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Quality assessment of Unbranded Palm Oil Distributed in Five Local Markets in Port Harcourt, Rivers State Nigeria

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Absracts: *The study was carried out to assess the microbiological and physicochemical characteristics of palm oil sold in 5 major markets (Rumuokoro, Choba, Oil mill, Mile 1, Mile 3) within Port Harcourt metropolis. A total of 50 samples were collected from the five different markets in sterile bottles. The bacterial and fungal loads were obtained by standard plate count method and isolates were tentatively identified based on their cultural, morphological and biochemical characteristics. Physicochemical parameters such as; free fatty acid value, iodine value, peroxide value, relative density and pH values were also determined using standard protocols. The mean Total heterotrophic bacterial count (THBC) ranged from 1.70×10^{9} to 9.0×10^{8} cfu/ml. Samples from Oil mill market had the highest bacterial load and samples from the Mile 1 market had the least. The mean value for lipid utilizing bacteria (LUB) ranged from 2.0×10^{7} to 9.0×10^{7} cfu/ml with Rumuokoro market having the lowest and Oil mill having the highest value. The total heterotrophic fungal count (THFC) ranged from 1.00×10^{6} to 8.60×10^{5} cfu/ml. Samples from the Mile 1 had the least fungal load and Samples from Choba market had the highest fungal load. The total lipid utilizing fungal count ranged from 1.0×10^{5} to 8.0×10^{5} cfu/ml. Samples from Choba market had the least value and Rumuokoro market had highest fungal load. Total coliform counts was; 7.0×10^{6}cfu/100ml (Oil mill Market), 3.0×10^{5}cfu/100ml (Choba Market), 8.8×10^{6}cfu/100ml (Mile 1 Market), 7.0×10^{6}cfu/100ml(Mile 3 Market) and 4.0×10^{5}cfu/100ml (Rumuokoro Market). isolated microorganisms found to be in associated with the palm oil samples were Enterobacter sp, Micrococcus sp, Pseudomonas sp, Klebsiella sp, Staphylococcus sp, Aspergillus niger, Aspergillus fumigatus , Fusarium sp and Mucor sp. Results obtained on the physicochemical quality of samples were as follows; Iodine value wijis 16.98-16.86, Relative density (g) 0.939-0.874, Peroxide value(meq/kg) 19.94-19.87, free fatty acids (mgKOH/g) 16.92-16.40 and pH 5.4-4.80. The bacterial load of palm oil samples from most of the markets and physicochemical qualities were found to greatly exceed the stipulated maximum acceptable linits compared to standards.*

Keywords: *Palm Oil, Unbranded, Microbial Load, Quality.*

I. Introduction

Oil palm is a common name for ornamental and economically valuable palm tree, native to Western Africa and widespread throughout the tropics. The oil palm and the American oil palm are members of the family Arecaceae formerly known as Palmae. The oil palm is classified as Elaeis guineensis and the American oil palm as Elaeis Oleifera.

The oil palm grows up to 9m (30ft) in height. It has a crown of feathery leaves that are up to 5m (15ft) long. The flower clusters is on a short thick spike at the base of the leaves. Flowering is followed by the development of a cluster of egg shaped, red, orange or yellowish fruits. Each fruit is approximately 3cm (1inch) long and contains from one to three seeds embedded in a reddish pulp.

Palm oil is derived from the mesocarp of the fruits of West African oil palm (Elaeis guineensis) or of E. Oleifera and E.odora which are natives of South America. The extracted oil contains a considerable proportion of water as well as soil, fibre and debris Palm oils rich content of saturated and monounsaturated fatty acids has actually been turned into an asset in view of current dietary recommendations. The use of palm oil in combination with other oils and fats facilitates development of a new generation of fat products that can be tailored to meet most current dietary recommendations (Sundram, 2003). Palm Vitamin E (30% tocopherols and 70% tocotrienols) has been extensively studied for its nutritional and health properties, including antioxidant activities, cholesterol lowering, anti-cancer effects and protection against artherosclerosis. A relatively new out-put from the oil palm fruit is the water soluble phenolicflavonoid rich antioxidant complex, which has potent antioxidant properties coupled with beneficial effects against skin, breast and other cancer. Its water solubility is being currentlytested for use as nutraceutical and in cosmetics with potential benefits against skin aging (Sundram, 2003).

Various microorganisms have been implicated in the deterioration of palm oil and other vegetable oils e.g. *Aspergillus flavus, A. niger, A fumigatus, Penicillum frequentans, Rhizopus stolonifer* etc. These moulds have lipolytic activities and their growth results in lipolysis (spoilage) of the palm oil. In general the rate of lipolysis is proportional to the amount of the free fatty acid already present in the oil. (Okechalu et al 2011)

Frying and cooking of the oil can reduce the microbial load to the minimum level, but the fact that some individuals consume this product raw is of concern as this may result in health problems in such individuals when the microbial load is high.(Okechalu et al., 2011)

II. Materials And Methods

2.1 Collection Of Sample:

Sampling were carried out using the modified method by Chabin et el (2009).

Palm oil (Elaeis guinensis) is derived from palm kernel mills. The sampling method employed samples of similarly collection 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},was made use of 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 (40^{0} C) as stock cultures for identification tests.

2.4.2 Enumeration of Total Heterotrophic Fungi

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 121c for 15 minutes. Oil samples were serially diluted in molten agar in test-tubes before pouring into sterile 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 oil, solid Macconkkey medium was employed for the enumeration of total coliforms. An aliquot of the prepared samples were plated onto Macconkey agar medium and incubated at

Isolation of Lipid Utilising 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 Utlizing Fungi (LUF)

Modified composed media (mainly 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 37^0C (Sirisha and Narasu. 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 Rhodamin B with fatty acid released during the enzyme hydrolysis of olive oil.

The fungal isolates were inoculated in media of the following composition (g/L). Potate dextrose agar, sodium chloride 4.0. The medium was adjusted to pH 7.0, autoclaved and cooled to 60^0C. 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 60^0C 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 36^0C 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).

III. Result And Discussion

The quality of palm oil 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).

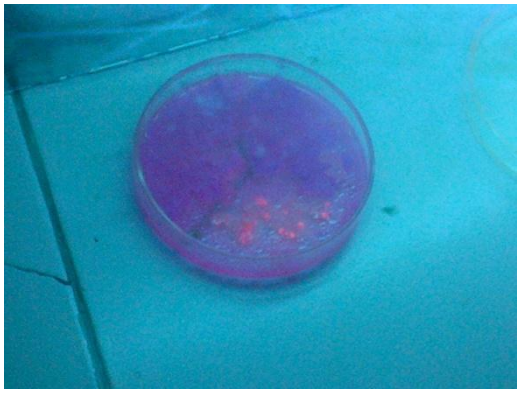
From the results obtained from the microbiological analysis, the sample exceeded the acceptable limit.

	OIL MILL	CHOBA	MILE 1	MILE 3	RUMUOKORO
THBC	2.72×10^9	2.30×10^8	1.7×10^9	9.0×10^8	2.0×10^8
THFC	1.5×10^6	8.6×10^5	1.00×10^6	1.64×10^6	3.3×10^5
TOTAL COLIFORMS	7.0×10^6	3.0×10^5	8.8×10^6	7.0×10^6	4.0×10^5

Table 2: Total Lipid Utilizing Bacteria And Fungi Load Of Samples

	OIL MILL MARKET	CHOBA MARKET	MILE 1 MARKET	MILE 3 MARKET	RUMUOKORO MARKET
Total Lipid Utilizing Bacteria Load	9.0×10^7	4.0×10^7	3.1×10^8	6.5×10^8	2.0×10^7
Total Lipid Utilizing Fungi Load	1.56×10^6	8.60×10^5	1.00×10^6	1.64×10^6	3.30×10^5

The presence of coliform is an indication of faecal contamination which could be become as a result of mishandling and poor processing of palm oil. The orange halo around the colonies on the Rhodamine B plates shows the presence of lipolytic organisms capable of liberating extracellular lipases.



The detection of free fatty acids(FFA) in palm oil is an indication of degree of spoilage.

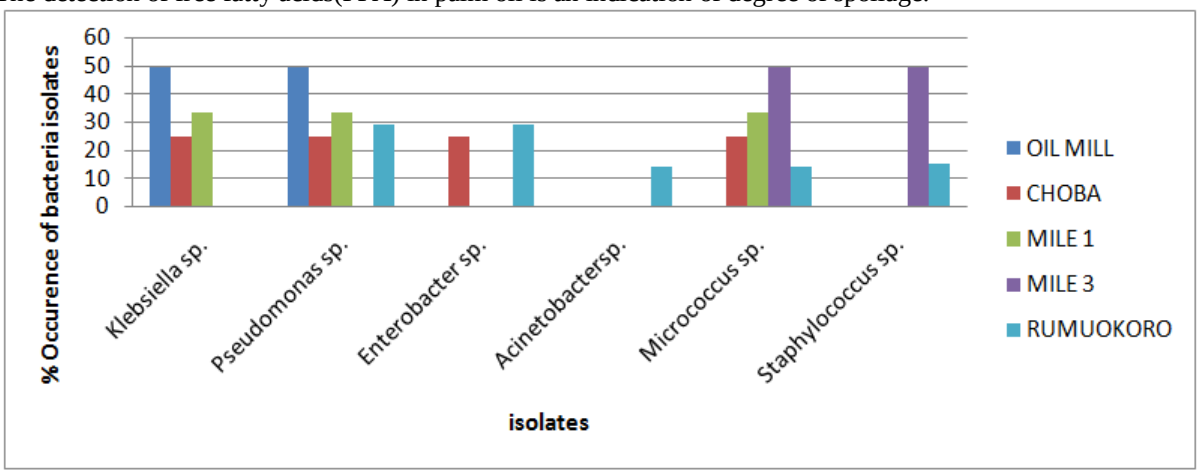


Fig 1: Percentage distribution of identified bacteria in all the markets

The microbial profile of Palm oil sample for each markets show they contain organism that are peculiar to a particular market. *Pseudomonas* sp was found in all the markets, as well *Klebsiella* sp were found in Choba and Mile 1 market. *Micrococcus* sp were found in Choba,Mile 1 and Mile 3 markets. . The presence of *Enterobacter* sp in the sample which is an indicator of contamination has been reported to cause Gastroenteritis (Okechalu et al,2011), and therefore pose serious health hazards. Although palm oil is consumed boiled, it should be noted that consumption without heating would be the safest measure to be employed against health implication.

Table 3: Frequency of occurrence of Fungi isolates.

ISOLATED GENERA	MARKETS					FREQUENCY OF OCCURENCE(%)
	MILE 1	OIL MILL	CHOBA	RUMUOKORO	MILE 3	
***Aspergillus* sp.**	+	_	+	_	+	**60**
***Mucor* sp.**	_	+	+	_	_	**40**
***Fusarium* sp.**	_	_	_	+	_	**20**

The presence of moulds in some of the samples may be because some of the palm oil sample are sold openly in the market exposed to the spores of the organism which are dormant and are highly resistant to the lethal effects of heat drying and ultraviolet radiation.

Table 4: Physicochemial Quality Of Samples.

Source of oil Sample.	iodine Value wijis.	relative Density Value(g)	peroxide value(Meq/kg)	free fatty acid yalue (%)	pH value
Oil mill Market	16.9507	0.8749	19.9068	16.50%	5.40
Mile 1 Market	16.8689	0.9391	19.8700	16.70%	4.94
Rumuokoro Market	16.9752	0.9198	19.8714	15.92%	5.02
Mile 3 Market	16.7500	0.9360	19.9481	16.26%	4.98
Choba Market	16.9804	0.9338	19.8124	16.40%	4.80

The maximum acceptable limit for free fatty acis(FFA) is 5% (Ngondo et al.,2011; Ohimain et al., 2012). Results obtained from the free fatty acid analysis is far above the maximum acceptable limit. The high (FFA) values obtained may be due to the exposure of samples to normal temperature at the market stores (Okechalu et al.,2011) or even due to the presence of very high load of both lipid utilizing fungi bacteria (Table 2) liberating extracellular lipases, which break down ester bonds in lipid molecules to liberate diglycerides, monoglycerides, glycerols and free fatty acids (Bora and Kalitha.,2007). Egan et al, (1981) stated that glycerides in oil can be decomposed by lipases or other actions and that decomposition can be accelerated by light heat.

Peroxide value determines the degree of oxidation in oil as well as an indication oof 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.

Iodine value is an index for detecting adulteration in Palm Oil and other vegetable Oil (Okechalu et al., 2011). Ekwenye reported that high iodine value is an indication of greater degree of unsaturated fatty acid and higher tendency to get rancid on the other hand low IV obtained is far lower than the minimum acceptable limit by codex standards; an indication of the presence of saturated fat and low tendency to get rancid.

Relative density.

The pH of samples was generally acidic and can be attributed to the presence of high FFa liberated by lipid utilizers. Relative index of samples was within the acceptable limits by international standards

IV. Conclusion

Most Palm oil distributed in Port Harcourt are produced by traditional extractors, according to Ngondo et al.(2011) it has been demonstrated that oil produced by traditional oil extraction methods re of lesser quality when compared with oil produced from industrial oil mills.

In general, samples from markets in Palm Oil samples from the local markets in Port Harcourt metropolis do not meet NAFDAC standard for food and International standards for oil and vegetable

The limits exceeded by the microbiological and physic-chemical characteristics of the oil sample purchased from these markets indicates the poor processing and poor handling of palm oil by extractors and local sellers.

Improvement in the production procedures and processes as well as good seed selection, good handling practices and clean environment can go as far improving the standard of palm oil sold in Port Harcourt.

V. Recommendation

Due to the importance and necessity of Palm oil as a food source and its Industrial value. I recommend that more stringent regulatory policies be enforced by Regulatory agencies such as Food and Agricultural organization(FAO), World health organization(WHO),National Agency for Food and Drug administration and control(NAFDAC) concerning its production, distribution and Quality control majorly in countries of its(Palm oil) International trade. Also (FAO) should create workshops for local manufacturers on the aseptic measures to employ during processing, handling, storage and transportation of Palm oil.

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