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Microbiological Quality Assessment of Unbranded Groundnut Oil Sold in Major Markets in Port Harcourt City, Rivers State, Nigeria

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ABSTRACT

Keywords

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The microbial quality of unbranded groundnut oil sold in Mile 1 and Mile 3 Markets in Diobu area of Port Harcourt, Rivers State was investigated. Five samples of different unbranded groundnut oil bought from both markets were used for the microbiological analysis. The isolation and enumeration of total aerobic heterotrophic bacteria, total fungi, and total coliform bacteria counts was done with nutrient agar, potato dextrose agar and MacConkey agar medium using standard microbiological techniques. The results revealed that the total heterotrophic bacteria counts ranged from 0 to 2.5×10^5 cfu/ml and 1.4×10^5 to 8.3×10^5 cfu/ml for the Mile 1 market and Mile 3 markets respectively. The total coliform count range from 0 to 2.3×10^4 cfu/ml for samples from Mile 1 markets, while samples from Mile 3 markets did show growth of coliforms. The total heterotrophic fungi counts ranged from 0 to 9.3×10^4 cfu/ml and 0 to 6.45×10^4 cfu/ml for Mile 1 markets and Mile 3 markets respectively. The counts obtained were beyond the standard limits of 5×10^3 cfu/ml. *Klebsiella*, *Micrococcus* and *Staphylococcus aureus* species were the bacteria isolates identified, while the fungi include *Aspergillus flavus*, *Aspergillus niger*, *Curvularia*, *Penicillium* and *Rhizopus* species. The analysis of variance (ANOVA) at $p \leq 0.05$ showed that there was no significant difference between the THB counts from the unbranded groundnut oil samples sold in Mile 1 and Mile 3 Markets, while there was significant difference between coliform counts. The high level of the microbial counts reveals severe contamination of the oil samples which poses a risks to the health of the consumers. Good manufacturing practices, education and enlightenment of the processors and dispensers as well as regular monitoring by regulatory agencies will ensure minimum contamination.

Introduction

According to Atasi *et al.*, (2009), groundnut (*Arachis hypogea*) or peanut is a member of the *Fabaceae* family, native of South and Central America. Though groundnuts are legumes, they are the most commonly eaten

“nut” in the world. The groundnut oil, also known as peanut oil or *Arachis* oil is light yellow transparent edible oil with clear colour, pleasant fragrance and good taste that can digest very easily (ABC Machinery, 2019). Groundnuts are usually enriched with some essential nutrients that play beneficial

roles to human health. Onawo and Adamu, (2018) stated that groundnut oil is one of the most important group of oil and is normally used to reduce cholesterol and prevent heart disease. Eshun *et al.*, (2013) reported that the risk of heart disease can be significantly lowered when groundnut oil, groundnut or groundnut butter is consumed daily. Nkafamiya *et al.*, (2010) and Mandloi *et al.*, (2014) noted that Pharmaceutical companies now incorporate groundnut oil in formulations for the production of various products intended for internal and external use.

Groundnut oil is a vegetable which contains only a small proportion of non-glyceride constituents. According to Hati *et al.*, (2009), unbranded vegetable oil (UVO) are those locally produced and laboratory extracted edible vegetable oils that are branded. Edible oil is one of the most valuable and widely used processed foods Tesfaye *et al.*, (2015). Vegetable oils are consumed domestically in Nigeria. Babatunde and Bello, (2016) reported the major sources of edible oils in Nigeria are groundnut and palm oil. These vegetable oils are used mainly as cooking oils, salad oil and for the production of soap, margarine and cosmetics. Therefore, the quality of the oil and its stability are two critical requirements for the consumers and the food processing industries (Wali *et al.*, 2015).

Vegetable oils contributes significantly to the improvement of the diet, providing a good source of lipid and fatty acids for human nutrition and functioning to repair worn out tissues, formation new cells as well as source of energy supply (Babatunde and Bello, 2016). According to Nkafamiya *et al.*, (2010), groundnut oil contains much potassium than sodium and is a good source for calcium, phosphorus and magnesium. The demand and consumption of unbranded groundnut oil has increased rapidly in recent time.

This may be influenced by the regular availability, accessibility and low cost of groundnut oil. There are basically four methods used for the production of groundnut oil, including pressing, solvent extraction, aqueous extraction, and aqueous enzymatic methods (ABC Machinery, 2019). Many people who use the traditional method of production for the extraction of edible oil have neither little nor no knowledge of the current production techniques nor the microbiological implication of engaging in unhygienic sanitary practises and employing poor storage methods.

Mandloi *et al.*, (2014) stated that the most essential characteristics of oils are the chemical properties, which constitute one of the vital components of balanced diet. Groundnut oil is composed of more than 80% unsaturated fatty acids such as oleic acid (41.2%) and 37.6% linoleic acid (37.6%). It is also contains 19.9% of palmitic acid, stearic acid, arachidic acid and other unsaturated fatty acids (ABC Machinery, 2019).

The fatty acid composition of peanut oil is relatively good and easily digested and absorbed by the human body. Nkafamiya *et al.*, (2010) in a study have found that groundnut oil contains more potassium than sodium, and a very good source of calcium, phosphorus and magnesium. Its content also includes thiamine, vitamin E, selenium, zinc and arginine. Barku *et al.*, (2012) noted that edible oils represent the highest source of energy per unit weight consumed notwithstanding the origin (animal, vegetable or marine).

Groundnut oil undergoes rancidity, which is the complete or incomplete oxidation or hydrolysis of fats and oils when exposed to air, light, or moisture or by bacterial action, resulting in unpleasant taste and odour. When these processes occur in food, undesirable

odours and flavours occur. Sunlight catalyses the reaction, this is due to nature of the free-radical reactions (Sergey, 2014). This breakdown is as a result of lipases, a class of hydrolases primarily responsible for hydrolysis of acyglycerides. Many microorganisms such as bacteria, yeasts, moulds and a few protozoa are known to secrete lipases for the digestion of lipid materials (Nagarajan, 2012; Abrunhosa *et al.*, 2013). Often times they are contaminated by mycotoxins and heavy metals (Ma *et al.*, 2015). The main aim of this study is to evaluate the microbiological quality of unbranded groundnut oils sold in two major markets in Port Harcourt city, Rivers State.

Materials and Methods

The study area

The study was carried out within the Diobu area of Port Harcourt city, Rivers State where the major markets are located.

Sampling sites

The two major markets selected for this study are the popular Mile 1 (Rumuwoji) Market and Mile 3 (Oroworukwo) Market. These markets are the major places where unbranded groundnut oil marketing takes place. The GPS coordinates of Mile 1 Market is Latitude 4° 47' 28.7376" N and Longitude 6° 59' 53.7756" E, while Mile 3 Market had coordinate of Latitude 4° 48' 8.0604" N and Longitude 6° 59' 27.3912" E (Figure 1).

Collection of samples

Five samples of the unbranded groundnut oil were bought from the vendors from each of the Mile 1 and Mile 111 markets, making a total of ten (10) samples. All the samples were collected in already labelled 100ml sterile bottles and transported to the Biology

Department Laboratory, Ignatius Ajuru University of Education for microbiological analysis.

Microbiological analysis of groundnut oil samples: isolation and enumeration of total heterotrophic bacteria

Nutrient Agar, MacConkey Agar and Sabouraud Dextrose Agar (SDA) media were used for the isolation and enumeration of total heterotrophic bacteria, total coliform bacteria and total fungal counts respectively. Preparation of the media was according to the manufacturers instructions. The bacterial isolates were identified on the basis of their cultural, morphological and physiological characteristics in accordance with the schemes and methods described by Holt *et al.*, (1994), Fawole and Oso (1995) and Cheeseborough (2006). The standard identification keys and atlas of De Hoog *et al.*, (2000) and Tsuneeo, (2010) was used to characterise and identify the fungal isolates based on their macroscopic and microscopic features. The data obtained from the colony counts was subjected to Analysis of Variance(ANOVA).

Results and Discussion

The results of the total aerobic heterotrophic bacteria (THB), total coliform and total fungal counts obtained from analysis of the unbranded groundnut oil samples in mile 1 and mile 3 markets are presented on the tables 1–12 and figures 1–3.

The focus of this study was to evaluate the microbiological quality of unbranded groundnut oil sold in two major markets (Mile 1 and Mile 3 markets) in the Diobu area of Port Harcourt city, Rivers State. This is because the microbiological contamination of edible oils is very prevalent in most areas and poses a great health risks to the unsuspecting

consumers. Tesfaye *et al.*, (2015) reported that the issue of microbial contamination is commonly observed in many edible oil markets. The result of the study shows that the total heterotrophic bacteria (THB) counts ranged between 0 cfu/ml to 2.5×10^6 cfu/ml and 1.4×10^5 cfu/ml to 8.3×10^5 cfu/ml for Mile 1 Market and Mile 3 Market respectively.

Although, the total heterotrophic bacteria counts were higher in mile 3 market than in mile 1 market, the analysis of variance (ANOVA) at $p \leq 0.05$ showed that there was no significant difference between the THB counts from the unbranded groundnut oil samples sold in Mile 1 and Mile 3 Markets. However, the THB counts are higher than the (4.53 to 12.13×10^3 cfu/ml) reported by Tesfaye *et al.*, (2015). The high bacterial load could be due to the unhygienic nature of the market, oil component, storage facility available and the storage conditions (Okechalu *et al.*, 2011).

The total coliform counts ranged from 0 cfu/ml to 2.3×10^4 cfu/ml in mile 1 market, while no coliforms was detected from the samples in mile 3 market. According to Chabiri *et al.*, (2009), edible oils do not exhibit significant growth of the coliform bacteria. This may probably account for the absence of coliforms in samples from mile 3 markets.

The analysis of variance (ANOVA) at $p \leq 0.05$ of coliforms counts indicates that there was a significant difference between the coliform from samples in Mile 1 and Mile 3 Markets. For total heterotrophic fungi (THF), the counts ranged between 0 cfu/ml and 9.3×10^4 cfu/ml, and 0 cfu/ml to 6.45×10^4 cfu/ml for mile 1 markets and mile 3 markets respectively. The differences in fungal counts may be due to the inability of the fungal species to effectively metabolise the oil. The

analysis of variance (ANOVA) at $p \leq 0.05$ of THF counts reveals that there was no significant difference between the THF counts obtained from unbranded groundnut oil samples sold in mile 1 and mile 3 markets.

The bacterial isolates identified in the study include *Micrococcus* sp, *Klebsiella* sp. and *Staphylococcus aureus*. Tesfaye *et al.*, (2015), in a study had also identified similar bacteria species. The fungi identified are *Aspergillus flavus*, *Aspergillus niger*, *Curvularia*, *Penicillium* and *Rhizopus* species. Sylvester and Eligha (2013) and Flora *et al.*, (2018) reported the isolation of similar fungal species. Tobin-West *et al.*, (2018) also isolated and identified *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus* and *Fusarium* species as the major fungal species associated with raw groundnut seeds sold in Port Harcourt metropolis.

According to CDC, (2006) the *Aspergillus* species isolated from the vegetable oils have shown ability to produce Aflatoxin B1, B2, G1 and G2 in in-vitro studies. There is also the probability of acquiring food borne disease due to consumption of the groundnut oil products contaminated with *Staphylococcus aureus*. It could cause gastroenteritis in the consumers especially when the groundnut oil is eaten raw. Faecal contamination of the product leads to the occurrence of coliforms, which is a good indicator of food spoilage (Dubey and Maheshwar, 2003). In terms of prevalence, *Staphylococcus aureus* (78.2%) and *Micrococcus* (69.9%) had the highest prevalence rate for the bacteria species, while for the fungi, *Aspergillus flavus* (47.1%) had the highest prevalence followed by *Rhizopus* species. The prevalence of these organisms is a reflection of the poor conditions of the markets and the unhygienic practices adopted by the processors and vendors.

Table.1 Total heterotrophic bacteria counts in unbranded groundnut oils samples sold in Mile 1 Market

SI	Total Heterotrophic Bacteria Count (cfu/ml)				
	M1M1	M1M2	M1M3	M1M4	M1M5
1 st	1.25×10 ⁵	2.50×10 ⁶	1.49×10 ⁵	2.83×10 ⁵	1.00×10 ⁵
2 nd	1.00×10 ⁵	1.12×10 ⁵	1.41×10 ⁵	2.92×10 ⁵	9.50×10 ⁵
3 rd	-	1.02×10 ⁵	1.38×10 ⁵	2.80×10 ⁵	1.05×10 ⁵

SI – sampling instances; M1M1 - Mile 1, market 1, M1M2 - Mile 1, market 2; M1M3 - Mile 1, market 3; M1M4 - Mile 1, market 4; M1M5 - Mile 1, market 5

Table.2 Total heterotrophic bacteria counts in unbranded groundnut oil samples sold in Mile 3 Market

SI	Total Heterotrophic Bacteria Count (cfu/ml)				
	M3M1	M3M2	M3M3	M3M4	M3M5
1 st	2.55×10 ⁵	1.50×10 ⁵	1.48×10 ⁵	8.30×10 ⁵	7.30×10 ⁵
2 nd	2.00×10 ⁵	1.55×10 ⁵	1.50×10 ⁵	8.00×10 ⁵	6.50×10 ⁵
3 rd	2.80×10 ⁵	1.49×10 ⁵	1.40×10 ⁵	7.80×10 ⁵	7.00×10 ⁵

SI – sampling instances; M3M1 - Mile 3, market 1, M3M2 - Mile 3, market 2; M3M3 - Mile 3, market 3; M3M4 - Mile 3, market 4; M3M5 - Mile 3, market 5

Table.3 Analysis of variance (ANOVA) of THB counts in sampled unbranded groundnut oil sold in Mile 1 and Mile 3 Markets

SUMMARY					
Groups	Count	Sum	Average	Variance	
Means for Mile 1 markets	3	1.08×10 ⁶	3.58×10 ⁵	6.53×10 ¹⁰	
Means for Mile 3 markets	3	1.22×10 ⁶	4.08×10 ⁵	2.53×10 ⁸	
ANOVA					
Source of Variation	SS	Df	MS	F _{cal.}	F _{tab.}
Between Groups	3.65×10 ⁹	1	3.65×10 ⁹	0.1114 ^{ns}	7.7086
Within Groups	1.31×10 ¹¹	4	3.28×10 ¹⁰		
Total	1.35×10 ¹¹	5			

SS - Sum of squares, df – degree of freedom, MS – Mean square, F_{cal.} – F calculated, F_{tab.} – F tabulated

Table.4 Total coliform counts in unbranded groundnut oils samples sold in Mile 1 market

SI	Coliform Count (cfu/ml)				
	M1M1	M1M2	M1M3	M1M4	M1M5
1 st	1.00×10 ⁴	-	2.50×10 ⁴	1.60×10 ⁴	-
2 nd	1.40×10 ⁴	-	1.80×10 ⁴	1.30×10 ⁴	-
3 rd	1.10×10 ⁴	-	2.00×10 ⁴	1.00×10 ⁴	-

SI – sampling instances; M1M1 - Mile 1, market 1, M1M2 - Mile 1, market 2; M1M3 - Mile 1, market 3; M1M4 - Mile 1, market 4; M1M5 - Mile 1, market 5

Table.5 Total coliform counts in sampled unbranded groundnut oils sold in Mile 3 Markets

SI	Coliform Count (cfu/ml)				
	M1M1	M1M2	M1M3	M1M4	M1M5
1 st	-	-	-	-	-
2 nd	-	-	-	-	-
3 rd	-	-	-	-	-

SI – sampling instances; M3M1 - Mile 3, market 1, M3M2 - Mile 3, market 2; M3M3 - Mile 3, market 3; M3M4 - Mile 3, market 4; M3M5 - Mile 3, market 5

Table.6 Analysis of variance (ANOVA) of Coliform counts in sampled unbranded groundnut oil sold in Mile 1 and Mile 3 Markets

SUMMARY					
Groups	Count	Sum	Average	Variance	
Means for Mile 1 markets	3	2.34×10 ⁴	7.80×10 ³	1.00×10 ⁷	
Means for Mile 3 markets	3	0	0	0	
ANOVA					
Source of Variation	SS	Df	MS	F _{cal.}	F _{tab.}
Between Groups	9.13×10 ⁷	1	9.13×10 ⁷	18.1392**	7.7086
Within Groups	2.01×10 ⁷	4	5.03×10 ⁶		
Total	1.11×10 ⁸	5			

SS - Sum of squares, df – degree of freedom, MS – Mean square, F_{cal.} – F calculated, F_{tab.} – F tabulated

Table.7 Total heterotrophic fungal counts in unbranded groundnut oil samples sold in Mile 1 Markets

SI	Total Heterotrophic Fungal Count (cfu/ml)				
	M1M1	M1M2	M1M3	M1M4	M1M5
1 st	1.00×10 ⁴	9.00×10 ⁴	-	1.00×10 ⁴	1.00×10 ⁴
2 nd	1.30×10 ⁴	7.80×10 ⁴	-	1.00×10 ⁴	1.30×10 ⁴
3 rd	1.00×10 ⁴	9.30×10 ⁴	-	1.60×10 ⁴	1.00×10 ⁴

SI – sampling instances; M1M1 - Mile 1, market 1, M1M2 - Mile 1, market 2; M1M3 - Mile 1, market 3; M1M4 - Mile 1, market 4; M1M5 - Mile 1, market 5

Table.8 Total heterotrophic fungal counts in sampled unbranded groundnut oils sold in Mile 3 Market

SI	Total Heterotrophic Fungal Count (cfu/ml)				
	M3M1	M3M2	M3M3	M3M4	M3M5
1 st	-	6.30×10 ⁴	1.00×10 ⁴	3.00×10 ⁴	1.00×10 ⁴
2 nd	-	6.45×10 ⁴	1.40×10 ⁴	2.50×10 ⁴	1.30×10 ⁴
3 rd	-	6.26×10 ⁴	1.00×10 ⁴	3.30×10 ⁴	1.70×10 ⁴

SI – sampling instances; M3M1 - Mile 3, market 1, M3M2 - Mile 3, market 2; M3M3 - Mile 3, market 3; M3M4 - Mile 3, market 4; M3M5 - Mile 3, market 5

Table.9 Analysis of variance (ANOVA) of THF counts in sampled unbranded groundnut oil sold in Mile 1 and Mile 3 Markets

SUMMARY					
Groups	Count	Sum	Average	Variance	
Means for Mile 1 markets	3	7.36×10^4	2.42×10^4	2.28×10^6	
Means for Mile 3 markets	3	7.04×10^4	2.35×10^4	9.44×10^5	
ANOVA					
Source of Variation	SS	Df	MS	F_{cal.}	F_{tab.}
Between Groups	7.92×10^5	1	7.92×10^5	0.4913 ^{ns}	7.7086
Within Groups	6.45×10^6	4	1.61×10^6		
Total	7.24×10^6	5			

SS - Sum of squares, df – degree of freedom, MS – Mean square, F_{cal.} – F calculated, F_{tab.} – F tabulated

Table.10 Identification of bacteria isolates from unbranded groundnut oil samples sold in mile and mile 3 markets

S/N	Gram Reaction	Coagulase	Catalase	Oxidase	Indole	Urease	Citrate utilization	Hydrogen sulphide	Motility test	Lactose	Bacterial isolate
1	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Non-motile	-	<i>Micrococcus</i> sp.
2	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	Non-motile	-	Coagulase- <i>Staphylococcus</i> sp.
3	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	Non-motile	-	<i>Staphylococcus aureus</i>
4	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Non-motile	+	<i>Klebsiella</i> sp.

N/B: +ve (positive), -ve (negative)

Table.12 Macroscopic identification of fungi isolates from unbranded groundnut oil samples sold in mile 1 and mile 3 Markets

Macroscopic Characterization and Texture	Inference Fungi
Green fungal colony that later turned greenish — yellow or pale green	<i>Aspergillus flavus</i>
Wooly velvet, whitish in colour but later turned fungal black	<i>Aspergillus niger</i>
Wooly colonies, the colour of the colony is white to pinkish gray initially and turns to olive brown or black	<i>Curvularia</i> sp.
Powdery whitish surface but later turned blush-green whitish reverse sides and edges	<i>Penicillium</i> sp.
Creamy powder growth that later turned black	<i>Rhizopus</i> sp.

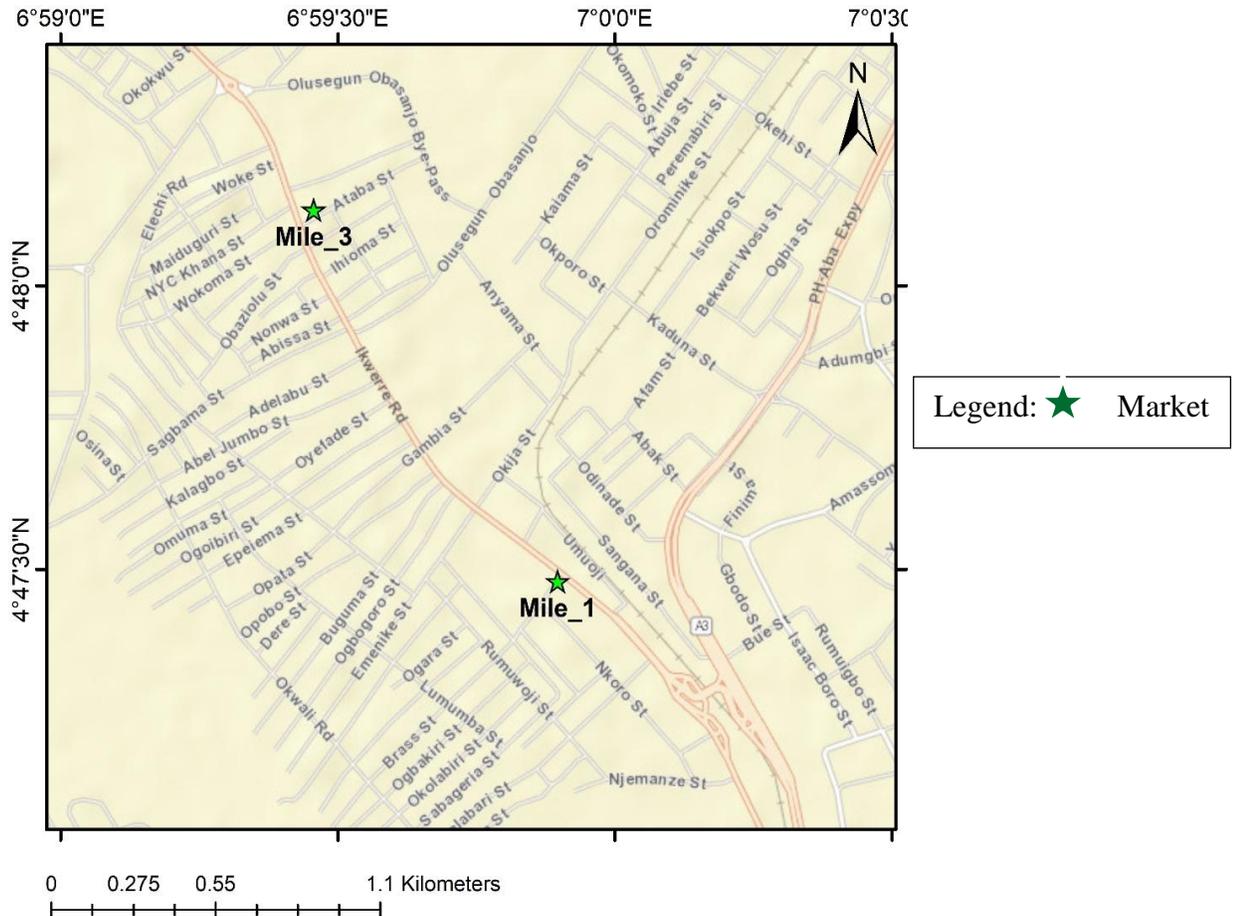
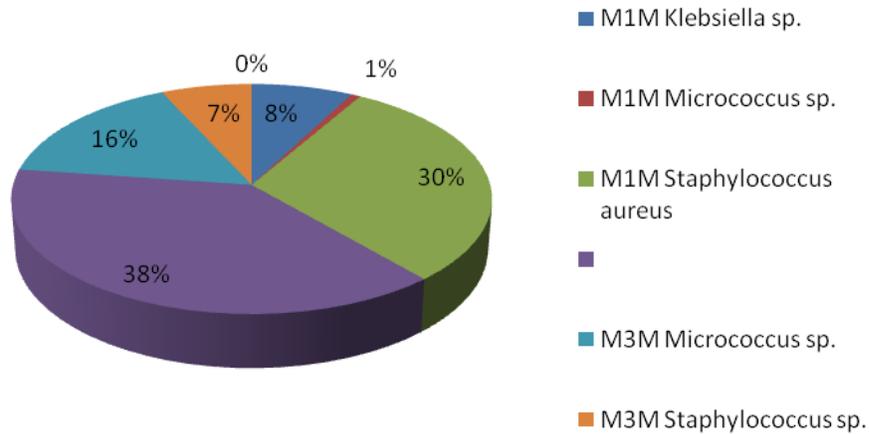


Figure.1 Map of Port Harcourt City Local Government Area showing the GPS coordinates of Mile 1 and Mile 3 Market



N/B: M1M (Mile 1 Market), M3M (Mile 3 Market)

Figure.2 Prevalence of identified bacteria isolates in the unbranded groundnut oil Samples

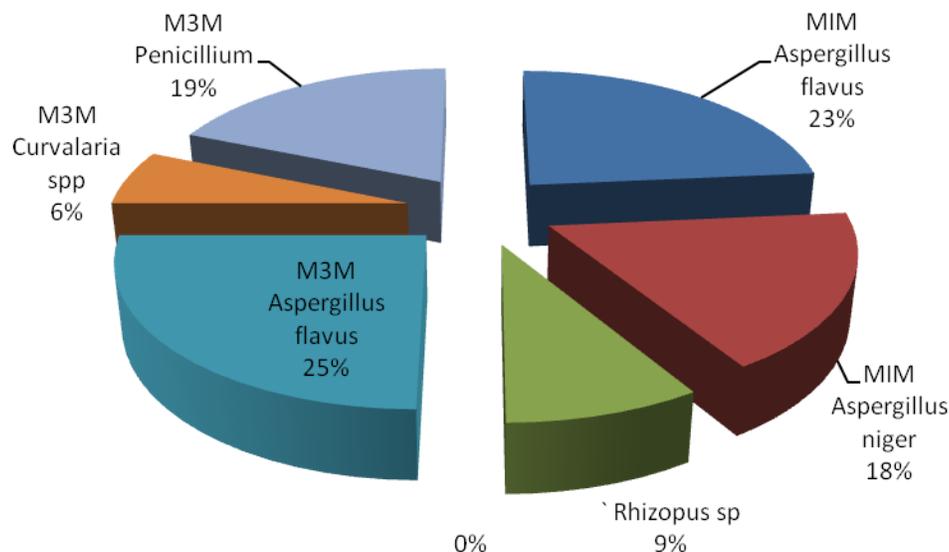


Figure.3 Prevalence of fungi isolates identified in the unbranded groundnut oil samples

The unbranded groundnut oil samples showed growth of both bacteria and fungi species, and the colony counts obtained were high above the recommended limit for edible groundnut oil. The high microbial counts could constitute a public health risks to the numerous consumers. The seeds used for production of groundnut oil should be of good quality and stored under conducive environment.

Local or small scale producers and retailers should be enlightened on the need to adopt Good Manufacturing Practices (GMP), while commercial producers should apply the Hazard Analysis Critical Control Points (HACCP) principles. Public health officials and the regulatory agencies should regularly visit the markets to monitor producers and dispensing of the oil to customers.

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