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MICROBIOLOGICAL SAFETY OF TOASTED *UKWA* (AFRICAN BREADFRUIT) SNACK SOLD IN ABA, SOUTH-EASTERN NIGERIA

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ABSTRACT

A microbiological assay was conducted to assess consumer safety of toasted ukwa (African breadfruit) snack in the Aba metropolis, South-eastern Nigeria. The microbial loads, isolation and identification were carried out by the spread plate agar dilution method. Results indicated that the counts of bacteria ranged from 3.12-7.22x10^{3}cfu/g. The fungal counts ranged from 2.11-5.48x10^{3}cfu/g while coliforms were detected in some of the market samples with values ranging from 0.00-0.16x10^{3}cfu/g. Staphylococcus aureus, Bacillus spp., Streptococcus spp Klebsiella spp. were bacteria isolated from the samples. Aspergillus niger, Fusarium spp., Mucor spp. and Aspergillus flavus were the isolated fungi. No Escherichia coli or other significant pathogens produced by food were identified from the samples. Coliform (Aerobic colony counts) were lower than the International Food Standards (≥ 105cfu/g) and the absence of microbial food-borne pathogens makes these ukwa samples safe for consumption.

Key Words: Ukwa, Snack, Microbial Loads, Spread Plate

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INTRODUCTION

African breadfruit (*Treculia africana*) is an evergreen forest tree, a native of many parts of tropical West Africa, which its seed is a grain legume. The tree is commonly cultivated in Southern Nigeria for the seed which is utilized as a source of protein especially by the poor people in south- eastern Nigeria (Nwokolo, 1996; Ugwu *et al*, 2001; Badifu and Akubor, 2001) where it is cherished in their diets and is often eaten as delicacy.

The process for its preparation before consumption are boiling, baking, roasting/toasting and frying. The seeds can be cooked as porridge or mixed with other food stuff such as sorghum (Onweluzo and Nnamuchi, 2009) or roasted/toasted and sold as snack on the roadside or market. *Treculia africana* seeds are very nutritious, making up a cheap supply of vitamins, nutrients, proteins, carbohydrates, and fats. The seed comprises 11% crude fat, 17-23 % crude protein and other important vitamins and minerals (Akubor *et al*., 2000).

The sale of snacks and beverages by vendors and hawkers in the markets, streets and motor parks in Nigeria is a daily activity of the urban poor as a source of income and employment. According to Aso *et al*. (2002), street food is appealing to consumers because it is inexpensive with good nutritional value and thus acts as a significant source of achieving their prescribed daily dietary allowance. Umoh and Odioba (1999) observed that, given the absence of

surveillance activities in Nigeria, laboratory studies have demonstrated the existence of pathogens and strong microbial loads in certain street foods. Previous research by Costarrica and Morin (1996) indicated that there is a strong risk for serious health issues connected with the processing and delivery of street foods in developing countries such as Nigeria. Aroyeun and Adegoke (2000) noted that the majority of local snacks sold along the streets in Nigeria are characterized by poor hygienic practices, starting from procuring the raw materials to the final products sold to consumers.

Toasted ukwa (African breadfruit) snack is sold along the streets, markets and major roads of Aba and little or nothing is known about the microbiological safety of this snack-food. The contamination of snack foods sold by hawkers and street vendors has become a significant public health issue. Health risks related to such foods are prevalent in Nigeria. Therefore, microbiological assessment of this snack-food (toasted ukwa) needed to be carried out to ascertain its safety for human consumption. The goal of this study was to evaluate the microbiological safety of toasted ukwa (African breadfruit) snacks sold in Aba, south-eastern Nigeria, as well as propose measures to improve their quality.

MATERIALS AND METHODS

Sample Collection

Five samples of toasted ukwa (African breadfruit) wrapped in polyethylene were purchased from sellers in ten markets in Aba metropolis. The obtained samples were sent to the laboratory for microbiological assay.

Microbial analysis

Sterilization of materials

All the glass wares used for microbial analysis were sterilized in an oven at 150^0C for one hour while all media diluents used were sterilized for 15 minutes using an autoclave at 120^0C.

Enumeration and isolation of microorganisms

In 10ml of sterile peptone water a 1 g quantity of each toasted ukwa sample was homogenized. Dilutions were carried out by combining 1.0ml of the homogenate in 9.0ml of sterile peptone water to get 10^{-1} dilution and this dilution was then carried out in sequence before 10^{-3} was collected and then used to inoculate each of the plates in triplicates and the result was recorded as count of colonies (Oshoma *et al*., 2009).

Isolation and identification of microorganisms

Microorganism isolation was achieved using the process reported by Ogbulie *et al*. (2005). Regarding bacteria isolation, the total viable bacteria counts were estimated by recounting the colony forming units (cfu/g) by pour plating 1.0ml of 10^{-3} diluent on nutrient agar (oxoid) plates and incubating at 37◦C for 48 hours.

For fungi isolation, sabouraud dextrose agar was used. The total number of fungi was determined by pour plating 1.0ml 10-3 diluent and incubating for 72 hours at 37◦C.

Pure colonies of bacterial and fungal isolates were obtained from nutrient agar and sabouraud dextrose agar

Characterization and identification of the isolates

Bacterial isolates were distinguished based on their cultural properties, morphological properties and subsequently subjected to biochemical examination using the Microbact Identification Kit (Microbact 24E Oxoid), accompanied by computer aided identification Package (Advanced Bacterial Identification Software, ABIS). Colonies typical of coliforms were subjected to Indole, Methyl red, Voges Proskaur and Citrate (IMVC) tests.

The fungal isolates were distinguished by their cultural attributes, stained with a cotton-

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blue lactophenol solution and viewed under a microscope's low power objective lens (AOAC, 2005). Various isolated fungi were identified using their gross morphologies according to the description of Collins and Lyne (1984).

Statistical analysis

Statistical analysis was carried out on all the data using descriptive statistics. ANOVA was also used to determine the significant differences. Means were separated using least significant differences (LSD). Significant differences were accepted at significance level of 5% (Ihekoronye, 1999).

RESULTS AND DISCUSSION

Total Microbial Count of Toasted *Ukwa* Samples

The total microbial count of toasted ukwa sold in Aba is shown in Table 1. There were substantial variations ($p<0.05$) in microbial counts between the samples from the ten Aba metropolitan markets. Total bacterial counts ranged from 3.12-7.22x10^3cfu/g with sample AWUK recording the highest bacterial count while sample CMUK had the lowest count. The fungal counts ranged from 2.11-5.48x10^3cfu/g. Sample EAUK (5.48x10^3cfu/g) recorded the highest count that was statistically different ($p<0.05$) from the value (5.0X10^3cfu/g) of sample AWUK. The lowest (2.11 x10^3cfu/g) recorded fungal counts for CMUK sample was statistically different ($p< 0.05$) from that of sample AEUK (3.0 x10^3cfu/g).

Also, the coliform counts ranged from 0.00-0.16x10^3cfu/g with sample AWUK (0.16x10^3 cfu/g) recording the highest count followed by sample ARUK (0.14 x10^3 cfu/g). The lowest coliform count was recorded for samples AMUK and EAUK with the value 0.10 x10^3cfu/g. There were no coliform bacteria found in samples AOUK, AEUK, ANUK, CMUK and AFUK.

The differences in microbial counts in these results could be due to different hygienic practices of the producers, preparation environment, air borne microorganisms, washing water, wrapping materials, etc. (Stainer and Ingram, 1990; Okaka, 2005; Ocheme *et al*., 2011). Buchanan (1991) and Ogbulie *et al*. (2005) have expressed the opinion that foods hawked in streets, especially in countries with high ambient temperatures, are a good medium for the propagation of bacteria that could contribute to food spoilage and disease. The high number of microbial counts recorded in the toasted ukwa samples could be attributed to poor post-preparation handling with progressive spoilage, or unsuitable storage conditions (temperature and humidity) which permitted the growth and escalation of these microorganisms. The high microbial loads obtained in this study could have undesirable effect on nutritional quality of the snack products. Furthermore, such products could become dangerous to human health due to toxic decomposition products. To remedy this, proper hygienic conditions must be observed during preparation, packaging, storage and hawking of toasted ukwa. Polyethylene wraps used for packaging must be clean, and the products must be stored in a clean, cool and dry place (Potter and Hotchkiss, 1995; Braide *et al*., 2018).

Isolation and Identification of Microorganisms in Toasted *Ukwa* Samples

The results of the isolation and identification of microorganisms in toasted *ukwa* samples are shown in Tables 2 and 3. The isolates of the bacteria (Table 2) indicated the existence of Staphylococcus aureus, Bacillus spp, Streptococcus spp and Klebsiella spp. as the prevalent microorganisms.

The fungi isolates (Table 3) showed the dominant microorganisms as *Aspergillus flavus, Aspergillus niger, Mucor spp. and. Fusarium spp.*

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Microbial safety of any food is determined by its microbiological quality. It is observed from this study that the toasted *ukwa* solid in Aba markets are not contaminated with food-borne pathogens like *Escherichia coli* and other pathogens but contains opportunistic microorganisms. The presence of these microorganisms in the toasted *ukwa* indicates their contamination must have been after the toasting and cooling. The sources of the contaminating microorganisms identified could be toasting pans, sieves, hands of handlers, water for washing and polyethylene wraps for packaging. Potter and Hotchkiss (1995) opined that the polyethylene packaging materials which are plastics, are not inert to food and permits permeation of gases and vapours in packaged foods. Therefore high degree of sanitation should be employed in the preparation of toasted ukwa (Braide *et a*l., 2018).

Staphylococcus aureus has been reported in soymilk and soyflour products hawked in Uyo metropolis by Brooks *et al*. (2002), corn-based street food sold in Abeokuta by Afolabi *et al*. (2011) and hawked retted cassava fufu sold in Aba by Udensi *et al*. (2011). Staphylococcus aureus suggests contamination from the food handlers' skin, mouth and nose, which shows poor personal hygiene (Omafuvbe *et al*., 2002) and its detection in the toasted *ukwa* samples is of serious public health importance. Staphylococcus spp. is widespread in nature (Buchanan, 1991) and are repeatedly implicated in food and water contamination. *Staphylococcus aureus* are leading causes of gastroenteritis. Nausea, abdominal cramping and vomiting are symptoms of staphylococcal food poisoning (Frazier and Westhoff, 2008).

The presence of Bacillus spp has been found in some of the samples and is an opportunistic human pathogen, a regular inhabitant of soil and its appearance in the samples could be from toasting utensils or wrapping material. *Bacillus spp*. has been implicated in food poisoning and produces toxins that cause pneumonia and broncho-pneumonia (Chessbrough, 2006).

Klesiella spp, gram negative bacilli of Enterobacterioceae family was present in some of the toasted *ukwa* samples and the bacteria is typically associated with fecal contamination and this suggests poor personal hygiene practices among the handlers (Uzeh, *et al*., 2006). Therefore, good hygienic measures must be observed during preparation, packaging and selling of toasted *ukwa.*

Aspergillus spp. are fungi. Fungi are spore formers and filamentous. They are ubiquitous and are found everywhere. The spores produced by these fungi are single to few cell reproductive structures that may be dispersed by wind, water, animal or equipment (Mahovic *et al*., 2004). They produce toxins that are carcinogenic and are aflatoxins. Ingestions of aflatoxins in mouldy foods has been implicated in the development of liver cancer (hepatoma) (Nester *et al*., 2004). *Aspergillus spp*. can also cause superficial infections of the external ear and occasionally infect the eye (Cheesbrough, 2002).

Mucor spp. present in some of the samples is a mold found in the soil and is a common contaminant of stored and processed food in the kitchen (https://www.moldbacteria.com). Fusarium spp., *Streptococcus spp., Mucor spp.,* etc., isolated from the toasted *ukwa* samples are ubiquitous in nature and are opportunistic microorganisms of human.

The isolated microorganisms in this study *Streptococcus spp., Klebsiella spp., Fusarium spp., Mucor spp., Aspergillus spp*., etc., have been implicated in the spoilage of food and beverages (Udensi *et al*., 2011, Afolabi *et al*., 2011, Adebayo *et al*., 2010). These microorganisms can lead to the spoilage of toasted *ukwa*.

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The results showed that the coliform counts (aerobic colony counts) of the toasted ukwa samples were lower than those of the International Food Standards ($\geq 10^5$ cfu/g) (Afolabi *et al*., 2011). The Standards Organization of Nigeria (SON) had also stated that there should be no coliform bacteria and pathogenic microorganisms in food. It has been documented that counts of 10^4 cells/ml for *Bacillus cereus* and 10^6 cells/ml for enterogenic *Staphylococcus aureus* are needed to pose a risk of intoxication (Ashiru *et al*., 2003), and lower counts have been observed in this study for these microorganisms in toasted ukwa samples, which is indicative of their safety.

Results from this study also revealed that the toasted ukwa samples were microbiologically healthy for human consumption as the overall microbial counts were below the acceptable limit of $< 10^5$ level recommended by the International Commission on Microbiological Specifications for Food (ICMSF) (Obadina and Ogundimu, 2011).

CONCLUSION

It is apparent from this study that the toasted *ukwa* sold in Aba Metropolis have high microbial load but they are not contaminated with pathogenic microorganisms like *Escherichia coli* and other pathogens of public health importance such as *Salmonella spp*., *Listeria monocytogenesis*, *Clostridium perfrigenes, Campylobacteria spp*. and other food-borne pathogens, but contain opportunistic microorganisms. Therefore, the need for proper sanitation during processing, packaging and selling/hawking of this product cannot be over-emphasized.

Furthermore, the State and Federal Ministry of Health should effectively monitor the microbial standard of snack-foods sold to the public as a protective measure of mitigating the health hazard that may arise from their consumption.

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APPENDICES

Table 1: Total microbial counts of toasted *ukwa* samples

Sample	Total Bacteria Counts (x 10^3cfu/g)	Fungi Counts (x 10^3cfu/g)	Coliform Counts (x 10^3cfu/g)
AOUK	6.02^{g}	3.10^{h}	NG
AEUK	5.01^{i}	3.0^{i}	NG
ANUK	6.10^{e}	3.15^{g}	NG
EOUK	6.15^{d}	3.85^{f}	0.12^{c}
CMUK	3.12^{j}	2.11^{j}	NG
AMUK	6.05^{f}	4.13^{e}	0.10^{d}
ARUK	6.20^{c}	4.30^{c}	0.14^{b}
AFUK	5.11^{h}	4.24^{d}	NG
AWUK	7.22^{a}	5.0^{b}	0.16^{a}
EAUK	7.01^{b}	5.48^{a}	0.10^{d}
LSD	0.00368	0.00489	0.00157

NG-No Growth; The mean counts are significantly different with different letters within the same column ($p<0.05$)

Key: AOUK=Ahia ohuu ukwa, AEUK= Ahia Ehere ukwa, ANUK= Ahia nkwo ukwa, EOUK= Eke oha ukwa, CMUK= Cemetry market ukwa, AMUK= Asannetu market ukwa, ARUK= Ariaria market ukwa, AFUK= Afo-ule market ukwa, AWUK= Ahia waterside ukwa and EAUK= Eke-akpara ukwa

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Table 2: Bacteria Isolates from toasted ukwa samples

Sample	*Staphylococcus aureus*	*Bacillus spp.*	*Streptococcus spp.*	*Klebsiella spp.*
AOUK	+	-	+	+
AEUK	+	-	+	-
ANUK	+	-	+	+
EOUK	+	+	+	+
CMUK	+	-	-	-
AMUK	+	+	+	+
ARUK	+	+	+	+
AFUK	+	-	-	+
AWUK	+	+	+	+
EAUK	+	+	+	+

Note: +=presence of the microorganism and - = absence of the microorganism.

Table 3: Fungi Isolates from toasted ukwa samples

Sample	*Aspergillus niger*	*Fusarium Spp.*	*Mucor Spp.*	*Aspergillus Flavus*
AOUK	+	+	-	-
AEUK	-	+	-	-
ANUK	+	-	+	-
EOUK	-	+	+	-
CMUK	+	-	-	-
AMUK	+	+	-	-
ARUK	-	+	+	+
AFUK	+	-	+	+
AWUK	+	-	+	+
EAUK	+	+	-	+

Note: +=presence of the microorganism and - = absence of the microorganism