



Microbiological Quality Assessment Of Unpeeled Groundnut Sold in Yenagoa Metropolis, Nigeria

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ABSTRACTS

This study assessed the microbial quality of unpeeled groundnut sold in locations in Yenagoa metropolis, the Bayelsa state capital, Nigeria. The samples were purchased in triplicates from six markets in Yenagoa metropolis. The microbial assessment was carried out using standard conventional microbiological procedure. Results ranged from 3.68 – 4.56 Log cfu/g, 1.92 – 2.57 Log cfu/g, 1.92 – 2.29 Log cfu/g and 2.61 – 3.94 Log cfu/g for total heterotrophic bacteria, total coliform, total *Staphylococci* and total fungi counts respectively. Analysis of variance showed that there were no significance difference ($P > 0.05$) among the microbial load of the samples from different locations. The microbial density is within International Commission on Microbiological Specification for Foods which is 10^3 cfu/g and 10^5 cfu/g for fungal and bacteria respectively in ready to eat food. The bacteria isolates tentatively identified includes *Staphylococcus aureus*, *E. coli*, *Enterobacter*, *Proteus*, *Bacillus*, *Micrococcus* and *Streptococcus* species. While the fungi diversity includes *Aspergillus*, *Penicillium*, *Mucor* *Fusarium* and *Rhizopus* species. Sorenson qualitative similarity index were 36.36 – 83.33% for the microbial diversity found in the different location of study, which is above critical level of significance = 50% apart from interaction for three location. The occurrence of coliforms and other toxin producing microbes suggest that they could be a source public health concern.

Keywords: Food, Groundnut, Microorganisms, Bayelsa state

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.) which belong to fabaceae family is an edible annual crop cultivated in several region of the world including Nigeria, India, Pakistan, China and the United States. In Nigeria, ground nut is cultivated in vast area of land. Akinnibosun and Osawaru (2015), Odu and Okonko (2012) reported that approximately 1.4 million hectares of land are used to cultivate groundnut especially in the northern region. According to Adebessin et al. (2001), Odu and Okonko (2012), groundnut is an essential crop in most developing countries. Like cashew nut, African breadfruit seed and conophor nut (African walnut or *Tetracarpidium conophorum* nut), groundnut is one of the major indigenous edible nuts consumed in Southern Nigeria (Nwabunnia and Ezeimo, 2015).

Groundnut is an essential oil crop and as such it's an important source of diets for human and animals. For instance, the oil derived from groundnut is used for cooking. Ocheme *et al.* (2014) also noted that groundnut can be roasted in oil and consumed as snacks and or food supplement. In addition, groundnut is used together with cereals such as maize, millets, sorghum for the formulation of weaning food (Ikeh et al., 2001). This could be due to nutrient contents i.e high protein content, omega 6 fatty acid (Akinnibosun and Osawaru, 2015), fats, protein and vitamins.

Groundnut are consumed in several consumed when boiled or roasted (Akinnibosun and Osawaru, 2015; Kayode *et al.*, 2011). Either of the preparation, the epicarp shell can be removed or cooked/roasted together. Groundnut roasted or boiled, peeled or unpeeled is sold in public places such as markets, offices, schools, motor parks, restaurants, supermarkets etc. Groundnut is also hawked along express way in both

rural and urban areas especially in southern Nigeria. Generally, Oranusi and Braide (2012) reported that ready to eat food including groundnut are sold in strategic locations including bus and trains, markets and shopping areas, commercial districts, outside schools and hospital, residential suburbs, factories, and construction sites. They are also used in entertaining visitors and as food (Akinnibosun and Osawaru, 2015). They are also service during ceremonies in some part of Nigeria and other public functions specifically with garden egg.

According to Oranusi and Braide (2012), groundnut is a ready to eat food because they do not require further processing prior to consumption. Most at times, these ready to eat food are processed, packaged and sold in unhygienic condition and the vendors practice little or no sanitary hygiene. Furthermore, several epidemiological reports have implicated ready to eat food as main route through which illness are caused by food- borne pathogens (Odu and Okonko, 2012).

The microbiological quality of groundnut consumed in several region of Nigeria has been reported. Some of the locations include Lagos state (Kayode et al., 2014), Benin-city, Edo state (Akinnibosun and Osawaru, 2015), Onitsha-Owerri (at different business activities - Oba, Okija, Ihiala, Mgbidi, Awomama and Ogbaku), South-Eastern Nigeria (Oranusi and Braide, 2012), roasted groundnut, Kulikuli, Yaji and Dankwa hawked in three major areas in Bauchi, Northeastern (Adebesin et al., 2001). Also microbial characterization of in dakuwa (a Nigerian cereal/ groundnut snack) have also been reported by Ocheme et al. (2014), Okeke et al. (2014), groundnut cake sold in an open market in Samaru, Zaria-Kaduna State (Oko et al., 2015), groundnut cake in major markets in four local governments (Kuto, Lafenwa, Sagamu, Owode) of Ogun state (Iboi, 2010), peanut cake (Kulikuli) (a groundnut-based snack) from agro-ecological zones and districts including humid forest (Oshodi, Mile 2, Ikorodu), derived savannah (Abeokuta, Sagamu, Ibadan), Southern Guinea Savanna (Minna) and Northern Guinea Savannah (Chencheya and Kaduna Central) (Ezekiel et al., 2011). Like most part of the country, groundnut is eaten by several people irrespective of socioeconomic status and age (i.e. children and adults) in Yenagoa metropolis, Bayelsa state, Nigeria. However, information about the microbial quality of groundnut vended in Yenagoa metropolis is scarce in literature, hence the need for this study.

2.0 MATERIALS AND METHODS

2.1. Field Sampling

Unpeeled groundnut vended in Yenagoa metropolis, Bayelsa State Capital was purchased from six markets including viz: Igbogene, Akenfa, Agudama-Epie, Tombia, Opolo and Swali. Three samples were purchased from eat market. A total of 18 samples were obtained from 6 markets. The samples were packaged in sterile Ziploc bag and transported to the laboratory for microbiological analysis within 24 hours.

2.2 Sample preparation

20g of unpeeled groundnut were macerated using electrical blender. 180 ml of de-ionized and sterilized water was added to the paste during bleeding. The blender was washed with sterile water prior to re-use.

2.3 Enumeration and identification of Bacterial and fungal counts

2.3.1 Total viable bacteria and fungi

The microbial density analysis was carried out using pour plate method previously described by Pepper and Gerba (2005), Benson (2002). The blended samples were serially diluted. The microbial population of the unpeeled groundnut were enumerated using five different media including Nutrient Agar (i.e. to enumerate obligate and facultative bacteria); Mannitol Salt Agar (i.e. to enumerate total *Staphylococci* counts), MacConkey Agar (i.e. to enumerate bacteria of the *Enterobacteriaceae* family), Salmonella-Shigella Agar (i.e for Salmonella and Shigella counts) and Potatoes Dextrose Agar (i.e. to enumerate fungi (yeast and mould). All the media were prepared according the manufacturers' guide. Approximately 0.1 ml of the dilutions was aseptically plated into petri dish and the medium prepared accordingly i.e. Nutrient Agar, MacConkey agar, Salmonella-Shigella Agar and Potatoes Dextrose Agar were separately prepared accordingly. The agar plate meant for bacteria were incubated inverted at 37 °C for 24-48 hours, while the Agar plate meant for fungal counts (i.e containing Potatoes Dextrose Agar) was incubated inverted at 30 °C for 3-5 days. The colonies that grew on the medium were counted and expressed as

cfu/g of the groundnut sample. The microbial colonies were isolated into pure cultures by streaking in a fresh medium depending on the microbes. The isolates were tentatively identified.

2.3.2 Identification of total viable bacteria and fungi counts

Based on the isolates that grew on the various medium, they were streaked in Levine's Eosin Methylene Blue (EMB) Agar and incubated at 37° C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli*, while absence of the sheen with large nucleated colonies suggests the presence *Enterobacter* sp (Benson, 2002). From the Mannitol salt agar plate, the presence of yellow pigment, which did not show hemolytic properties on blood agar indicate *Staphylococcus* sp. The presence of black colonies indicates Salmonella, while pink colonies indicate the presence of Shigella (Odu and Adeniji, 2013). The different isolates grown on Nutrient Agar plates was sub cultured into Blood Agar plate and incubated inverted at 37°C. For 24 hours. The presence of swarming and haemolytic characteristics indicates the presence of *Proteus* and *Streptococcus* species respectively. Some basic biochemical test were carried out on the different microbial isolates from nutrient agar slant including gram reaction, citrate, catalase, oxidase, Indole, coagulase, motility, methyl red were carried out using the guide of Cheesbrough (2004) and Benson (2002). The resultant characteristics in this study were compared with those of known taxa using scheme of Cheesbrough (2004) and Bergey's Manual of Determinative Bacteriology by Holt *et al.* (1994).

The fungal isolates were identified by macroscopic and microscopic techniques. The microscopic morphology was determined using Lactophenol cotton blue stain following the method previously described Pepper and Gerba (2005), Benson (2002). The resultant morphology viewed under light microscope and colonial characteristics was compared with the scheme provided by Pepper and Gerba (2004), Ellis *et al.* (2007) and Benson (2002).

2.4 Statistical Analysis

The statistical analysis was carried out on log transformed data using SPSS software version 20. The data were expressed as Mean \pm standard error. The data were subjected to ANOVA at $\alpha = 0.05$. The source of the observed difference was determined using Tukey Honestly Significant Difference test statistics. Similarity index was determined at Critical level of significance = 50% for microbial diversity similarity between samples from different locations using Sorenson qualitative index previously described by Ogbeibu (2005). The charts with error bars, which was determined at 95% interval level was plotted using Paleontological statistics software package by Hammer *et al.* (2001). The chart without error bar was plotted with Microsoft excel.

3.0 RESULTS AND DISCUSSION

Figure 1- 4 presents the microbial population found in unpeeled groundnut sample sold in major markets of Yenagoa metropolis, Bayelsa state, Nigeria. For the different bacterial groups, their density ranged from 3.68 – 4.56 Log cfu/g (Figure 1), 1.92 – 2.57 Log cfu/g (Figure 2) and 1.92 – 2.29 Log cfu/g (Figure 3) for total heterotrophic bacteria, total coliform and total Staphylococci counts respectively. Basically there was no significance difference ($P > 0.05$) among the various location in each of the different bacteria groups. The salmonella-Shigella was not detected in the various samples. The fungal density in the samples ranged from 2.61 – 3.94 Log cfu/g, being not significantly different ($P > 0.05$) among most of the locations of study (Figure 4).

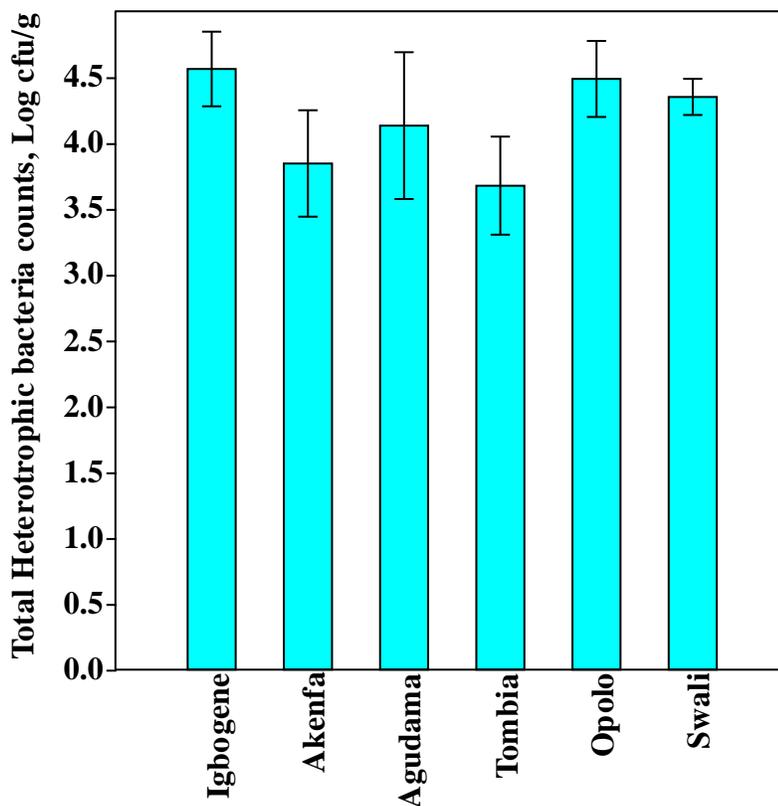


Figure 1: Total Heterotrophic bacteria count of unpeeled groundnut sold in some major markets in Yenagoa metropolis, Nigeria

The absence of significant variation among the microbial parameters studied could be due to the fact that groundnut is homogenous (i.e. most of vendor sells ground nut in more than one market the samples were purchased from, which is basically transacted at different days in the same week. Furthermore, no significant variation among the various locations in each of the microbial parameters could also be due to similarity in handling processes (Orutugu et al., 2015). The apparent difference in the samples from some locations could be due to the hygienic condition of the area. However, the microbial density (bacteria and coliform) of the groundnut samples is with 10^3 cfu/g and 10^5 cfu/g for fungal and bacteria respectively in ready to eat food as recommended by International Commission on Microbiological Specification for Food (1998) cited in Iboi, (2010).

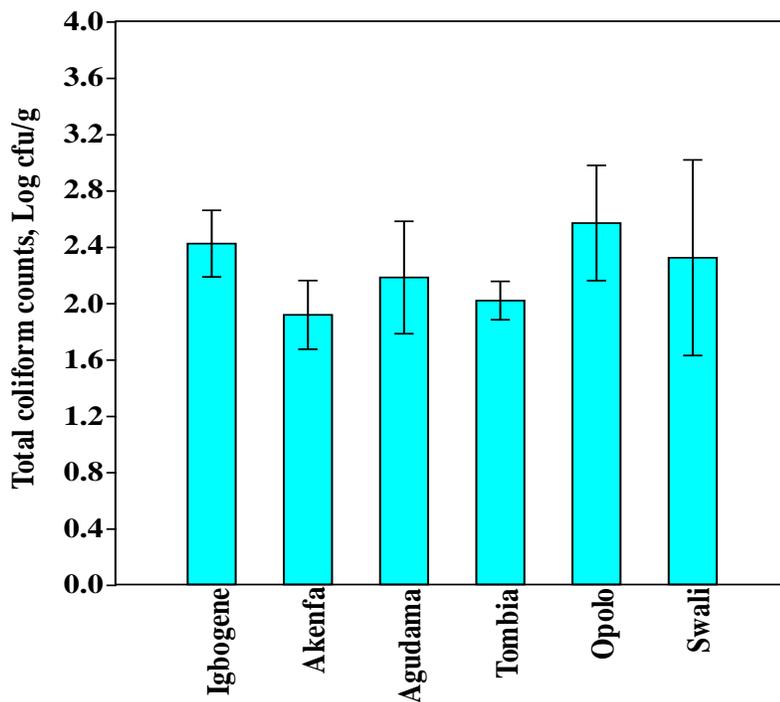


Figure 2: Total coliform count of unpeeled groundnut sold in some major markets in Yenagoa metropolis, Nigeria

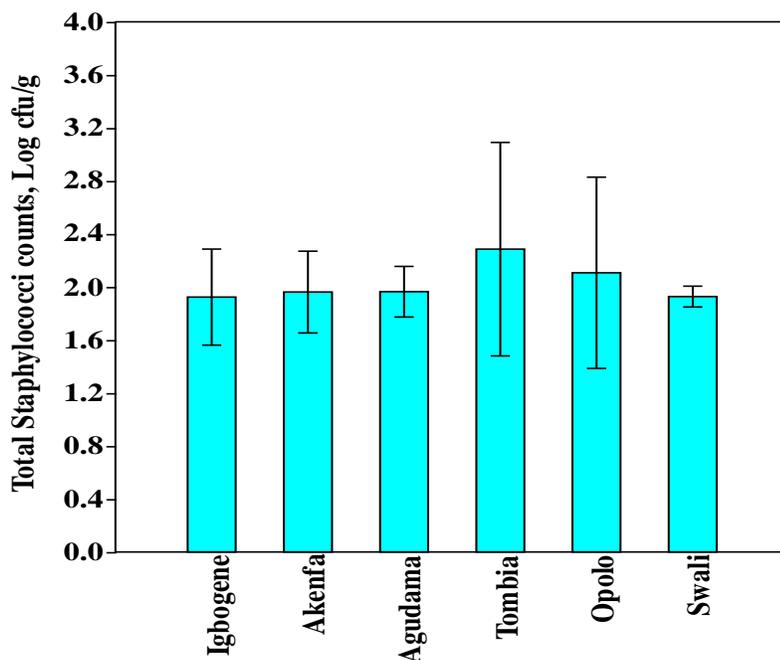


Figure 3: Total Staphylococci counts of unpeeled groundnut sold in some major markets in Yenagoa metropolis, Nigeria

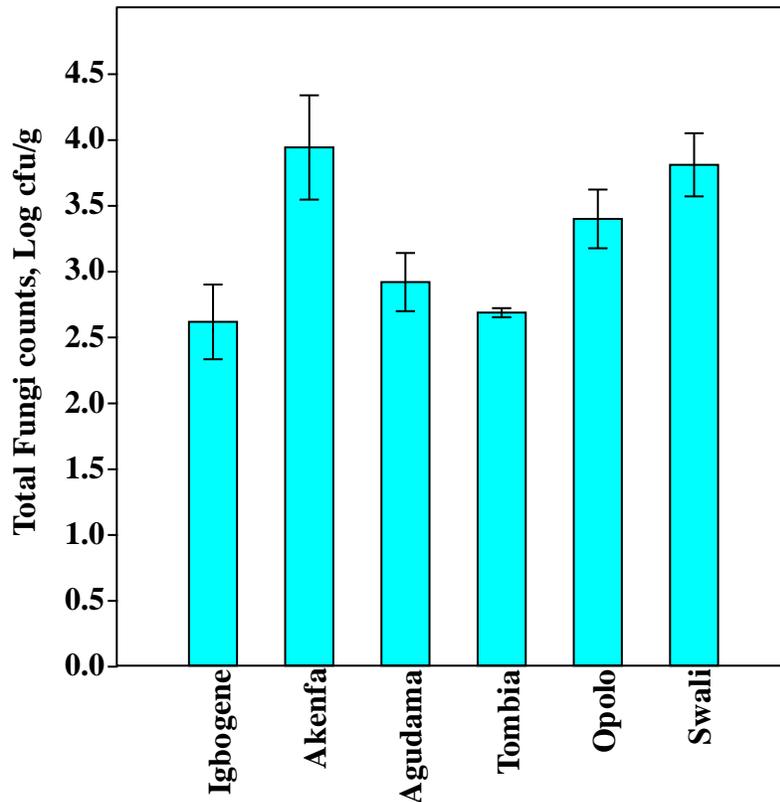


Figure 4: Total fungi counts of unpeeled groundnut sold in some major markets in Yenagoa metropolis, Nigeria

The microbial density from this study has some similarity with work of other authors on groundnut sold vended in other location in Nigeria. For instance, Akinnibosun and Osawaru (2015) reported total heterotrophic bacteria and total fungal counts in unpeeled ground nut sold in Benin City in the range of $0.5 - 2.1 \times 10^4$ cfu/g and $3.4 - 6.6 \times 10^4$ cfu/g respectively. Oranusi and Braide (2012) reported total heterotrophic bacteria, total coliform and total fungal counts in groundnut sold along Onitsha-Owerri express way in the range of $1.1 - 58.0 \times 10^4$ cfu/g, $3.5 \times 10^2 - 4.3 \times 10^4$ cfu/g and $2.6 \times 10^2 - 4.3 \times 10^4$ cfu/g respectively. Adebessin et al. (2001) reported total heterotrophic bacteria and total fungal counts in roasted groundnut sold in some market in Bauchi town ranged from $4.25 - 5.82 \times 10^5$ cfu/g and $7.0 - 8.6 \times 10^4$ cfu/g respectively. This slight difference with the findings of this study with previous work could be due to variation in socio-economic, life style, and demographic criteria.

Table 1 presents microbial diversity found in unpeeled groundnut sold in some location in Yenagoa metropolis, Bayelsa state, Nigeria The bacteria diversity include *Staphylococcus aureus*, *E. coli*, *Enterobacter*, *Bacillus*, *Proteus*, *Micrococcus* and *Streptococcus* species. While the fungi diversity include *Aspergillus*, *Rhizopus*, *Mucor*, *Fusarium* and *Penicillium* species. Figure 5 present the similarity index of the microbial diversity between each of the location based on Sorenson qualitative index. Based on the microbial diversity, the similarity interaction between each location ranged from 36.36 – 83.33%, with the similarity index above critical level of significance = 50% apart from interaction between

Akenfa-Tombia, Agudama-Epie-Opolo and Opolo - Swali. This similarity index showed that majority of the microbes found in groundnut from the various location are basically related.

Table 1: Microbial isolates found in unpeeled groundnut sold in some locations in Yenagoa metropolis, Bayelsa state, Nigeria

Microorganisms	Igbogene	Akenfa	Agudama- Epie	Tombia	Opolo	Swali
<i>E. coli</i>	+	+	+	+	-	+
<i>Streptococcus</i> sp	-	+	-	-	+	-
<i>Proteus</i> sp	-	+	-	-	-	+
<i>Staphylococcus aureus</i>	+	+	+	+	+	+
<i>Micrococcus</i> sp	-	-	-	+	+	-
<i>Enterobacter</i> sp	-	-	-	+	-	+
<i>Bacillus</i> species	+	+	+	-	-	+
<i>Aspergillus</i> sp	+	+	+	+	+	+
<i>Penicillium</i> sp	-	-	+	+	-	-
<i>Fusarium</i> sp	-	+	-	-	-	-
<i>Mucor</i> sp	+	+	-	-	+	+
<i>Rhizopus</i> sp	-	-	-	+	+	-

+ = Present; - = Absent (some of the microbes are only present in one out of the three samples obtained from each market)

The occurrence of microorganisms in the samples could be associated to poor sanitary practices during processing, packaging, handling and distribution to the final consumers (Oko et al., 2015). Some of the various microbes tentatively identified are organisms of public interest due to their ability to cause disease condition (Adebesin et al., 2001). For instance, *S. aureus* is known to cause enterotoxigenicity due to the production of enterotoxin (Adebesin et al., 2001). Diseases such as bacteremia/septicemia, endocarditis and respiratory tract can be caused by *Bacillus* species, sinusitis, sore throat are caused by some species of *Streptococcus* (Orutugu et al., 2015; Izah et al., 2015a), urinary tract infection can be caused by some species of *Proteus* species (Izah and Ohimain, 2016; Izah et al., 2015a). Also, the presence of *Escherichia coli* and *Enterobacter* sp indicates the presences of fecal contamination (Izah et al., 2015a). Many *E.coli* and *Enterobacter* sp strains are enterotoxigenic (Ezekiel et al., 2011). *Bacillus* sp is typically found in the soil (Odu and Okonko, 2012), they would have entered the samples when they come in contact with soil.

Some of the fungi diversity found in the groundnut in this study is toxic producing microbes. For instance, some species of *Penicillium*, *Fusarium* and *Aspergillus* sare known to produces mycotoxins in food (Izah et al., 2015a). According to Dubey and Maheshwari (2013), *Aspergillus* species (produces aflatoxins and ochratoxins), *Fusarium* species (produces Moniliformin and Fumornisins), *Penicillium* species (produces Citrinin and Cyclopiazonic acid). Ezekiel et al. (2012) reported that groundnut contain *Aspergillus flavus* and *A. tamari* which produced aflatoxin B1 (AFB1), AFB2 and AFG1. Aflatoxins are mycotoxins produced by *Aspergillus* species that grow in many cereals and oilseeds, and are known to be hepatotoxic, carcinogenic and teratogenic (Ezekiel et al., 2012). Several diseases have been attributed to the mycotoxins produced by these moulds. For instance, vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and death are assoicated with acute aflatoxicosis in humans (Akinyemi et al., 2011).

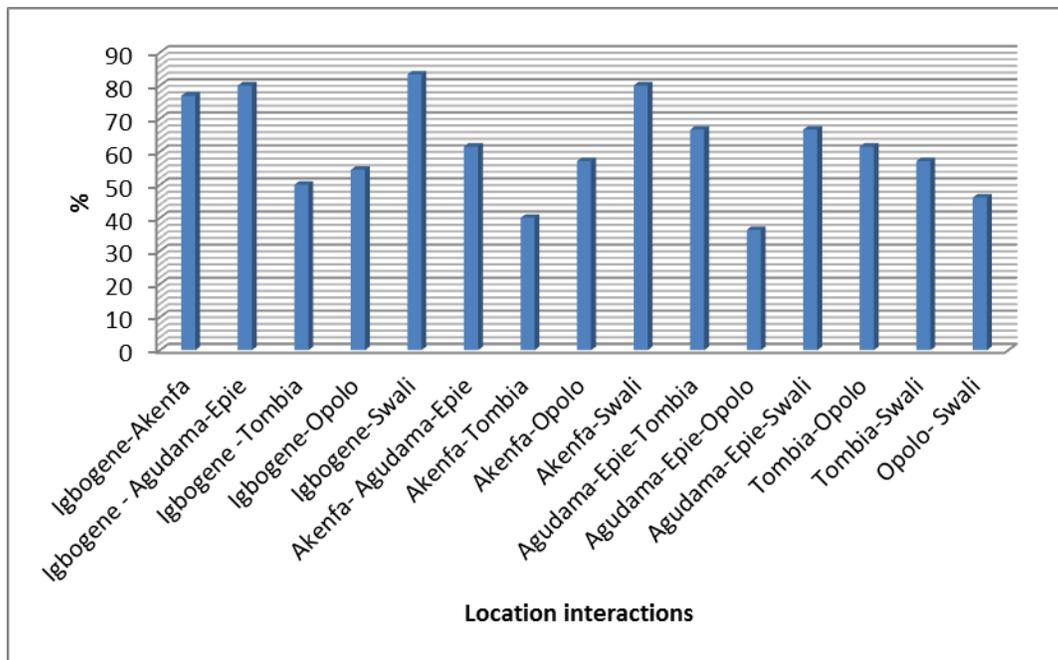


Figure 5: Similarity index of microbial diversity found in unpeeled groundnut sold in some major markets in Yenagoa metropolis, Nigeria

The microorganism identified in this study has been reported from ground nut from ground nut vended in other location in Nigeria. For instance, Adebessin et al. (2001) reported the occurrence of *Staphylococcus aureus*, *Micrococcus* sp. and *Bacillus cereus* (bacteria), *Aspergillus flavus*, *A. niger*, *A. tamarii*, *P. citrinum*, *R stolonifer*, *M. phaseolina* (fungi) from roasted groundnut sold in markets from Wunti, Yelwa and Railway areas in Bauchi town. Oranusi and Braide (2012) reported that *Enterobacter* sp, *Proteus* sp, *S. aureus*, *S. epidermidis* *S. liquefaciens*, *B. Megaterium*, *Aspergillus*, *Fusarium* spp as microbes associated with groundnut vended along Onitsha-Owerri express way at Oba, Okija, Ihiala, Mgbidi, Awomama and Ogbaku communities. Akinnibosun and Osawaru (2015) reported *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus* sp., *Streptococcus* sp. and *Proteus vulgaris* (bacteria) and *Aspergillus flavus*, *Aspergillus niger*, *Neurospora* sp., *Mucor* sp., *Rhizopus* sp., *Penicillium* sp., *Trichoderma* sp. and *Fusarium* sp (fungi) as microbes associated with ground nut sold in Benin city Nigeria.

Furthermore, the microbes isolated from this study have been identified in other read to eat food including Zobo (Izah et al., 2015b, 2016a; Alo et al., 2012; Amusa et al., 2005; Nwachukwu et al., 2007; Ezearigo et al., 2014; Ezeigbo et al., 2015; Risiquat, 2013; Omemu et al., 2006; Onuoha and Fatokun, 2014), Kunu drink (Orutugu et al., 2015; Ayo et al., 2004; Amusa and Odunbaku, 2009; Amusa and Ashaye, 2009; Mbachu et al., 2014; Essien et al., 2009; Ogbonna et al., 2011; Edward and Ohaegbu, 2012; Musa and Hamza, 2013), Suya (Kigigha et al., 2015a; (Hassan et al., 2014; Afolabi and Odubanjo, 2015; Onuorah et al., 2015; Manyi et al., 2014; Egbibi and Seidu, 2011), gari (Kigigha et al., 2015b; Olopade et al., 2014), sliced fruits i.e. Pawpaw, Pineapple, Watermelon (Izah et al., 2015b), apple and cucumber (Izah et al., 2016b), smoked fish (Ineyougha et al., 2015)

The group of microbes identified in the samples have been reported in different frozen food material and its handling including ready to-eat frozen sea-foods processed in Ijoraolopa, Lagos State (Okonko et al., 2008a,b), frozen shrimps processed in Ibadan and Lagos (Okonko et al., 2008c), seafood handlers hygiene in Ibadan and Lagos (Okonko et al., 2009a), Seafood processors and water used in processing (Okonko et al., 2009b). The presence of microbes in ready to eat food is associated with are improper handling during processing and hawking (Adebessin et al., 2001)

4.0 CONCLUSION

Groundnut is consumed by several people irrespective of their socio-economic status and sex. Groundnut is sold in public places. This study assessed the microbial quality of unpeeled groundnut sold in some locations in Yenagoa metropolis, Bayelsa state Nigeria. The study found that the microbial density is with the limit specified by International Commission on Microbiological Specification for Food. Furthermore, some of the microbes tentatively identified are toxin producers as such they are microbes of public health importance. The density and diversity of microorganisms found in ground nut sold in Yenagoa metropolis could be reduced with improved handling processes.

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