

# Bacteriological And Nutritional Analysis Of Groundnut Cake Sold In An Open Market In Samaru, Zaria-Kaduna State

Oko, J.O., Ariba, C., Audu, J.A., Kutman, N.A., Okeh, Q.,

**Abstract:** Bacteriological and nutritional analysis of groundnut cake powder sold in open market at Samaru-Zaria was studied. The samples collected from four zones of the study area were analysed for possible microbiological contamination and its nutritional quality. The results indicated a microbial load of  $1.93 \times 10^5$  cfu/g and  $1.94 \times 10^5$  cfu/g for zones A and B respectively,  $1.01 \times 10^5$  cfu/g for zone C and  $2.37 \times 10^5$  cfu/g for zone D. The bacterial isolates found to be associated with the groundnut cake powder in this study included *Klebsiella oxytoca*, *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *P. aeruginosa* and *Streptococcus faecalis*. The nutrients content of the sample included carbohydrates 55.15%, moisture 12.65%, lipid 15.40%, protein 12.60%, ash 3.95% and crude fibre 0.25%. Groundnut cake sold in the study area is highly contaminated with bacteria except for samples from zone C which is within the Food and Drugs Agency (FDA) recommendation of  $1.0 \times 10^5$  cfu/ml as allowable microbial contamination for food. The high level of microbial contamination is a serious cause for concern as it may trigger epidemics. However, the product is highly nutritious.

**Keywords:** Bacteriology, Groundnut cake, 'Kuli kuli' Microbial load, Nutrition, 'Oko', Proximate analysis

## 1 INTRODUCTION

Groundnut cake is a by-product obtained after extraction of oil from groundnut. The cake is believed to be rich in proteins, carbohydrates, crude fibre and minerals [10]. Groundnut (*Arachis hypogea* L.) occupies an important position in the economy of developing nations. The major groundnut producing countries are India, China, and the United States. It was introduced into Nigeria in the 16<sup>th</sup> Century and it has been estimated that about 1.4 million hectares are cultivated for groundnut in Nigeria [18], [14]. Groundnut is a nutrient dense agricultural produce, which is very high in energy due to its high fats and proteins content. The carbohydrates content of groundnut is relatively low, being under 30% of the whole nut. The nut has relatively high content of fibre. It is an industrial crop whose major utilization is a source of oil [11]. Groundnut is widely consumed in Nigeria as roasted or boiled nuts, which could be cooked or eaten with boiled maize in the western and southern part of the country. "Kulikuli" (groundnut cake) is the residue obtained after the extraction of oil and it is high in protein and used as supplement in feed and food. The groundnut cake is usually fried in oil and is used as delicious snacks or food supplements.

Groundnut flour is obtained by grinding groundnut cake into fine powder which can be used in making soup or stew, sauces, confectioneries, puddings and bakery products. "Yaji" is groundnut flour that has been mixed with different portions of groundnut spices like ginger, alligator paper and salted to taste. "Dakuwa" is groundnut flour that has been mixed with roasted cereal flour in which some spices like dried alligator paper, sugar and salt are added to the mixture before being pounded and moulded into small balls that can be eaten without further processing [2]. As a result of improper processing and storage conditions, groundnut and its products may be contaminated with microorganisms. The number and type of microbes presents in the produce are important in deterioration and numerous molds may be involved, but most common are species of *Aspergillus*, *Penicillium*, and *Fusarium* [12]. Abalaka and Elegbede [1] isolated species of *Bacillus*, *Salmonella*, *Pseudomonas* and *Escherichia coli* from groundnut. Groundnut cakes popularly called "kulikuli" in the northern states of Nigeria is produced by expelling the oil from the groundnut kernals. Groundnut oil is extracted by solvent or hydraulic method and residual groundnut cake has protein content of 38-47% depending on the extraction process [2]. It is a popular snacks in Nigeria and often eaten with meals such as "Gari", "Akamu" and sometimes put into salad. However peasant processing and packing methods are still commonly adopted for "kulikuli" production. The latter predispose the groundnut cake to microbiological contamination especially during hawking of the product which is often exposed or packaged in hand knotted thin polythene bags. There are no labels to indicate vital information such as name and address of producers, nutritional content and recommendations for storage and best before date for human consumption despite the fact that mycotoxicoses are becoming increasingly implicated in human and animal pathology [7]. The situation may be worsened by consumer reluctance to discard fairly molding food samples such as groundnut cake due to it is irresistible flavour. As a result of improper processing and storage conditions, groundnut cake may be contaminated with microorganisms. 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*, *Penicillium*, *Fusarium* [12]. The presence of contaminating

- Oko, Joseph Odey, and Audu, Jeremiah Adeiza are lecturers in Microbiology Division, Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology Samaru Zaria, Nigeria, E-mail: [odeyadebe@yahoo.com](mailto:odeyadebe@yahoo.com)
- Ariba, Clement is a lecturer in the Department of Microbiology, University of Calabar, Calabar-Nigeria.
- Okeh, Queendaline is a lecturer in Chemistry Division, Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology Samaru Zaria, Nigeria.
- Kutman, Nanpan Ali is a Technologist in Microbiology Division, Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology Samaru Zaria, Nigeria

microorganisms in a product as the groundnut cake patronized by wide range of people of different social and economic class as well as status is of public health concern. Therefore this study was aimed at determining the bacteriological quality and nutritional value of groundnut cake powder sold in open market in Samaru, Zaria.

## 2 MATERIALS AND METHODS

### 2.1 Bacteriological Analysis

#### 2.1.1 Media Preparations

Nutrient agar, MacConkey agar and Mannitol salt agar were used for the culturing. The media were prepared according to manufacturers' instructions and sterilized in an autoclave at 121°C for 15 minutes. The surfaces of the agar plates were dried in an oven before the inoculation of the samples [8]. Buffered peptone water was prepared by weighing 0.5g of BPW using analytical weighing balance and dissolving it in 500ml distilled water. It was then autoclaved at 121°C for 15 minutes.

#### 2.1.2 Collection and Preparation of Samples

The food samples (groundnut cake powder) were bought from hawkers in the Samaru open market. The market was divided into four zones according to the four cardinal points, zones A, B, C and D. Samples were purchased from three different hawkers in each zone, homogenized and identified according to the zone. Using an analytical weighing balance, exactly 25g of each of the homogenized samples from each zone was aseptically weighed and dissolved in sterile 225ml of buffered peptone water in a 500ml conical flask. The food sample was thoroughly mixed on a shaker and was diluted in a tenfold serial dilution up to the dilution of  $10^{-6}$  using buffered peptone water as the diluent [3].

#### 2.1.3 Inoculation and Incubation

For the nutrient agar plates, pour plate technique was employed. 0.2 ml of the sample was pipetted and dispensed into a petri dish and 15 ml of freshly prepared molten nutrient agar was added to the sample in the dish, swirled and allowed to set prior to incubation. For the MacConkey and Mannitol salt agar plates, spread plate method was used. 1ml of the dilution was transferred onto a dried agar surface of the plates and a sterile glass rod spreader was used to spread the sample suspension on the surface of the agar plates. The spreader was sterilized by dipping in absolute ethanol and flamed by passing it through a Bunsen flame and allowed to cool for 20 seconds. All cultures were prepared in duplicates. The plates were then incubated at 37°C for 24 hours [8]. All the colonies appearing on the surfaces of the duplicate media plates after the incubation period were counted using a digital illuminated colony counter and the count expressed as colony forming unit per gram (cfu/g) of the samples. The actual number of colonies was determined using the formular

$$\text{Actual number of colonies} = \frac{\Sigma C}{(N1 + 0.1N2)D}; \quad (1)$$

Where C is the sum of colonies counted on all the dishes retained, N1 is the no. of dishes retained in the first dilution, N2 is the no. of dishes retained in the second dilution and D is the dilution factor corresponding to the first dilution plated [4].

### 2.1.4 Characterization and Identification of Isolates

The colonies of the bacteria developing on the surface of the plates were purified by sub-culturing until pure isolates are obtained. Representative surface colonies were identified using colonial morphology, microscopical examinations and biochemical characteristics based on standard procedures [6], [9], [13].

### 2.2 Proximate Analyses

The proximate analyses were carried out according to the standard official methods of Analysis of the Association of Official Analysis of Chemist [5] and those of Onyeike and Osuji [17].

#### 2.2.1 Determination of Moisture Content

A clean crucible was dried to a constant weight in a hot air oven at 105°C, cooled in a desiccator and weighed ( $W_1$ ). Two gram of finely ground sample (groundnut cake powder) was accurately weighed into the previously labelled crucible and reweighed ( $W_2$ ). The crucible containing the sample was dried in the oven to a constant weight ( $W_3$ ). The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100; \quad (2)$$

#### 2.2.2 Determination of Ash Content

The porcelain crucible was dried in an oven at 100°C for 10 minutes, cooled in a desiccator and weighed ( $W_1$ ). Two grams of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed ( $W_2$ ). It was first ignited and then transferred into a furnace which was then set at 55°C. The sample was then left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed and then cooled in the desiccators after which it was then weighed ( $W_3$ ). The percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100; \quad (3)$$

#### 2.2.3 Determination of Crude Lipid Content

A clean, dried 500ml round bottom flask containing few anti-bumping granules was weighed ( $W_1$ ) and 300ml of petroleum ether (40-60°C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing 20.0g of the sample was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractors and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for six hours. The solvent was recovered and the oil was dried in the oven at 70°C for one hour. The round bottom flask and oil was cooled then weighed ( $w_2$ ). The lipid content was calculated thus:

$$\% \text{ Crude lipid content} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100; \quad (4)$$

#### 2.2.4 Determination of Nitrogen and Crude Protein

One and a half (1.5) grams of the groundnut cake powder in an ash less filter paper was dropped into 300ml Kjeldahl flask. Twenty-five millilitres conc.  $H_2SO_4$  and 3g of digesting mixed

catalyst (weighed separately into an ash less filter) was also dropped into the Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained. The digestion cooled and diluted to 100ml with the distilled water. 20 ml of diluted digest were measured into 500ml kjeldahl flask containing anti-bumping chips and 40ml of 40% NaOH was slowly added by the side of the flask. A 250ml conical flask containing a mixture of 50ml 20% boric acid and 4 drops of mixed indicator was used to trap the ammonia being liberated. The conical flask and the kjeldahl flask were then placed on kjeldahl distillation apparatus with the tubes inserted into the conical flask and the kjeldahl flask. The flask was heated to distil out the NH<sub>3</sub> evolved. The distillate was collected into the boric acid solution. When the boric acid turned to green, it was allowed for 10 minutes for complete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1M HCl.

#### Calculation:

$$\% N_2 = \frac{14 \times M \times V_t \times TV}{\text{weight of sample(mg)} \times V_a} \times 100; \quad (5)$$

Therefore,

$$\% \text{ crude protein} = \% \text{ nitrogen (N}_2) \times 6.25; \quad (6)$$

Where M = actual molarity of acid  
TV = titre volume of HCl used  
Vt = total volume of diluted digest  
Va = aliquot volume distilled

#### 2.2.5 Determination of Crude Fibre

Two (2.0) grams of the fine groundnut cake powder was weighed out into a round bottom flask containing 100ml of 0.25M sulphuric acid solution and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100ml of hot 0.31M sodium hydroxide solution was added and the mixture boiled again under the reflux for 30 minutes and quickly filtered under suction. The soluble residue was washed with boiling water until it was base free and was dried to constant weight in the oven at 100°C and recorded as C<sub>1</sub>. The weighed sample (C<sub>1</sub>) was then incinerated in a muffled furnace at 550°C for 2 hours, cooled in the desiccator and reweighed (C<sub>2</sub>).

#### Calculation:

The loss in weigh on incineration = C<sub>1</sub> - C<sub>2</sub>. The calculation was carried out thus.

$$\% \text{ Crude fibre} = \frac{C_1 - C_2}{\text{weight of sample}}; \quad (7)$$

#### 2.2.6 Determination of Carbohydrates (By Difference)

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100.

#### Calculation,

$$\% \text{ total carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ protein} + \% \text{ fibre}) \quad (8)$$

[5].

### 3 RESULTS

The plates inoculated with the dilutions of 10<sup>-3</sup> and 10<sup>-4</sup> were retained for the aerobic viable plate count. The results of the viable aerobic plate count are shown in Table 1. It indicated a highest count in zone D and lowest in zone C whereas zones A and B had the count of 1.93 x 10<sup>5</sup> cfu/g and 1.94 x 10<sup>5</sup> cfu/g respectively. The bacterial load of samples in all the zones exceeded the FDA allowable microbial contamination of 1.0 x 10<sup>5</sup> cfu/g except for zone C that is within the limit having 1.01 x 10<sup>5</sup>.

TABLE 1: Viable aerobic count of groundnut cake

Zone	Dilution factor	Colonies counted		Aerobic Plate Count (cfu/g)
		Plate I	Plate II	
A	10 <sup>-3</sup>	120	98	1.93 x 10 <sup>5</sup>
	10 <sup>-4</sup>	101	105	
B	10 <sup>-3</sup>	128	131	1.94 x 10 <sup>5</sup>
	10 <sup>-4</sup>	78	89	
C	10 <sup>-3</sup>	77	62	1.01 x 10 <sup>5</sup>
	10 <sup>-4</sup>	50	33	
D	10 <sup>-3</sup>	134	130	2.37 x 10 <sup>5</sup>
	10 <sup>-4</sup>	126	131	

The result from this study shows that Escherichia coli is the dominant contaminant as shown in Figure 1 followed by Klebsiella oxytoca. Pseudomonas aeruginosa was the least contaminant.

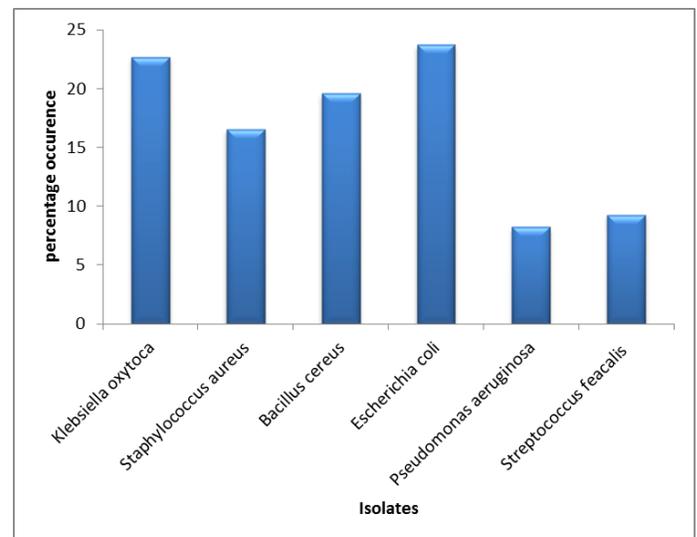
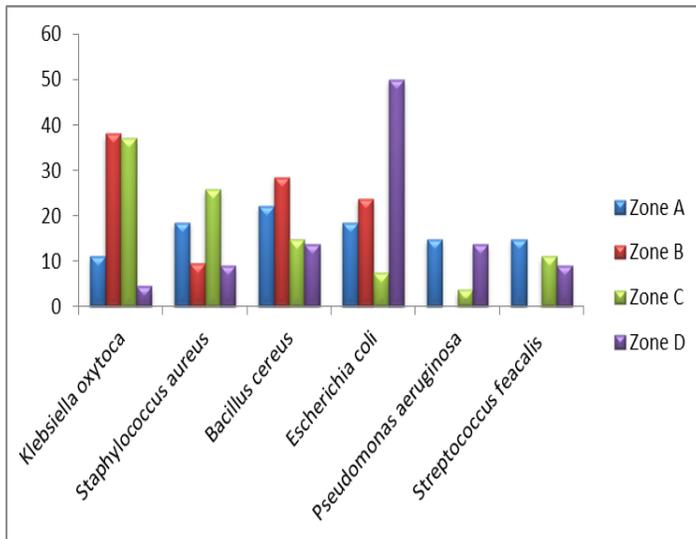


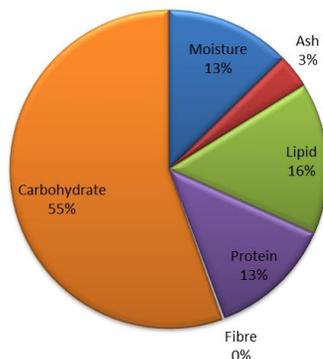
Figure 1: Percentage occurrence of bacterial isolates in the sample

The distribution of bacterial species according to zones shows that all bacteria isolated were present in all the zones except Pseudomonas aeruginosa and Streptococcus faecalis that were absent from zones C and D (Figure 2). Zone B has the highest prevalence of Klebsiella oxytoca and Bacillus cereus whereas zones C and D recorded the highest occurrence of Staphylococcus aureus and Escherichia coli respectively. P. aeruginosa and S. faecalis were less prevalent.



**Figure 2:** Percentage occurrence of bacteria by zones

This study has revealed that groundnut cake is rich in carbohydrate (55.15%), moisture and proteins (12.65% and 12.60% respectively), lipid (15.40%) and ash (3.45%) whereas it is poor in fibre content as the study indicated only 0.25% ash content (Figure 3).



**Figure 3:** Percentage nutritional composition of groundnut cake

## 4 DISCUSSION

Food safety in any society is nothing to be compromised. This study was aimed at analysing the bacteriological safety and nutritional quality of groundnut cake sold in open market in Samaru which is consumed in various forms by the majority of the populace. The result of the bacterial count as presented in Table 1 shows that the product is highly contaminated by bacteria. This may be as a result of unhygienic practices during the processing of the product. This may range from methods of obtaining and quality of raw materials including sources of water to the packaging, handling and distribution of the finished product to the final consumers. This observation is in agreement with those of Frazier and Westhoff [12], Jacquelyn [15] as well as Betcha and Akoma [7] who reported that improper processing and storage conditions of groundnut and its products may lead to contamination by microorganisms. There is no doubt to this as per information from interview with the product handlers revealed that there are no adequate sources of potable water, inadequate storage

facilities and lack of electricity to power modern appliances that may reduce contamination. The aerobic count for zone C indicated a better and acceptable bacterial load ( $1.01 \times 10^5$  cfu/g, Table 1). This is evident in the way and manner majority of marketers in this zone packaged and displayed their products. Some were sealed in clean polyethylene containers and prevented from dust particles while others were left exposed. Consumption of such unwholesome products may lead to enteric fever and diarrhoea [16]. However, this research has revealed a number of bacteria associated with the contamination of groundnut in the study area to include *Pseudomonas aeruginosa*, *Streptococcus feacalis*, *Klebsiella oxytoca*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. This is in agreement with Abalaka and Elegbede [1] who isolated species of *Bacillus*, *Salmonella*, *Pseudomonas* and *Escherichia coli* from groundnut in their study. The zone by zone prevalence of the isolates indicated that all except *P. aeruginosa* and *S. feacalis* were isolated from all zones with zone D having the highest prevalence of *E. coli* which is an indicator microorganism. The proximate analysis showed that the cake is rich in carbohydrate, proteins and lipid (Figure 3). There are essential to body mass development in humans and animals. The results indicated slightly lower nutritional contents, as compared to those of other researchers such as Desai et al [10] who recorded 45-60% proteins, 20 – 30% carbohydrate, and 4 – 6% crude fibre. Relatively there are no significant differences between their results and those of this study except that our study had higher carbohydrate and lower fibre values when compared to what Elegbede [11] reported from whole groundnut. Finally, this research indicated that the product is contaminated with several bacterial species but highly nutritious and can even be used as additive in animal feeds.

## 5 CONCLUSION

Peasant processing and packaging methods are still commonly adopted for production of groundnut cake in the study area. This leads to lack of labels to indicate vital information such as name and address of producers, nutritional content and recommendations for storage and best before (expiry) date for human consumption and most importantly microbial contaminations. Therefore considering the nutritional importance of groundnut cake and its suitability for incorporation into traditional and conventional products, it is hereby suggested that a small to medium scale production of groundnut cake powder be carried out under standard hygienic operation procedures. This will drastically reduce contamination and epidemics that may result in the consumption of improperly produced food and food products as well as popularize the nutritional benefits of the product.

## References

- [1] Abalaka, J.A. and Elegbede, J.A. "Aflatoxin Distribution and Total Microbial Content in an Edible Extracting Plant", *J.f.d Toxic* 20:43-46, 1981.
- [2] Akano, D.A., and Atanda, O. "Present Level of Aflatoxin in Nigerian groundnut Cake ('Kulikuli')", *Letters in Applied Microbiology*, 10(4):187-189, 1990.
- [3] Aneya, K.R., *Experiments in Microbiology, Plants Pathology and Biotechnology*, New Age Publishers, ISBN 81-224-1494-X, P.69, 2005.

Harvest Biotechnology of Food Legumes CRS Press Inc.,  
p.160, 1985.

- [4] Arlington, V.A., Bacteriological Analytical Manual, 6<sup>th</sup> Edn. Association of Analytical Chemists for FDA, Washington, D.C. pp. 17-21, 1992.
- [5] Association of Official Analysis of Chemist (AOAC), "Standard Official Methods of Analysis of the Association of Official Analysis of Chemist", SW (Bd), Washinton D.C., 1984.
- [6] Barrow, G.I. and Feltham, R.K.A., Cowan and Steel's Manual for the Identification of Medical Bacteria (2<sup>nd</sup> Edn). Cambridge University Press, United Kingdom, p1330, 2003.
- [7] Betcha, B.J.O. and Akoma, O., "Microbial Studies on Nigerian Groundnut Product, Kwoka Supplement with Soyabean", J. Food Safety 17:249-259, 1988.
- [8] Cheesbrough, M., District Laboratory Practice in Tropical Countries (2<sup>nd</sup> edn) pp.63-75, 2000.
- [9] Collins, C.H and Lyne, P.M., (1987). Microbiological methods, 5<sup>th</sup> edition Butterworth and Co. Publ. Ltd London pp.31-345, 1987.
- [10] Dessai, B.B., Kotecha, P.M., Salunke, D.K., "Groundnut Protein" In Basic and Applied Aspects of Seed Biology. Ed. By R.H. Ellis, M. Black, A.J.Murdoch and T.D. Hong. Kluwer Academic Publishers, Netherlands. Pp.540-582, 1996.
- [11] Elegbede, J.A., Legumes: In Nutritional Quality of Plant Food. A.U. Osagie and O.V. Eka, Editors. Post-Harvest Research Unit, Biochem. Dept. University of Benin, pp.53-83, 1998.
- [12] Frazier, W.C. and Westhoff, D.C., Food Microbiology 3<sup>rd</sup> edition. Tata Mcgraw Hill Pub. Co. Ltd New Delhi, Indian, pp.17-34, 1978.
- [13] Holt, J.G., Koieg, N.R., Sneath, P.H.A., Starley, J.T., Williams, S.T., Bergey's Manual of Determinative Bacteriology (9<sup>th</sup> Edn). Wiliams and Wilkins, Boltimore, p.767, 1994.
- [14] Irvine, F.R., West African crops (3<sup>rd</sup> edn) Oxford University press, pp.193-196, 1970.
- [15] Jacquelyn B., Microbiology Principles and Exploration, 8<sup>th</sup> edition. John Wiley and sons, ISBN 978-0-470-54109-8, p.68, 2012.
- [16] Oko, J.O., Ariba, C. and Itah, A.Y., Epidemiologic Study of the Prevalence of Diarrhoeic Agents amongst Hospitalized Children in Some Hospitals in Calabar, Nigeria. J. Microbiol. Biotech. Res., 4(3):25-31, 2014.
- [17] Onyeike, E.N. and Osujji, J.O., Research Techniques in Biological and Chemical Science, Springe field publishers Ltd. Owerri, Imo states, 2003.
- [18] Salunkhe, D.K., Kadam, S.S. and Chevan J.K., Post-