was observed. This could be caused by the fermentation process involved,

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yielding unwanted organisms, therefore causing smell to the final products (Obadina *et al.,* 2009). Therefore due to other previous research and findings, Garri is known to have high microbial content, which may be detrimental to human health.

Microorganism, especially bacteria vary from species to species in nutritional requirement (Asegbeloyin and Onyimonyi, 2007). Their presence in food at any stage depends on the nutritional status of the food at that stage, temperature, water content, pH as well as the nature of the organism. The bacteria that cause food poisoning have a similar nutritional requirement with that of human (Baine, 2000). Baine, (2000) also states that food poisoning could have been minimized, if the food producers and processors are trained in safe-food-handling and consumers are better advised in the choice of food. It is therefore with this facts in view, that this study was carried out on the isolation of bacterial species associated with Garri sold in Ekpoma and its environ.

MATERIALS AND METHOD

Area of Study: This study was carried out in Ekpoma, the administrative headquarter of Esan West Local Government Area of Edo State which makes up one of the five local government areas of Esan land. This area is located between latitude '6^0 10 and 6^0 45' north of the equator and between longitudes 6^0 10' and 6^0 30' east of the Greenwich Meridian (Dic-Ijiewere *et al*., 2016).Ekpoma had a population of 89,628 in 1991 and 127,718 in 2006(NPCN, 2012), Projected to 2017 at 2.8 percent national growth rate, the 2017 population of the study area is 167,055 people ((Dic-Ijiewere *et al*., 2016), majority of which are civil servants, traders, business men/women, transporters, farmers, teachers/lecturers and students. The town is home to the Ambrose Alli University. The main source of water in the locality is rainfall. It has 2 distinct seasons, wet and dry seasons. The wet season occurs between April and October with peak in August, average rainfall ranging from 150cm to 250cm. The dry season occurs between November and March with cold harmattan between December and January, withaverage temperature of about 25^{o}C (Edo state of Nigeria, 1992).

Location and Collection of Sample: Two samples of each of White, Red and Yellow Garri were purchased from the main Ekpoma market and new Ekpoma market in Edo State, Nigeria. These samples were studied over a period of 4-5 weeks. The samples were put into clean nylon bags and taken to the laboratory for immediate analysis.

Apparatus: The apparatus that were used for the experiment are syringe (2ml and 10ml) disposable Petri dish, test tube rack, conical flasks, beakers, digital balance, foil paper, masking tape, spatula, cotton wool, spirit lamp, marker and measuring cylinder.

Reagents: The reagents used for this experiment were peptone water, Nutrient Agar and MacConkay Agar.

Preparation of Samples from Different Markets/Microbial Count and Isolation: 7g of Nutrient agar dissolved in 250ml of distilled water and 12.13g of MacConkay agar dissolved in 250ml of distilled water was measured separately into clean conical flask and allowed to dissolve, it was corked immediately and transferred to the autoclave for sterilization at 121^{o}C for 15mins. 0.9g of peptone water in 60ml distilled water was measured in a clean conical flask and stirred properly to avoid particles of the peptone water. Using 10ml syringe, 9ml of peptone water was pipetted and dispensed into clean test tubes and autoclaved at 121^{o}C for 15mins.A gram of each sample was measured and dispensed after sterilization from the stock and a tenfold serial dilutions was done. 1ml each of the diluents (101 to 106) was dispensed in duplicates for each dilution on petri dishes. After which the nutrient agar was poured. Same was done for the MacConkay agar in a sterile environment and mixed properly and was incubated at 37^{0}C for 24hours.

Identification of Isolates: Bacteria isolates were identified using Gram staining technique, microscopy and biochemical tests such as Catalase test, Oxidase test and Coagulase test.

Gram Reaction: Gram reaction was used to classify the isolates into gram positive and gram negative bacteria after examining the agar plates. A thin smear

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of young bacterial culture (24hours old) was made on a clean grease free glass slide, it was allowed to air dry, then heat fixed by passing it through a Bunsen burner flames about 3 times. The heat-fixed smear was then covered with crystal violet stain for 30-60seconds. The stain was quickly washed off with clean water. The water was tipped off and the smear was covered with Lugol's iodine for 30-60seconds. The iodine was washed with clean water. The smear was decolourized rapidly for about 20seconds with 95% ethanol. The smear was quickly washed with clean water and then covered with dilute carbolfuschin for 30 seconds.Thestain was washed off with clean water and the slide was allowed to dry at room temperature. The gram stained slide was examined first with x40 objective lens to check for the staining and distribution of the gram stained bacteria, then with oil immersion objective lens (x100) to look for the bacteria. Gram positive bacteria appeared purple while gram negative appeared red or pink.

RESULTS

Table 1 below shows the prevalence of bacterial contamination distribution among the different garri samples; Ijebu garri recorded a 66.6% contamination, followed by white garri with 40% contamination while the yellow garri was the leastcontaminated with 33.3% contamination.

Table 2 represents the morphological characteristics of isolates from Main Ekpoma Market. A1 – A6 represents the number of the isolates, isolates was recorded patterning to their colour, shape, size and elevation. All the isolates (A1 – A6) are cream coloured, shapes isolated includes; three round, two undulating and one rhizoid. From the isolates the following isolates (A1 – A6) sizes were discovered as follows; 0.25mm, 0.3mm, 0.5mm, 0.45mm, 0.4mm and 0.3mm respectively. And the elevation from A1 – A6 respectively as follows: flat, flat, raised, raised, flat, and flat.

Table 3 represents the morphological characteristics of isolates from Ekpoma new Market. B1 – B6 represents the number of the isolates, isolates was recorded patterning to their colour, shape, size and elevation. All the isolates (B1 – B6) are cream coloured, shapes isolated includes; three round, two undulating and one rhizoid. From the isolates the following isolates (B1 – B6) sizes were discovered as follows; 0.35mm, 1.1mm, 0.7mm, 0.3mm, 0.3mm, and 0.9mm respectively. And the elevation from B1 – B6 respectively as follows: flat, raised, raised, flat, flat, and raised.

Table 1 Prevalence of Bacterial contamination of different garri types

Parameter	White Garri	Yellow Garri	Ijebu Garri
No of sample collected	15 (15.00)	15 (15.00)	15 (15.00)
No of samples that yielded	6(6.00)	5 (5.00)	10 (10.00)
Column total	21	20	25
Prevalence	40%	33.3%	66.6%

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Table 2: Morphological characteristics of isolates from Main Ekpoma Market.

Number of the isolates	Colour of isolates	Size of isolates	Elevation
A1	Cream	0.25mm	Flat
A2	Cream	0.3mm	Flat
A3	Cream	0.5mm	Raised
A4	Cream	0.45mm	Raised
A5	Cream	0.4mm	Flat
A6	Cream	0.3mm	Flat

Table 3: Morphological characteristics of isolates from Ekpoma New Market

Number of the isolates	Colour of isolates	Size of isolates	Elevation
B1	Cream	0.35mm	Flat
B2	Cream	1.1mm	Raised
B3	Cream	0.7mm	Raised
B4	Cream	0.3mm	Flat
B5	Cream	0.3mm	Flat
B6	Cream	0.9mm	Raised

Table 4: Biochemical Characterization of Isolates

Gram reaction	Catalase	Coagulase	Oxidase	Cit	Indole	Urease	Mot	Lact	Man	Glu	Suc	isolates
GPC	+	-	-	-	-	+	-	+	+	+	+	*Staphylococcus epidermdis*
GPC	+	+	-	+	-	+	-	+	+	+	+	*Staphylococcusaureus*
GNR	+	-	-	-	+	-	-	+	-	+	-	*Escherichia coli*

Key: Positive = +; negative = - ; Gram positive cocci = GPC; Gram negative rod= GNR

Table 4 above shows the biochemical characteristics of isolates from Ekpoma new market, while table 5 below shows specific sites of bacterial contaminants. This indicates that *Staphylococcus aureus*and *Escherichia coli* were the predominant isolates while *Staphylococcus epidermdis* was isolated from two types of garri.

Table 5: Specific site of isolation

Bacterial isolates	White garri	Yellow garri	Ijebugarri
Staphylococcus aureus	+	+	+
Staphylococcus epidermdis	-	-	+
Escherichia coli	+	+	+

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DISCUSSION

The fermentation of Garri is by mixed microbial cultures, this could have accounted for the diverse microbial population contaminating the product. Similarly post process contamination specifically associated with sieving of products after heat treatment and the spreading of products in the open to air dry, coupled with the practice of leaving Garri open for sales could have accounted for the diverse microbial population. The isolation of diverse microbial species from this ready to eat food (Garri) corroborate the findings of Nichols *et al.*,(1999), Mensachet and Baine, (2002), Idowu, (2006), Tauloand Nwogu, (2008), Oranusi and Braide, (2012).

In this study microorganisms were isolated, majority of them were cream coloured, round and flatly elevated. The presence of microorganism causes deterioration of food and can adversely affect the health of humans. It also influences the biochemical characters and flavours of the product (Garri) and their appearance is commercially undesirable and often results in downgrading of the product. Bacteria and fungi can also adhere to particles of grain dust and be transported through air. Grain dust is generated during the process of farming and secondary processing of grains (sacking, milling, handling of powdered grains, sorting, etc.) in markets and can play a role as an effective infectious aerosol because its organic materials provide essential nutrients for airborne microorganisms adhered to their surfaces (Kim *et al.*, 2009). Despite the apparent weaknesses of the gravitational sampling technique used in this study as opposed to the use of air sampling impellers, it is not surprising that the

atmospheric bioloads of these markets were high considering that these markets were in close proximity to grain milling centers, farms, refuse dumps, road construction sites, abattoirs and grain processing centers.

The total bacterial identified recorded the highest prevalence with Ijebu Garri with the least being Yellow garri which is an indication of the sanitary quality, safety and utility of foods; it may reflect the conditions under which the product is manufactured such as contamination of materials, the effectiveness of processing and sanitary conditions of equipment and utensils at the processing plants (International Commission on Microbial Specification for foods, 1986).

Conclusion and Recommendation

In conclusion, results obtained from this study have shown that airborne contaminants in market areas may contribute considerably to the microbial burden of garri sold in markets in Ekpoma and its surrounding communities. This is worsen by the unhealthy but accepted mode of selling and distributing garri in open basins, trays and mats in Nigerian markets. As a result of that, the focus on garri should not only be on surface cleaning and hygienic handling during processing, but also on reducing or eliminating microbial air contamination during marketing and packaging of products for sale and distribution as safety is of particular concern with ready-to-eat food products. Environmental characteristics of the region should be considered while addressing marketing strategies.

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