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Microorganisms and Aflatoxin Content in Ready-To-Eat Groundnut Paste from Some Markets in Anambra and Edo States, Nigeria

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Abstract- This study was undertaken to quantitatively and qualitatively estimate microbial and aflatoxin content in ready – to – eat groundnut pastes sold in some markets in Anambra and Edo States, Nigeria. A total of 100 samples of ready- to eat – groundnut pastes packaged in plastic cans and low density polyethylene were purchased from some marketsin Anambra State (Head Bridge, Ogbaru, Agulu, Mgbuka and Awka) and Edo State (Oba market, Santana, New Benin, Uselu and Oregbeni). The samples were analyzed microbiologically and physicochemically using standard procedures. Aflatoxin detection was done using Enzyme-Linked Immunosorbent Assay (ELISA). Bacteria species associated with the samples were identified as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus roseus*, *Esherichia coli* and *Pseudomonas aeruginosa* while fungi include *Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, Aspergillus fumigates* and species of *Penicillium* and *Fusarium.*

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Abstract- This study was undertaken to quantitatively and qualitatively estimate microbial and aflatoxin content in ready – to – eat groundnut pastes sold in some markets in Anambra and Edo States, Nigeria. A total of 100 samples of ready- to eat – groundnut pastes packaged in plastic cans and low density polyethylene were purchased from some marketsin Anambra State (Head Bridge, Ogbaru, Agulu, Mgbuka and Awka) and Edo State (Oba market, Santana, New Benin, Uselu and Oregbeni). The samples were analyzed microbiologically and physicochemically using standard procedures. Aflatoxin detection was done using Enzyme-Linked Immunosorbent Assay (ELISA). Bacteria species associated with the samples were identified as *Staphylococcus aureus*, *Bacillus cereus* *Bacillus subtilis*, *Micrococcus roseus*, *Esherichia coli* and *Pseudomonas aeruginosa* while fungi include *Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, Aspergillus fumigates* and species of *Penicillium* and *Fusarium.* Total bacterial count ranged from $2.50 \pm 0.61 \times 10^4 - 5.54 \pm 0.50 \times 10^4$ cfu/g. Lowest bacterial count($2.50 \pm 0.61 \times 10^4$ cfu/g) was obtained in Awka market while the highest($5.54 \pm 0.50 \times 10^4$ cfu/g) was from Oba market. The fungal counts ranged from $1.70 \pm 0.99 \times 10^3 - 5.60 \pm 0.65 \times 10^3$ cfu/g with Uselu market having the lowest counts ($1.70 \pm 0.99 \times 10^3$ cfu/g) and Ogbaru market in Anambra State having the highest counts($5.60 \pm 0.65 \times 10^3$ cfu/g). Aflatoxin content of the samples ranged from $1.1 \pm 0.07 - 143.9 \pm 2.72$ ppb. The presence of pathogenic bacteria and aflatoxin in the ready-to-eat groundnut paste pose a potential health challenge to the consumers in some parts of Anambra and Edo States, Nigeria.

Keywords: ready-to-eat groundnut paste, microbial quality, aflatoxin content, ELISA method.

I. Introduction

Groundnut (*Arachis hypogaea*) which is also known as peanuts, monkey-nut and goobers, are edible seeds of legume plant that grow to maturity in the ground rather than on aerial part of the plant. As a legume, groundnut belongs to the botanical family Fabaceae (also known as Leguminosae, and commonly known as the bean or pea family. The initial domestication of groundnut may have taken place in North – Western Argentina or in South – Eastern Bolivia where the peanut landraces with the most wide – like features are grown today (Hepper, 2001).

Groundnut is widely grown in the tropics and sun tropics, being important to both small holder and large commercial producers. It was introduced into Nigeria in the 16th century and it has been estimated that about 1.4 million hectares is cultivated for groundnut in Nigeria. Nigeria is the 4th largest producer of groundnut with a proportion of 4.5% of the total world production. It follows China, India, and USA with 45.5%, 18.2% and 6.8% respectively of total world groundnut production. In West Africa, Nigeria produces 41% of the total groundnut production (Taru *et al*., 2008).

Groundnut contains high quality edible oil (50%), easily digested protein (25%) and carbohydrate (20%) (Taru *et al*., 2008; Muhammad – Lawal *et al*., 2012). Groundnut seeds are nutritionist source of vitamin E, felacin, calcium, zinc, iron, phosphorus, niacin, magnesium, potassium, riboflavin, and thiamine (Surendranatha *et al*., 2011). It is widely consumed in Nigeria as roasted or boiled nuts. Groundnut processing is basically the transformation of raw groundnuts and would yield edible oil which can be refined to get vegetable oil.

Groundnut paste is a food paste made primarily from ground dry roasted groundnut and is popular in the North America, United Kingdom, Phillipines and Netherlands. It is mainly used as sand – wish spread, sometimes in combination (peanut butter and jelly sand – wish). In Nigeria, ready – to – eat groundnut paste is consumed in combination with bread, kolanuts and garden egg (*Solanum melongena*). It is rich in protein, fat and oil and is a very good source of vitamins (Achu *et al.,* 2005). Groundnut paste has been frequently associated with food illness in which initial contamination is traceable to food handlers. Numerous epidemiological reports and studies have implicated food of ready – to –eat origin as the major vehicles associated with illness caused by food – borne pathogen (Sokari, 1991).

Due to unhygienic practices during processing, groundnut pastes can be contaminated with microorganisms. Species of *Proteus, Serratia, Micrococcus, Bacillus, Staphylococcus, Samonella,* and *Escherichia* were isolated from peanut butter samples obtained in Port Harcourt (Odu NN and Okonko *et al.,*2012). Also, Elzupir *et al.,* (2011) reported total aflatoxin concentration in peanut butter ranging from 26 .6 -853µg /Kg in Sudan. Microbial and aflatoxin contamination is a major concern in food safety as they

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affect humans, animal and economic growth of any nation (Hwang *et al.,* 2004).

II. Materials and Methods

Samples of ready – to – eat groundnut pastes were collected randomly between December, 2015 and February, 2016 from five (5) major markets each in Anambra State namely Head Bridge, Ogbaru, Agulu, Mgbuka and Akwa and in Edo State which include Oba market , Santana , New Benin, Uselu and Oregbeni in 2015 and 2016, respectively. Ten samples were bought from each market making the total number of samples collected 100. They were packaged in low density polyethylene.

a) Bacteriological Analysis

Ten (10) grams of ready – to – eat groundnut paste was weighed aseptically into a conical flask and 90ml of sterile distilled water was added. A ten – fold serial dilution was made using 1ml aliquot from stock solution. Pour plate method was used to cultivate the organism. An aliquot of 1ml of the ten – fold serial dilution was poured into the petri – dishes before the molten agar was poured over it. The plates were incubated at 37^0C for 24 hours. At the end of incubation, total viable counts were expressed in cfu/g and colonies of isolates were purified by sub-culture into fresh plates of nutrient agar and thereafter stored in nutrient agar slant for characterization and identification using the method of Holt (1994).

b) Mycological Studies

A ten - fold serial dilution of 1ml of each of the sample was made. An aliquot of 1ml was plated on Sabouraud dextrose agar (SDA) amended with chloramphenicol to inhibit bacteria growth and alcohol for fungi isolation. All plates were incubated at room temperature (28^0C) for seven (7) days. After incubation, the colonies were counted and expressed in cfu/g. Isolated pure fungal colonies were identified according to Robert *et al.,* (2004).

c) Physicochemical Analysis

The physicochemical parameters analysed were pH and titratable acidity. pH value of the groundnut samples was determined with a single electrode pH meter (Hanna calibrated pH Tester). The sample was prepared by homogenizing 10g of ready – eat groundnut paste in 90ml of sterile distilled water. The pH of the solution was taken.

The titratable acidity of the sample (ready – to – use groundnut paste) was determined by introducing 10ml of the sample solution into a conical flask with three (3) drops of phenolthalein indicator. Sodium hydroxide (0.1M solution) was placed in a burette and titrated against the supernatant in the flask. The appearance of a pinkish colour indicated the end point of titration. The procedure was carried out in triplicates and average values noted.

d) Determination of Aflatoxin Content In The Ready – To – Eat Groundnut Paste Samples

Aflatoxin content of samples was determined using Enzyme – Linked Immunosorbent Assay (ELISA) method. The analysis was done using competitive ELISA method and Agraquant Total Aflatoxin Assay Kit (from Roman Singapore Company).

e) Elisa Testing Procedure

i. *Extraction of ready – to – eat groundnut paste samples*

5gm of sample was weighed into a suitable plastic container that was covered and 25ml of ethanol / tween water (70: 30) was added. It was placed on the shaker set at 250 rotations for 110 seconds and was filtered using the folded filter paper.

ii. *Sample Analysis*

A multichannel pipette set at 200μl was used to pipette aflatoxin enzyme conjugate into the uncoated antibody microplate wells. 100μl of each standard solution and samples extract were added to the coated antibody microplated wells and incubated at 20 – 25^0C for 10minutes.

Toxins in samples and control standards compete with enzyme conjugate for binding to solid phase antibody. Content were discarded and washed with de – ionized water for 5minutes and dried using absorbent paper towel. Thereafter, 100μl of enzyme substrate was added to wells and incubated at room temperature for 5minutes until blue colour was observed in wells. Then 100μl of stop solution was added to the wells and the blue colour change into yellow. Dye concentration is inversely related to concentration of toxin in the sample and standard. ELISA reader in the wavelength of 450 – 630nm was used to analyzed toxin concentration and wells absorbance. Toxin concentration in the sample was compared with standard concentration curve.

III. Results and Discusssion

The microbial and aflatoxin contamination in food commodities and its association with health risk in both animals and humans continue to raise increasing concern over years. Groundnuts pastes are a rich source of protein and edible oil; it has been reported to be mostly contaminated by microorganisms and aflatoxin. In this study, microbial counts as well as aflatoxin content of this product were determined. The bacterial counts ranged from $2.50 \pm 0.61 \times 10^4$ – $5.54 \pm 0.50 \times 10^4$ cfu/g and fungi counts ranged from $1.7 \pm 0.99 \times 10^3$ – $5.6 \pm 0.65 \times 10^3$ cfu/g as shown in Table 1. Fungal load of the ready – to – eat groundnut pastes had lower counts than bacterial counts. Work done by Adebesin *et al. (*2001) indicated similar microbial load

from groundnut products hawked in Bauchi. The bacterial count ranged from 1.16 ± 0.35 x 10^5 to 5.92 ± 0.59 x 10^5(cfu/g), while the fungal counts ranged from 1.91 ± 0.32 x 10^4 - 8.60 ± 0.22 x10^4 cfu/g in the samples. However, Akinnibosun and Osawaru, 2015 reported higher fungal than bacterial load in Benin Metropolis, Edo State, Nigeria.

Samples from Anambra had higher microbial contamination than samples from Edo State. This could be attributed to poor processing and prolonged storage as the pastes were produced in bulk in Anambra State and might not be readily sold out because of bulk product overshadowing the demand, forcing the bulk producers to embark on long storage which can be termed lag storage resulting in proliferation of organisms contrary to the practice in Edo State, where due to low patronage, the pastes were produced in low quantities and almost on demand.

The bacteria and fungi isolates from the samples are shown in table 2. The presence of these bacterial species in the product was of particular interest because of their possible involvement in food infections. The presence of *Staphylococcus* sp and *Micrococcus* sp in samples could be attributed to contamination from human handling, the surrounding air and environment during processing and display for sale in the markets. Most groundnut pastes samples are stored at temperature between 26^{0}C – 30^{0}C which favours the growth of the organisms. *Staphylococcus* sp in the groundnut pastes may result if the grinding machine and other utensils are contaminated. The sanitary conditions of the environment may also lead to contamination of the products. The presence of these microbes is an indication of the use of non – portable water, which is mostly used in local food processing (Adebayo-Tayo *et al.,* 2009; Adebayo-Tayo *et al.,* 2012). *S. aureus* is known for production of heat stable enterotoxin and has potentials for multiple antibiotic resistances when they get into living tissue (Scot, 2002) making the product an immense epidemiological danger (Adebayo-Tayo *et al.,* 2009; Adebayo-Tayo *et al.,* 2012). Heat stressed microorganisms that survived roasting process are capable of growing if samples were not preserved under appropriate temperature (Sokari, 1991).

Bacillus sp and *Pseudomonas* sp are normal inhabitants of the soil and capable of causing disease in humans and animals. These organisms are capable of surviving harsh conditions such as roasting and blending processes (Doyle, 2007). The presence of *E.coli* indicated unhygienic handling of the products right from the source, storage and packaging. This might have adverse effect on the health of the consumers (Okonko *et al,* 2008a,b,c). The isolation of *E.coli* in the pastes was in line with the findings of Odu and Okonko, 2010 who also isolated *E.coli, Bacillus* sp, *Serratia* sp, *Proteus* sp, *Micrococcus* sp, and *Staphylococcus* sp.

All the samples showed fungal growth with Ogbaru market being the most contaminated sample (5.6 ± 0.65 x10^3 cfu/g) although this is below the maximum tolerance limit of 10^4 cfu/g recommended by International Commission on Microbiological Specification for foods (Elliot, 1980; Da Silver, 2000). Fungal species isolated includes; *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. tamarii, Fusarium* sp and *Penicillium* sp. *Fusarium* species are plant pathogens that contaminate crops in the field or immediately after harvest while *Aspergillus* species are predominantly storage contaminants (Sweeney and Dobson, 1999). The presence of these organisms in the groundnut pastes might be due to improper handling during processing, hawking and display in open trays for sales. Some of these fungi especially *Aspergillus* sp is able to survive in situations where free water is not available (Reddy *et al.,* 2012). Their presence in the product may result in production of toxic substances such as aflatoxins and fumonisins.

Table 3 revealed the pH and titratable acidity values of the samples. The pH values of the groundnut paste samples ranged from 5.9 ± 0.01 (Oba market in Edo State) to 6.9 ± 0.03 (Agulu market in Anambra State). The pH values obtained could be as a result of the presence of bacteria in the groundnut samples whose pH optimal for growth is near neutrality (7.0), showing no erosive potential to the body as acidic pH in the body can occur from an acidic forming diet which negatively affects blood body's ability to absorb minerals and other nutrients, decrease the energy production in cells, and decrease its ability to repair damaged cells.

Titratable acidity measurement has also been used to assess food erosive potential. The titratable acidity ranged from 0.02 to 0.05 with Oba markets having the highest titratable acidity. This may be due to temperature in storage material which may have increased the activities of lactic acid bacteria breaking down sugars to produce lactic acid among the groundnut paste samples.

Total aflatoxin content (Table 4) obtained from samples from different open markets ranged from 1.1 ppb – 143.9 ppb. The samples obtained from Anambra State indicated high levels of aflatoxin B1 compared to samples from Edo State. This could be attributed to groundnut species, processing, handling and storage. The mean aflatoxin concentration of the samples obtained from Anambra State was five times higher than the specification of National Agency for Food, Drug Administration and Control in Nigeria whose permitted aflatoxin limit is 4 ppb in ready – to – eat foods. Akano and Atanda (1990) found aflatoxin B1 concentrations in the range of 20 - 455μg/kg in groundnut cake purchased from market in Ibadan, Oyo State, Nigeria.

Similarly, Adebanjo and Idowo (1994) reported that most of the corn groundnut snacks, contained aflatoxins above 30μg /kg immediately after preparation. Isibor *et al*. (2010) also reported high levels of aflatoxin contamination in groundnut and groundnut products while Okwu *et al*., (2010) revealed high incidence and alarming level of naturally produced aflatoxin in ready – to –use food thickeners sold in South – East geopolitical zone in Nigeria. The fungi that produce mycotoxins proliferate in the tropics where climatic and crop storage such as temperature, humidity, and water activity are conducive for fungal growth (D Mello and Mavdonald, 1997). The incidence of fungal contamination has also been linked to high rainfall and high relative humidity (Gamanya and Sibanda, 2001).

High levels of aflatoxin and fungal contamination result in a decrease in quality and nutritional value of ready – to – eat groundnut pastes (Gong *et al.,* 2002). Furthermore, children are most vulnerable to the detrimental effects of aflatoxin (Williams *et al*., 2004; Cullen and Newberne, 2003). Hence the detection of high levels of aflatoxins in ready – to – eat groundnut is a cause for concern. Also the threshold dose for aflatoxin leading to malnutrition and growth stunting in children is very low (Shephard, 2003). In Togo and Benin, chronic aflatoxicosis was also linked to growth stunting and underweight in infants under 5 years old (Gong *et al.,* 2002). It should also be noted that underweight children are also prone to child mortality and acute morbidity due to diarrhoea, malaria, measles, pneumonia and other selected infectious diseases (Williams *et al.,* 2004).

The commercial ready – to – eat groundnut pastes were contaminated. This might be due to several factors including poor policing of set regulations by the authorities and lack of effective quality control systems to cover unpackaged food products (e.g. factory inspection for aflatoxin contamination, hand sorting to remove shriveled nuts, and proper cleaning of equipments). However, manufacturing process can reduce aflatoxin contamination to levels that are acceptable in many countries. It is imperative that effective system for policing unregistered and unpackaged food products be put in place by constituted authorities. This will prevent manufacturers from churning out ready – to – eat groundnut pastes that have aflatoxin levels above the maximum tolerable limits.

IV. Conclusion

Tackling the problem of microbial contamination and aflatoxin in developing countries is very difficult and complex. Policy makers and the general public in the regions with the highest contamination levels generally lack full knowledge of aflatoxins and the scale of adverse health effects they cause (Gong *et al.,* 2004). A vast majority of the people mostly affected produce and consume their own food, rendering regulatory measures to control exposure ineffective (Shephard, 2003). In general, public health programmes like vaccination, malaria prevention and control, improved sanitation and clean drinking water supply are perceived to be more valuable than aflatoxin control (Gong *et al*., 2003). Furthermore, the aflatoxin problem sits at the interface of agriculture, health and international trade and it is impossible to tackle it without first tackling the insurmountable problems affecting these sectors in the developing countries (Gong *et al*., 2003; Shephard, 2003.).

However, manufacturing process can reduce microbial and aflatoxin contamination to levels that are acceptable in many countries. It is imperative that effective systems for policing unregistered and unpackaged food products be put in place by constituted authorities. This will prevent manufacturers from churning out ready – to – eat groundnut paste that has microbial and aflatoxin levels above the maximum tolerable limits.

Table 1: Total Microbial Counts In Ready –To - Eat Groundnut Paste Samples Obtained From Ten Open Markets In Anambra And Edo States, Nigeria

Sample	Market	Total Bacterial Counts CFU/g ± SEM x 10^4	Total Fungal Counts CFU/g ± SEM x 10^3
Anambra	Head Bridge	4.20 ± 0.42	4.0 ± 0.45
	Ogbaru	5.00 ± 0.40	5.6 ± 0.65
	Agulu	4.80 ±0.48	4.63±0.57
	Awka	2.50 ± 0.61	5.20± 0.61
	Mgbuka	3.66 ± 0.32	4.20 ±0.70
Edo	Oba	5.54± 0.50	4.03± 0.50
	Satana	2.90 ±0.18	1.78± 0.13
	Oregbeni	3.53±0.24	2.71±0.18
	Uselu	2.99 ± 0.32	1.70±0.99
	New Benin	3.75 ±0.32	2.29±0.18

SEM = Standard Error Mean

Table 2: Bacteria and Fungi Isolated From Ready – To – Eat Groundnut Pastes From Ten Markets

Market	Bacterial Isolates	Fungal Isolates
Head Bridge	*Staphylococcus aureus, Bacillus* sp *E.coli*	*A. flavus, Penicillium* sp *A. tamarri*
Ogbaru	*Bacillus* sp, *Staphylococcus aureus*	*A. flavus, Penicillium* sp *A. tamarri*
Agulu	*Pseudomonas* sp, *Bacillus* sp, *Staphyloccocus aureus*	*A. flavus*, *A. niger*
Mgbuka	*Staphylococcus aureus, Bacillus* sp, *Pseudomonas* sp	*A. flavus, Penicillium* sp, *A. niger*
Akwa	*Psudomonas* sp, *Bacillus* sp	*A. flavus, A. niger*
Oba	*Staphylococcus aureus, Micrococcus roseus, Bacillus cereus*	*A. niger,Fusarium* sp, *A. flavus*
Satana	*Bacillus cereus, Bacillus subtilis*	*A. flavus, A. fumigates*
Oregbeni	*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Micrococcus roseus*	*A. niger, A. flavus*
Uselu	*Staphylococcus aureus, Bacillus subtilis Pseudomonas aeruginosa*	*A. niger, Fusarium* sp *A. flavus*
New Benin	*Micrococcus roseus, Bacillus cereus, Staphylococcus aureus*	*A. niger, Fusarium* sp, *A. flavus*

Table 3: Physicochemical Characteritics (Mean Ph And Titratable Acidity) Of The Ready – To – Eat Samples From Ten Different Open Markets In Anambra And Edo States.

Market Place Sample Collected	pH ± SEM	Titratable Acidity ±SEM
Head Bridge	6.0 ±0.03	0.03 ±0.00
Ogbaru	6.7 ±0.00	0.04 ±0.005
Agulu	6.9 ±0.03	0.03 ± 0.01
Mgbuka	6.5 ±0.03	0.02 ± 0.02
Awka	6.4 ±0.29	0.02 ± 0.01
Oba	5.9 ±0.01	0.05 ± 0.00
Santana	6.2 ±0.01	0.03 ± 0.05
Oregbeni	6.5 ± 0.03	0.02 ± 0.00
Uselu	6.7 ± 0.11	0.02 ±0.01
New Benin	6.3 ±0.02	0.04 ±0.01

SEM = Standard Error Mean

Table 4: Aflatoxin Content (Ppb ± Sem) In Ready – To – Eat Groundnut Paste Samples Sold In Markets In Anambra and Edo States.

Sample Location	Market	Total Aflatoxin content (ppb) ± SEM
Anambra	Head Bridge	143.9 ± 2.72
	Ogbaru	108.8 ± 1.59
	Agulu	55.0 ± 1.80
	Awka	43.3 ± 0.73
	Mgbuka	76.8 ± 1.38
Edo	Oba	1.1± 0.07
	Santana	27.8 ± 0.21
	Oregbeni	3.0 ± 0.07
	Uselu	43.3 ±1.80
	New Benin	3.5 ± 0.23

SEM = Standard Error Mean

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