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Bacteriological Evaluation Of Ready-To-Eat Roasted Peeled Groundnut Seeds Sold At A Community Market

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Abstract
Background*: Groundnut provides the body with energy and nutrients which are vital to health and at the same time a reservoir for bacteria which are of public health importance. This study evaluates the bacteriological quality of ready-to-eat roasted peeled groundnut seeds sold at Eke Agbani in Enugu East L.G.A. of Enugu State, Nigeria.*
Materials and Methods*: A random sampling method was used to collect ready-to-eat roasted peeled groundnut seeds from different vendors. A total of ten (10) wraps of different ready-to-eat roasted peeled groundnut seeds were purchased from different vendors and were screened using standard microbiological methods.*
Results: *The mean bacterial load of the samples ranged from $1.2x10^7$ to $4.0x10^7$ cfu/g. There were significant difference ($p<0.05$) between bacterial loads of each sample. The samples had 100% contamination. The bacterial density revealed Escherichia coli 6(27.2%), Klebsiella spp 8(36.4%) and Staphylococcus aureus 8(36.4%). There was no significant difference ($p>0.05$) in the percentage occurrence of isolates.*
Conclusion*: The presence of bacteria in the ready-to-eat roasted peeled groundnut seeds are indication of poor handling techniques, poor personal hygiene and environmental contamination, hence, groundnut vendors are advised to observe sanitary procedures to reduce contamination.*
Key Words*: Ready-to-eat roasted peeled groundnut, bacteria, Eke Agbani, contamination, vendors, bacterial load.*

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I. Introduction

Groundnut (*Arachis hypogaea* L.) is a leguminous plant which belongs to fabaceae family. This plant is an edible annual cash crop cultivated in many locations of the world including Nigeria[1]. Groundnut occupies an important position in the economy of developing nations[2]. Groundnut is nutrient dense agricultural produce, which is very high in energy due to its high fat and protein content [3]. The carbohydrate content of groundnut is relatively low, being under 30% of the whole nut. The nut has relatively high fiber content [4]. It is an essential oil crop and as such, it is an important source of diets for humans and animals[1]. Ocheme *et al*.[5] noted that groundnut can be roasted in oil and consumed as snacks and or food supplement. Moreover, Ike *et al*.[6] noted that groundnut is consumed together with cereals such as maize, millets, sorghum for the formulation of weaning food for children. This could be due to its nutritional contents like protein, omega-6 fatty acids[7], fats, vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium[8].

Groundnut, is an important food and fodder crop in the farming systems of developing countries [9]. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil[10]. It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer).Peeled, unpeeled or boiled/roasted groundnuts seeds are sold in public places such as markets, offices, schools, motor parks, restaurants, streets, buses and supermarkets[1]. They are also used in entertaining visitors where they are served with garden egg and as food where they are eaten with fermented food like garri [7]. These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries[11]. Oranusi and Braide [12] noted that groundnut is a ready to eat food when they do not require further processing prior to consumption. Most times, these vendors process, package, peel and sell in unhygienic condition and observe little or no sanitary practices. Another worrying situation is that there is cross contamination where the same hand used in peeling the groundnut is used to receive money from their customers or even wipe their noses, thereby risking the health of the consumers.

One of the major constraints facing the productivity and availability of healthy groundnut produce worldwide are the losses and spoilage caused by fungi, bacteria, viruses, insects, nematodes and parasites[13]. Seed-borne disease has been found to affect the growth and productivity of crop plants[14]. A seed-borne pathogen present

externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of growth by systemic or local infection[15].

As a result of improper processing and storage conditions, groundnuts and its products may be contaminated with microorganisms[16]. The number and type of microbes present on the produce is important in deterioration and numerous molds may be involved, but most common are species of *Aspergillus*, *Penicillum* and Fusarium[17]. Tobin-West *et al.* [18] isolated species of *Bacillus*, *Salmonella*, *Pseudomonas* and *Escherichia coli* from groundnuts; *Streptococcus spp*., *Staphylococcus spp*., *Proteus vulgaris*, *Micrococcus spp*. and *Bacillus spp*. have been isolated from groundnuts[19,7], as well as *Enterobacter spp*. and *Escherichia coli* [1]. Contamination of groundnut by these organisms can be due to poor personal hygiene by the vendors, exposure to polluted environment and poor environmental sanitation [20]. Elegbede [21] reported that the activities of microorganisms sometimes lead to the release of toxins that are harmful when consumed by humans and animals, thereby posing risks to the life of consumers. Although nut-associated outbreaks of infection are relatively uncommon, recent outbreaks associated with the consumption of groundnut and groundnut products have raised awareness of nuts as a potential vehicle for foodborne illness [22].

The microbiological quality of groundnut consumed in several regions of Nigeria has been reported. Some of the locations include Lagos State [14], Benin-city, Edo State[7], Onitsha-Owerri, South-Eastern Nigeria [12]. However, information about the bacteriological evaluation of ready-to-eat peeled roasted groundnut seeds vended at Eke Agbani in Enugu East L. G. A., Enugu State, Nigeria is scarce in literature, hence the need for this study.

II. Materials And Methods

Study Area: This study was carried out in Applied Microbiology and Brewing Departmental laboratory, Enugu State University of Science and Technology. The roasted peeled groundnut seeds were collected from different vendors at Eke Agbani market.

Collection of Samples: Ten (10) wrap samples of ready-to-eat peeled roasted groundnut seeds were purchased from different vendors at Eke-Agbani market in Enugu East LGA of Enugu State. The samples were labelled and taken to the laboratory unit of Applied Microbiology and Brewing, Enugu State University of Science and Technology (ESUT) and were analysed immediately for bacteria contamination.

Sample Preparation: A total of 20g of each of the wrap roasted peeled groundnut samples were macerated using sterile porcelain pestle and mortar. One-gram (1g) of each of the blended samples was suspended in 10 ml of sterile peptone water in a test tube. This was mixed properly to homogenize the mixture and was labelled as stock. A ten- fold serial dilutions of the samples were then made [23]. A total of 9 ml of the sterile normal saline was measured into six test tubes. One millilitre (1 ml) of the stock was collected using a pipette and serially diluted into the first test tube till the sixth test tube. 10^{-5} was used as the dilution factor and 0.1 ml was added into a sterile duplicate petri-dishes before the sterile media were poured. This was swirled properly and allowed to gel. The petri-dishes containing mannitol salt agar, nutrient agar, MacConkey agar and Salmonella Shigella agar were incubated at 37^0C for 24 hr to obtain the bacterial counts. After incubation, the colonies on the plates were counted using colony counter which were then transferred into nutrient agar slants for identification.

Characterization and Identification of Bacterial Isolates

All the bacteria isolated were sub-cultured into nutrient agar plates to obtain pure cultures. The bacteria were characterized using Gram staining and biochemical tests (catalase, coagulase, oxidase, citrate, indole, methyl red test and sugar fermentation tests) [23].

Gram Staining Procedure: The prepared smear was air-dried and heat-fixed. The slides were flooded with crystal violet for 60 sec and washed off with water, then each smear again was flooded with iodine solution for 1 minutes and was washed off with water. Thereafter, the slide was decolorized with acetone until the solvent draining from the slide appeared colorless and was immediately washed with water. It was counterstained with safranin for 30sec and washed off with water. The slides were blotted and air dried and observed under oil immersion objectives lens (x100).

Biochemical Analysis of the Isolates

The biochemical test performed were as follows;

Catalase Test : A drop of 3% hydrogen peroxide was made on one side of clean microscopic slide and a drop of water on the other side as the control. A colony was then collected with a sterile applicator stick and smeared on the slide containing hydrogen peroxide and was also done for the control. The presence of bubbles within 10sec which indicated positive result was observed and recorded.

Oxidase Test: A drop of prepared oxidase reagent (tetra methyl p-phenylenediamine dihydrochloride) was made on whatman filter paper. A colony of each isolate was collected with a sterile applicator stick and smeared on the soaked filter paper. The presence of purple colour which indicated a positive result was observed and recorded.

Indole Test (kovac's method): Peptone water (5m1) was dispensed into test tubes and sterilized at 121^0C/15p.s.i, a loopful of each isolate was into each test tube and incubated for 3 days. Kovac's reagent (3 drops) was placed into the test tubes after incubation. The presence of red ring which indicated a positive result was observed and recorded.

Citrate utilization tests: Simmon citrate agar (2.4g) was added to 100ml of distilled water in a 250ml conical flask. It was sterilized by autoclaving at 121^0C for 15minutes. It was allowed to cool to 50^0C. It was properly shaken, poured into sterile petri dishes and allowed to solidify. Saline preparation of the organisms was inoculated inside the medium by streaking and incubated at 37°C for 24hours. A change in colour from green to blue indicates a positive result.

Coagulase Test: A drop of normal saline was made on a clean slide, a colony was then collected with a sterile applicator stick and smeared on the slide. Test suspension were treated with a drop of plasma and mixed well with the applicator. Observations for clumping was made and recorded.

Sugar fermentation: Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially gram-negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water. One hundred milliliters of peptone water were prepared in five different conical flasks, for the four sugars, 0.5ml was discarded from each flask (which serves as control for the test) and 0.5ml of Andrade indicator was added in each conical flask; then 1g of each sugar was added into the flask and was shaken properly for the sugars to dissolve. 3ml from each flask and 5ml from glucose was measured out into 5 different test tubes. Durham's tube was added into the tubes containing the sugars for gas collection, the tubes with their content were then sterilized by autoclaving at 121^0C for 10minutes. The tubes were then inoculated with 24hour old culture of the isolates and incubated at 37^0C for 24hours. Acid production was indicated by colour change from orange to pink colour while gas production was indicated by the presence of gas in the Durham's tube, the control tube was not inoculated.

III. Results

Total Bacteria Count from Roasted peeled Groundnut seed Samples Sold at Eke Agbani.

Roasted peeled groundnut seed samples from Eke-Agbani was analysed for the presence of bacteria. The bacterial populations of the roasted peeled groundnut seed samples were presented in table 1. The total bacterial load ranged from $1.2x10^7$ to $4.0x10^7$ cfu/g. The enumeration of highest population of bacteria count was seen in sample F with a total count of $4.0x10^7$cfu/g and with least count seen in sample J with total count of $1.2x10^7$ cfu/g.

Table 1. Total Bacteria Count from Roasted Peeled Groundnut seed Samples Sold at Eke-Agbani.

Samples	Mean of bacterial counts (cfu/g)
A	1.6×10^7
B	2.3×10^7
C	1.4×10^7
D	3.1×10^7
E	1.5×10^7
F	4.0×10^7
G	1.7×10^7
H	1.8×10^7
I	2.4×10^7
J	1.2×10^7

Occurrence of Bacteria on Roasted Peeled Groundnut seed Samples Sold at Eke-Agbani

Roasted Peeled groundnut seed samples sold at Eke-Agbani were analysed for the presence of bacteria. All ten roasted peeled groundnut seed samples were prevalent of bacterial contamination (table 2).

Table 2: Occurrence of Bacteria on Dried Groundnut Samples Sold at Eke-Agbani

Samples	Number of samples	Number of positive samples	Percentage (%)
Roasted Peeled groundnut	10	10	100

Morphology, Microscopy and Biochemical Properties of Bacterial Isolates

The presence of bacteria on roasted peeled groundnut seed samples sold at Eke-Agbani were examined. The bacteria isolates showed varying biochemical, microscopy and morphological characteristics on different media. The result is shown in table 3.

Table 3: Morphology, Microscopy and Biochemical Properties of Bacterial Isolates

Isolates	Colonial Appearance on Media	Biochemical Tests											Possible organisms
		Gram reaction	Cat	Ox	Coa	Ct	Ind	Sugar fermentation					
								Glu	Fru	Mal	Man	La	
A	Greyish white, smooth, mucoid and medium sized colonies on Nutrient agar	Gram -ve short rod in pairs	+ve	-ve	-ve	-ve	+ve	AG	AG	AG	AG	AG	*Escherichia coli*
B	Large mucoid glistering pink on MacConkey agar	Gram -ve short rod in pairs	+ve	-ve	-ve	+ve	+ve	A	A	A	A	A	*Klebsiella* sp.
C	Yellow coloured large colonies Mannitol salt agar	Gram +ve cocci in clusters	+ve	-ve	+ve	-ve	-ve	A	A	A	A	A	*Staphylococcus aureus*

KEY: Ind = Indole test, Ct = Citrate utilization Test, Cat = Catalase test, Coa = Coagulase test, Oxi = Oxidase test Ma = Mannitol, La = Lactose, Glu = Glucose, Fru = D-Fructose, Mal = Maltose, +ve = positive, -ve = negative, A = Acidic, AG = Acidic and Gas, G = Gas,

Frequency Occurrence of the Bacterial Isolates

The presence of bacteria on roasted peeled groundnut seed samples sold at Eke-Agbani were examined. A total of 22 bacterial isolates were seen in all the samples. The result is shown in table 4.

Table 4: Frequency Occurrence of the Bacterial Isolates

Organisms identified	Number of samples	Occurrence (10)	Total (%)
Escherichia coli	10	6	27.2
Klebsiella sp.	10	8	36.4
Staphylococcus aureus	10	8	36.4
Total (%)	**30**	**22**	**100**

Statistical Analysis: Statistical Package for the Social Science (SPSS) was used for the data analysis. Analysis of variance (ANOVA) was used to arrive at statistical decision.

IV. Discussion

Food safety in any society is nothing to be compromised. Roasted peeled groundnut is consumed by several people irrespective of their socio-economic status and gender. The presence of bacteria in ready-to-eat roasted peeled groundnut sold in the market is of public health importance to the consumers and to the general public as a whole. This study therefore evaluates the bacteriological quality of ready-to-eat roasted peeled groundnut sold at Eke Agbani in Enugu East L.G.A, Enugu State, Nigeria. In this study, the mean bacterial load of the samples ranged from $1.2x10^7$ to $4.0x10^7$ cfu/g (table 1). The enumeration of highest population of bacteria count was seen in sample F with a total count of $4.0x10^7$ cfu/g and least count in sample J with total count of $1.2x10^7$ cfu/g. The differences in the bacterial load could be due to handling procedures and hygienic practices of the vendors. The bacterial 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[7] reported total heterotrophic bacteria counts in unpeeled groundnut sold in Benin City in the range of $0.5\text{-}2.1x10^4$cfu/g respectively. Oranusi and Braide [12] reported total heterotrophic bacteria and total coliform counts in groundnut sold along Onitsha-Owerri express way in the range of $1.1 – 5.8x10^4$cfu/g and $3.5x102 – 4.3x10^4$cfu/g. Adebesin *et al.* [8] reported that total heterotrophic bacteria in roasted groundnut sold in some markets in Bauchi town ranged from $4.25 – 5.82\ x10^5$cfu/g. This slight difference in the findings of this study with previous work could be due to variations in socio-economic, life style and demographic criteria.

The bacterial diversity found in roasted peeled groundnut sold at Eke-Agbani market includes *Staphylococcus aureus* 8(36.4%), *Escherichia coli* 6(27.2%) and *Klebsiella* species 8(36.4%) (table 4) . The occurrence of these bacteria in the samples could be associated with poor sanitary practices during processing, packaging, handling and distribution to the final consumers [24]. Some of the various bacteria tentatively identified are organisms of public interest due to their ability to cause disease condition [8]. For instance, *S. aureus* is known to cause enterotoxigenicity due to the production of enterotoxin [8]. Also, the presence of *Escherichia* coli indicates

the presence of fecal contamination[25]. Ezekiel *et al.* [3], reported that many *E. coli* strains are enterotoxigenic, hence, this calls for public health attention.

The bacteria identified in this study has been reported from groundnut vended in other locations in Nigeria. For instance, Adebesin *et al.* [8] reported the occurrence of *Staphylococcus aureus, E. coli, Klebsiella* sp. and *Bacillus cereus* from roasted groundnut sold in markets at Wunti, Yelwa and Railway areas in Bauchi town. Oranusi and Braide[12] reported *Klebsiella* sp., *Proteus* sp, *S. aureus*, *S. epidermidis*, *S. liquefaciens*, *B. Megaterium*, as bacteria associated with groundnut vended along Onitsha-Owerri express way at Oba, Okija, Ihiala, Mgbidi, Awomama and Ogbaku communities. Akinnibosun and Osawaru[7] reported *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, *Micrococcus* sp., *Streptococcus* sp. and *Proteus vulgaris* as bacteria associated with ground nut sold in Benin City Nigeria. Hence, there is need for community health officers to educate various vendors on importance of healthy living.

V. Conclusion

This study evaluates the bacteriological quality of ready-to-eat roasted peeled groundnut seeds sold at Eke Agbani market in Enugu East L.G.A., Enugu State, Nigeria. The results revealed that the samples were contaminated with different bacteria which could be due to personal hygiene, poor environmental sanitation and handling procedures. Therefore, proper handling procedures, personal hygiene and public enlightenment should be recommended to reduce the contamination of roasted peeled groundnut sold by vendors.

Conflict of interest statement

The author declares that she has no conflict of interest.