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Quality Assessment Of Unbranded Refined Palmkernel Oil In Distribution Within Five Local Markets In Port Harcourt, Rivers State Nigeria

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Abstract: *The study was conducted to evaluate the quality of groundnut oil sold in the five major markets in Port Harcourt River state, Nigeria. Samples were collected from Rumuokoro market, Choba market, Mile 1 market, Mile 3 market and Oil mill market. Microbial quality such as; Total heterotrophic bacterial load(THBC),Total Heterotrophic Fungal count(THFC) Total lipid utilizing bacterial and fungal counts (TLUB and (TLUF) , Total coliform count as ,well as physicochemical qualities such as; iodine number, relative density, pH, peroxide value and free fatty acid(ffa) content were investigated by standard methods. The results obtained shows that the total heterotrophic bacterial load ranged from 1.2×10^{7} - 1.5×10^{10}. Total heterotrophic fungal count ranged from 3.3×10^{5} -1.64×10^{6}, lipid utilizing bacteria (LUB) ranges from 6.0×10^{6} -1.4×10^{9} while the lipid utilizing fungi ranges from $3.0\times10^{5}\times10 - 7.0\times10^{6}$. Bacteria isolates tentatively identified and their frequency of occurrence in all the samples were Staphylococcus sp. (100%)., Alcaligenes sp.(60%)., Enterococcus sp. (100%)., Salmonella sp. (100%)., Achromobacter sp. (40%)., Micrococcus sp. (40%)., and Klebsiella sp. (20%). Fungal isolates identified as Aspergillus sp., Penicillium sp. and Fusarium sp. Results obtained from the physicochemical analysis showed that Iodine number(wiji's) was between 17.80-15.86, Relative density(g) 0.94-0.905, pH 5.3, Peroxide value(Meq/kg) 59.92-59.48 and free fatty acid (%) 1.57-1.29. Results on the microbiological and physicochemical qualities were found to exceed acceptable limits, this suggests that palm kernel oil in distribution within Port Harcourt metropolis are of low quality and requires some improvement in the production process.*

Keywords : *Groundnut oil, Quality, Microbial, Physicochemical.*

I. Introduction

Palm kernel oil is obtained from palm kernel seeds and grows widely in certain locations such as Niger Delta and South Eastern parts of Nigeria (Komolafe and Adegbola, 1980; Samuel and Alabi, 2012). The palm kernel oil is extracted from the walnut which has a hard hill or shell called kernel. It is also called lauric oil because it has high content of lauric acid (Deutheux, 2004;Macaire et al., 2010) the extraction of palm kernel oil is made by various techniques and the obtained oil is used in the food domain as well as non-domains. In the food domain, it is used in the preparation of certain traditional dishes and enter also in the constitution of food fats. In the non-food domain, its higher proportion of lauric acid gives to the oil an important characteristics used in the industries of beauty care and soap factory. This property also characterizes, its use in traditional pharmacopoeia (Salmiah et al., 1998; Macaire 2010). The acceptability of the products at world edible oil market depends on its ability to satisfy basic standard tests for fats and oil (Takakura, 2002; Samuel and Alabi, 2012).

II. Materials And Methods

2.1 Collection of Sample:

Sampling were carried out using the modified method by Chabiri et al., (2009).Palm kernel oil (Elaeis guinensis) is derived from palm kernel mills. Samples were similarly collected from different local sellers within the same market in Port Harcourt. Five local markets were selected, these include; Rumuokoro market, Oil mill, Mile one (1), Mile three (3) and Choba markest. Fifty mililitres of palm oil are collected from 10 different spots from different local sellers to make a composite of 500ml collected in a well labeled sterile bottle. For each market the sum total of 50 sample were composited into 5 samples, each per market.

2.2 Maintenance of Sample:

Samples were maintained at ambient temperature in sterile containers and were quickly transported in sterile canisters to the laboratory for analysis.

2.3 Preparation of Sample:

Samples were placed in sterile labeled bottle and were first diluted in Ringers solutions(0.12g/l calcium carbonate ; 0.105g/l potassium chloride; sodium bicarbonate 0.05g/l; and sodium chloride 0.05g/l) for emulsification of oil sample. The diluted samples were then employed for subsequent analysis for microbial content.

2.4 Microbiological Analysis of Sample

All the samples were analyzed for their viable bacterial and fungal load, fungi and bacteria that can degrade palm oil, extracellular lipase producers as well as total coliform.

2.4.1 Enumeration of Total Heterotrophic Bacteria

The molten nutrient agar medium was employed and autoclaved at 121^{o}c for 15minutes.Oil samples were serially diluted in the media in test tube before spreading onto sterile petri-dishes. One mililitre of the dilutions 10^{-2},10^{-3} ,10^{-4}.10^{-5},Were employed during plating. The agar medium was allowed to cool and solidify before plates were incubated at room temperature for 24 – 48hrs. Colonies were picked from the plates to purify. Pure culture of the bacteria were streaked on nutrient agar slant and stored in the refrigerator (4^{0}C) as stock cultures for identification tests.

2.4.2 Enumeration of Total Heterotrophic Fungal

Potato Dextrose Agar (PDA) and Ringers solution was employed in this analysis. Rifampicin (an antifungal agent) was added to PDA to suppress bacterial growth the agar medium was autoclaved at 121^{0}C for 15 minutes. Oil samples were serially diluted in molten agar in test-tubes before pouring into sterie petri dishes, one milliliter of 10^{-5},10^{-7} dilution were used. The agar medium was allowed to cool and solidify before the plates were incubated at room temperature (28^{0}c) for 72 – 120hrs.The counts for Fungal load was taken. A portion of each fungal colony which developed was picked and sub-cultured onto Potato Dextrose Agar plates using inoculating needle which was then kept as stock cultures for identification.

Enumeration of Total Coliform

Due to the immiscible nature of Palm kernel oil with an aqueous solution, solid Macconkkey medium was employed for the enumeration of total coliforms. An aliquot from each prepared samples were plated onto MacConkey agar medium and incubated at 37^{0} C for 2 days

Isolation of Lipid Utilizing Bacteria (LUB)

Samples that have been serially diluted on a palm oil medium by a modification of the medium by Sirisha et al., (2010) containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) palm oil (sole carbon) and 2% agar by spread plate method. Plates were incubated at 37^{0}C for two days. Pure cultures of the isolate were maintained on nutrient agar (yeast extract) NaCl, peptone and 2% agar, pH 7.0) and were sub cultured every 15 days.

Isolation of Lipid Utilizing Fungi (LUF)

Modified composed medium (15g peptone, 5g NaCl, Ig $CaCl_2$, 10ml palm oil, 15g agar) was employed. An aliqout of the sample was plated onto the medium which has palm oil as the sole source of carbon and incubated for four days at 37^{0}C (Sirisha et al., 2010)

Screening Fungi for Lipase Activity Using The Rhodamin-B Agar Dye

Modified method by Savitha et al (2007). This method involves measurement of fluorescence caused by the fatty acid released due to the action of lipase on olive oil. The quantitative fluorescence assay is based on the interaction of Rhodamine B with fatty acid released during the enzyme hydrolysis of olive oil.

The fungi isolates were inoculated in media of the following composition (g/L). Potato dextrose agar, and sodium chloride 4.0. The medium was adjusted to pH 7.0, autoclaved and cooled to 60^{0}C. Olive oil (13.25ml) and 10ml of Rhodamin-B solution (0.001% w/v) was added with stirring and emulsified by mixing for 1 min. The medium was allowed to stand for 10min at 60^{0}C to reduce foaming. 20ml of the medium was poured into sterile petri dishes.

Screening Bacteria for Lipolytic Using The Rhodamin Bdye Agar

A solid medium composed of 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% olive oil and 10ml of Rhodamin-B stock, 0.001% (w/v). The mixture was well homogenized by mixing. Cultures were incubated for 18-2hrs. For Rhodamin-B medium at 36^{0}C the lipolytic activities of isolates was monitored by fluorescence with

UV light at 350nm. The isolates that produce extracellular lipase would hydrolyze the substrate and produce halo zone surrounding the holes of the gar plates (Sirisha et al., 2010).

Identification of Isolates:

Identification of Bacterial Isolates

All bacterial isolates on the PCA plates were identified based on biochemical characteristics as described by Cheesbrough, (2000) and U.S.FDA manual online (2001).

Identification of Fungal Isolates

All fungal isolates were identified based on their macroscopic and microscopic appearance with reference to manual of Barnett and Hunter, (1972), Larone (1995) and Mycology online of Ellis (2006).

Physico-Chemical Analyysis of Samples:

Peroxide value, Iodine value, relative density Free-fatty acid and pH of each sample was analyzed using methods employed by Pearson (1976) Ohimain et al., (2013) and Ohimain et al., (2012

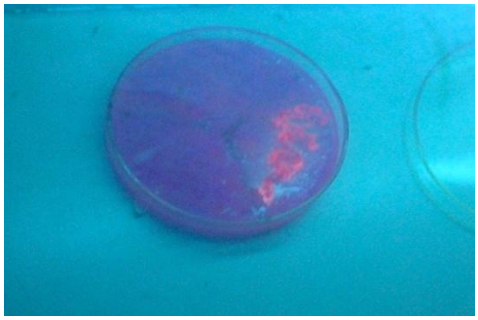

Plate 1: Bacterial Colonies Under Uv Illumination. Orange Halo Is Due To Extracellular Lipase Production By The Colony

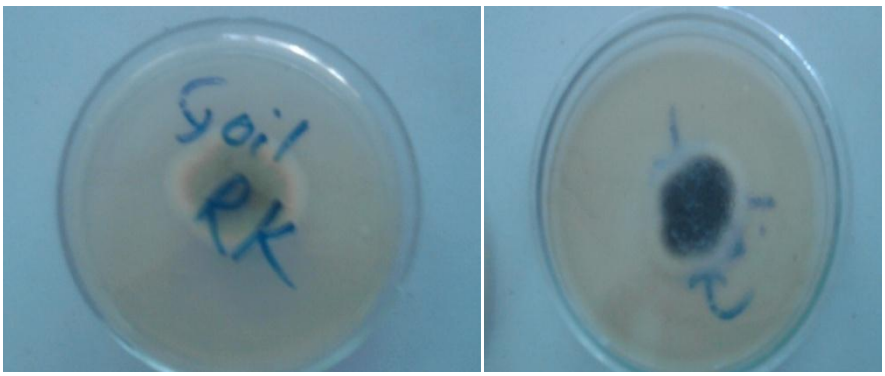

Plate 2: Microscopic Characteristics Of Aspergillus Niger Isolated From Palm Kernel Oil From Rumuokoro Market.

III. Results And Discussion

The quality of palm kernel oil (PKO) depends on its physicochemical state and characteristics and also on its microbiological quality. According to the stipulated standard by National Agency for Food and Drug Administration and Control(NAFDAC).The acceptable limit of microorganism in palm oil and other vegetable oils are 10^5ml for Aerobic mesophillic bacteria or total heterotrophic Bacteria.10^4ml for Aerobic mesophillic fungus, 10^2ml for coliforms,(Okechalu et al.,2011). The differences in the results obtained for the total heterotrophic bacteria and fungi load and the total lipid utilizing bacteria and fungi load may be due to the presence of non lipid degrading microbes co-existing with lipid degraders for survival.

Table 1: Total heterotrophic bacterial and fungal count.

	OIL MILL	CHOBA	MILE 1	MILE 3	RUMUOKORO
THBC	3.0×10^8	3.3×10^8	1.5×10^{10}	1.2×10^8	1.2×10^7
THFC	1.56×10^6	2.2×10^6	9.2×10^5	1.4×10^6	5.5×10^5
TOTAL COLIFORMS	1.6×10^8	5.0×10^8	3.0×10^5	5.0×10^7	4.2×10^8

Results obtained on the microbiological analysis (Table 1) carried out showed that the refined palm kernel oil samples from all the five markets in Port Harcourt were above the microbiological quality standard of the National Agency for food and drug administration and control.

Table 2: Lipid utilizing bacterial count

	OIL MILL MARKET	CHOBA MARKET	MILE 1 MARKET	MILE 3 MARKET	RUMUOKORO MARKET
TOTAL LIPID UTILIZING BACTERIA	6.0×10^{7}	1.6×10^{8}	1.4×10^{9}	3.3×10^{7}	6.0×10^{6}
TOTAL LIPID UTILIZING FUNGI	2.0×10^{5}	3.0×10^{5}	7.01×0^{4}	6.0×10^{5}	4.0×10^{4}

The presence of coliform is an indicator of fecal contamination which could be as a result of the use of untreated water or contaminated water and also as a result of bad handling during processing and packaging.

Growth on Rhodamine B agar and subsequent of exhibition of the characteristic yellow hyper orange halo around the colony under ultra violet light signifies the secretion of extracellular lipase by the isolate. Upon inoculation of isolates obtain from samples from market the presence of the orange halo which was expressed on the Rhodamine B plate under the ultra violet light indicate the ability of the organisms to liberate extracellular lipase. The orange halo on the Rhodamine plates (PLATE 1) shows the ability of the isolates to liberate extracellular lipase which is known for its ability to degrade lipid liberating free fatty acids and glycerol.

Table 3: Physico-chemical characteristics of samples

SAMPLE SITE	IODINE VALUE(wijis)	RELATIVE DENSITY(g)	PEROXIDE VALUE(Meq/kg)	FREE FATTY ACID(%)	pH
RUMUOKORO	16.15	0.939	59.85	1.36	5.34
CHOBA	16.05	0.941	59.48	1.29	5.30
MILE 3	17.81	0.949	59.55	1.57	5.38
OIL MILL	15.87	0.905	59.93	1.41	5.32
MILE 1	15.93	0.918	59.76	1.35	5.37

The liberation of free fatty acids is an indication of spoilage. According to the food and Agricultural organization (FAO) and world health organization (WHO) stipulate the acceptable free fatty acid value (Ffa) not to exceed 1.376%. Most of these sample falls within the standard except for samples from Mile 3 and Mile 1 markets because of the high number of lipid utilizing bacteria isolated from the sample from these markets. The low value obtained could suggest the onset of the breakdown of lipid molecules by exogenous lipases liberated by lipolytic microorganisms. Acidic pH obtained may be due to the presence of liberated fatty acids.

Iodine number measures the degree of unsaturation of free fatty acids indicating that the higher the degree the greater the likely-hood of rancidity. According to the FAO/WHO and CODEX standards, the limits value for iodine number in grams is 50.0-55.0. The iodine value for these samples, where found to fall within the limits (TABLE 3); an indication of the presence of saturated fat and a very low tendency to get rancid.

Peroxide value, reflects the state of oxidation and therefore the stability and quality of the oil. The peroxide value of samples is found to exceed the stated standard this shows the high oxidation state of the samples this could be the reason for the constant development of bad tastes in the palm kernel oil over a short period of time. Peroxide value determines the degree of oxidation in oil as well as an indication of level of deterioration of oil and fats(Nwanekezi and Onyeagba (2007); Okechalu (2011) . The high Peroxide value of the samples indicates an onset of oxidation, which agrees with the reports by Ekpa and Ekpe (1996) who stated that lipid degrading enzymes such as peroxidases and lipooxygenases (Onyeka et al., 2005). Oxidation takes place also, when microorganisms are capable of utilizing fatty acids in the absence of other simple sources of carbon through a catalytic pathway known as ß-oxidation.

Relative density, is a physical measures of adulteration of vegetable oils, since different oils have characteristic density and refractive index. Studies have shown that the contamination of vegetable oils with particulate matters and other chemical adulterants such as potassium hydroxide brings chemical reaction with fatty acids of vegetable oils with the production of soap i.e. carboxylic acid ester which alter the optical activity of the vegetable oils and increases the susceptibility of the vegetable oils to become rancid or spoiled (Williams, 1990; Chabiri et al., 2009). The maximum acceptable limit of Relative density for palm kernel is within 0.899-0.914 according to CODEX (1992).The results obtained on this were relative exceeding with slight differences.

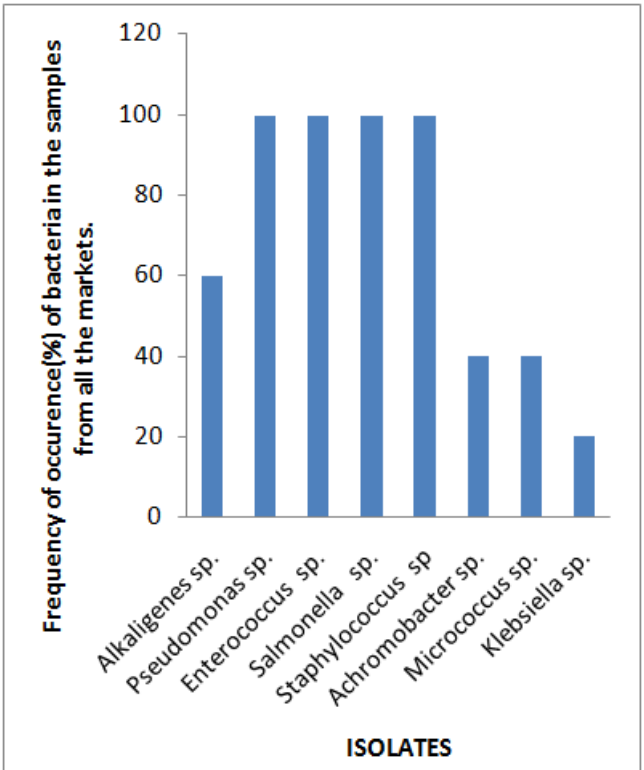

Figure 1: Frequency Of Occurrence Of Bacteria in Samples from All The Markets.

The study on the microbial profile of palm kernel oil carried out suggests the peculiarity of some isolated sample from different markets *Alcaligenes* sp were found to be peculiar to Mile 1, Mile 3 and Oil mill markets. *Enterococcus, Pseudomonas and Staphylococcus* species in all the markets which could be attributed to poor handling and human contact.

Achromobacter sp were found in Mile 1 and Oil mill markets only, Micrococcus sp in Rumuokoro markets where as *Klebsiella* sp was found in only Rumuokoro markets; in addition to bacteria, lipolytic fungi such as *Penicillium* sp, *Aspergillus niger* and *Fusarium* species were found in all the samples from all the markets.

IV. Conclusion

The microbiological quality of samples which exceeded the standard quality suggests how poor the traditional / local production process and poor handling of products have been. The presence of coliforms and other indicates the possible presences of organism that could pose health hazards. But can be overcome over boiling, interestingly, these products have been found to have the tendency to resist rancidity.

Therefore, more improvement is required in the production and distribution of groundnut oil in order to meet the stipulated standards.

Reference

[1]. Barnett H.L and Hunter, B. B. Illustrated Genera of Imperfect fungi. Burgess Publishing Company, Minneapolis, **1972**; Pp 62 – 63
[2]. Chabiri, S.A., Hati, S.S.,Dimari, G.A. and Ogugbuaja, V.O.(2009). Comparative Quality Assessment of Branded and Unbranded Edible Vegetable Oils in Nigeria. Pacific Journal of Science and Technology,10(2):927-934.
[3]. Cheesbrough, M. District Laboratory Practice in Tropical Countries part 2 Cambridge. Cambridge University Press. **2000**; Pp 290-320.
[4]. Codex A., 1992 Programme mixte sur les norms alimentaires FAO Rome.
[5]. Deutheux, B.,(2004): Utilisation de l'huile de palme comme combustible dans les moteurs diesels. Travaux de fin d'etude pp.31-33.
[6]. Egan H, Kirk RS, and Sawyer R (1981). Pearson's Chemical Analysis ofFood. 8th ed. New York, Churchill, Livingstone. 507-546
[7]. Ekpa OD,and Ekpe UJ (1996). Effect of coconut oil concentration on the melting point profile and free fatty acid formation of palm oil. Nig. J.Chem. Res. 8:12
[8]. Ellis.D. Mycology online. The University of Adelaide, Australia. **2006**.
[9]. Komolafe., M.F and A.A Adegbola 1980 AgriculturalScience for West Africa Schools and Colleges" Oxford University Press Oxford UK pp 130

[10]. Larone. D.C Medically Important Fungi: A guide to identification. American Society ofMicrobiology. Washington, DC. 3rd Ed. **1995**
[11]. Macaire, WH, Tienchen B., Tengyang W.,Tchonanyuep, ME, Kapseu C., Linder M and Fanni.J.(2010). Extraction of Palm kernel oil In Cameroon: effects of Kernel drying on the Oil quality. Journal of Foods Technology8(1);1-7
[12]. Ngando EGF, Mpaondo MEA , Dikotto EEL, Koona P (2011).Assessment of the quality of crude palm oil from smallholders in Cameroon. J. Stored Prod. Postharvest Resource 2 (3):52-58
[13]. Nwanekezi EC,and Oyeagba RA (2007). Effect of Spoilage Microorganisms on the physicochemical properties of African Pear Fruit Oil. J. Food Agri. Environ.5:90-93
[14]. Okechalu JN, Dashen MM, Lar PM, Okechalu B, Gushop T (2011). Microbiological quality and Chemical Characteristics of Palm Oil sold in Jos Metropolis, Plateau State, Nigerian. Journal Microbial Techechnological Resource 1(2):107-112.
[15]. Ohimain E.I, Daokoru-Olukole C.,Izah S.C and Alaka E.E (2012). Assesment of quality of crude paim oil, produced by small holdernprocesses in River State. Nigerian Journal of Agriculture, Food and Environment. 8(2)20-24.
[16]. Ohimain E.I, Izah S.C and Famari A.D(2013). Quality Assesment of crude Palm oil produced by semi-mechanised processes in Bayelsa State Nigeria. Discourse Journal of Agricultural and Food Sciences. 1(11):171-181.
[17]. Onyeka EU, Onugbu NI, Onuoha NU, Ochonogor F (2005). Effect of extracting pre-treatment on the Composition and Characteristics of seed and pulp oil of African Black pear (Dacryodes edules). Nig. Food J. 23:13-20
[18]. Pearson D (1976). The Chemical Analysis of Food. Churchill Livingstone, Edinburgh, London 121-150
[19]. Savitha. J.; S. Srividya, S, R. jeyat, Pauyal, S. Priyanki, G.W. Rashmi, K.T Roshini and Y.MShantala (2007). Identification of potential fungi strains for the production of Inducible, extracellular and alkalophilic lipase. African Journal of Biotechnology.
[20]. Salmiah A.I.,Zahariah and Y. Mohtar(1998). Oleochemicals for soaps and detergents international seminar and exposition world scenario in Oils. Oleochemicals and Surfactants Industries OTAL LUKNOW India.
[21]. Samuel O.D and AGP Alabi(2012) Problems and solution in Oil processing from kernel seeds. The Pacific Journal of Science and Technology. 18:373-381.
[22]. Sirisha E, N. Rajasekar and M.Lakshmi Narasu (2010) Isolation and Optimization Of Lipase Producing Bacteria from oil contaminated soil. Adv. Bio, Res 4(5) 249-252.
[23]. Takakura T. (2008). Food Production strategy in East-Asia-Engineering Perspective.in the Third Millenium" CIGR Journal of Scientific Research and Deevelopment Chicago IL
[24]. United States Food and Drug Administration, Bacteriological Analytical Mannual Online. FDA/Center for Food Safety and Applied Nutrition. **2002**
[25]. Williams, K.A. 1990. Oils, Fats and Fatty Foods, Their Practical Examination (4thEdition). Longman Publishers: London, UK. 41-33.