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Microbial and Physicochemical Qualities of Edible Palm Oil Sold in Major Markets of South-Eastern Nigeria

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ABSTRACT

Physicochemical and microbial characteristics of edible palm oil sold in major markets of South-Eastern States of Nigeria were studied. This study was undertaken to determine the wholesomeness or otherwise palm oils sold in South-Eastern States of Nigeria. Replicate 45 samples were collected from fifteen markets across the states. Analyses were by standard analytical methods. Microbial analyses indicated that some samples had microbes; *Aspergillus niger*, *Aspergillus flavus, Mucor sp., Penicillium chrysogenum, Saccharomyces cerevisiae*, *Bacillus sp*, *Micrococcus sp*, *Pseudomonas sp*, *Staphylococcus aereus* and *Proteus sp*. Bacterial loads ranged from 2.0 x 10^4cfu/ml to 9.0 cfu/ml, with the sample from Eke Emene, having the highest load. Mould counts ranged from1.0 x10^4 to 10.0 x 10^4 cfu/ml. The highest count was for samples from Imo and Enugu States. Physicochemical assay showed mean values ranging from 0.130 to 0.20%; 0.82 to 0.92; 6.5 to 8.30meq/Kg; 46.42-50.31Wij's; 2.60 to 2.90 mgKOH; 194.3 to 204 mgKOH and 0.08 to 1.05% for the moisture content, specific gravity, peroxide value, iodine value, free fatty acids, saponification value and impurity content, respectively. Though the microbial loads fall within the permissible range of Standards Organization of Nigeria (SON) and National Industrial Standards (NIS) the regulatory agencies in Nigeria, some of the isolated microorganisms are known to be of human health significance. Some others like *Mucor sp* may enhance deterioration and spoilage thus reducing product shelf life. The low moisture and free fatty acids indicate the oils could have extended shelf life maintaining good quality for food and industrial uses. The assayed palm oils were within the SON/NIS recommended standards and thus are of acceptable quality.

Keywords: Palm oil contamination, Microbial quality, Physicochemical parameters and South-Eastern Nigeria

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INTRODUCTION

The oil palm tree (*Elais guineensis*) is an ancient tropical plant indigenous to a number of West African countries. It is indeed a very resourceful and economic oil-bearing plant which has been widely cultivated in the humid tropical and subtropical regions of the world including Indonesia, Malaysia, Thailand, Columbia, and Nigeria (Iza and Ohimain, 2016). Malaysia and Indonesia are the leading producers of palm oil which

accounts for 86% of global production; other palm oil-producing countries of the world include Guinea, Cote d'Ivoire, India and Brazil (Mancini et al., 2015; Sulaiman et al., 2012; Madubuike et al., 2015). Frank et al. (2011) had noted that Palm oil has become the world's most important edible oil because of its vital qualities and properties, representing 25% of the total oils and fats production. Palm oil and palm kernel oil are two different types of oil produced from oil palm fruits. The fruit of oil palm yields about 20 to 22% of its oil from the fibrous mesocarp and 10% of the total yield is from the white kernel flesh (Norizzah et al., 2014). Bahadi et al. (2016) stated that 'Though the two types of oil are extracted from the same palm fruit; both types of oil have different physical and chemical properties. Palm oil generally comprises mainly of acylglycerols and small quantities of non-acylglycerol components which include free fatty acids (FFAs), trace metals, moisture, impurities and minor components. The fatty acid composition of palm oil comprises 50% saturated and 50% unsaturated fatty acids, and the triacylglycerols composition of palm oil consist of 6.4% tri-saturated, 44.7% di-saturated, 37.7% monosaturated and 6.5% tri-unsaturated. Palm oil contains minor components; 3 to 8% diglycerides, less than 3% monoglycerides, 3 to 4% free fatty acids and approximately 1% of the major components which include carotenoids, vitamin E (tocopherols and tocotrienols), sterols, phospholipids, glycolipids, and terpenic and aliphatic hydrocarbons. Extensive studies have been conducted on the vitamin E content of palm oil (30% tocopherols and 70% tocotrienols) due to its nutritional and health qualities, including antioxidant activities, cholesterol-lowering, anti-cancer effects and protection against artherosclerosis.

The water-soluble phenolic flavonoid-rich antioxidant complex is another relatively new output from palm oil which has antioxidant properties and also beneficial effects against skin, breast and other cancers. Currently investigations are being carried out on its use as a nutraceutical and in cosmetics with potential benefits against skin aging due to the water solubility of this compound (Ezediokpu et al., 2015). A number of microbial species have been implicated in the spoilage and deterioration of palm oil as well as other vegetable oils. These include Proteus *sp*, *Bacillus* sp, *Staphylococcus* sp., *Enterobacter* sp., *Bacillus* sp (Onifade and Bolarinwa, 2016), *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Penicillum frequentans, Rhizopus stolonifer* etc. The fungal species exhibit lipolytic activities and their growth results in lipolysis (spoilage) of the palm oil. Studies have shown that the rate of lipolysis is proportional to the amount of free fatty acid already present in the oil (Ezediokpu et al., 2015).In Nigeria, oil palm processing industry is mostly dominated by smallholders who occupy about 80% of the sector, and by lesser extent semi-mechanized and mechanized processors who cover about 16 and 4%, respectively (Izah and Ohimain, 2016). Palm oil is prone to contamination by microorganisms found in the environment, raw materials and equipment used for the processing, as well as those used for storage and distribution. Traditional methods and rudimentary equipment are usually employed for palm oil processing by the smallholders who have little or no knowledge either of modern aseptic production techniques or of the microbial implication of poor sanitation and storage methods (Okechalu et al., 2011; Izah and Ohimain, 2016). Palm oils are used domestically mainly for cooking of various dishes and industrially in the production of soap, margarine, cosmetics, pharmaceuticals and a number of other industrial products. Therefore, oil quality and its stability are considered very pertinent for the consumers and for industrial applications (Babatunde and Bello, 2016). Several factors are known to influence not only the qualities but the shelf life of the palm oil. These include handling of the harvested palm fruits before processing, time interval between harvesting and processing for oil production, method of processing/extraction and storage conditions.

In spite of the strict regulations and enforcement by the Standards Organization of Nigeria (2000), National Industrial Standards (1992) and National Agency for Food and Drug Administration and Control (NAFDAC) on the standards for production of palm oil for domestic, industrial and exportation purposes, rumors of widespread sale sub-standard and adulterated palm oil being sold in the South-Eastern Nigeria markets still abound. This creates the need to validate or refute these speculations. The results obtained in this study would help further sensitize regulatory agencies and promote awareness as to the quality of palm oils obtainable from the markets in South-Eastern Nigeria. The wide range of applications of palm oil underscores the import for stringent quality control measures, continual checks and monitoring with the aim of ensuring that high-quality products are released into the market by the producers. It is in recognition of these that this research work, assessment of the microbial and physicochemical characteristics of palm oil sold in three major markets in five states of the South- Eastern Nigeria was conceived and carried out.

MATERIALS AND METHODS

Sample Collection

A total of 45 samples of palm oil were obtained from three (3) open markets in five (5) States of South-Eastern geo-political zone of Nigeria namely, Abia, Imo, Anambra, Enugu and Ebonyi States in Nigeria. Three (3) samples were from each market sampled in the five (5) states. In Abia State, samples were collected from Ahiaohuru market in Aba, Ubakala market in Ubakala and Ubani market in Umuahia. For Imo State, samples were collected from Imo International market in Orlu,

Ekeonunwa market in Owerri and Afo Ogbe in Mbaise. In Anambra State, samples were collected from Onitsha International Market in Onitsha, Nkwo Nnewi in Nnewi and Nkwo Ogbe in Ihiala. Samples were also collected from three different markets in Abakiliki, Kpirikpiri and Ishieke in Ebonyi State. Finally, in Enugu State, samples were collected at Akpugo, Emene and Ngwo markets. All the samples were collected aseptically in sterile opaque glass bottles at ambient temperature (30°C to 32°C) and immediately conveyed to the laboratory in ice flasks (0 to 4°C) for analysis.

Physicochemical Analysis

Moisture Content

Moisture content was determined by using the methods described by AOAC (2000). The crucible was first weighed and 5g of each of the palm oil samples were added and weighed (W_1) again. They were subsequently dried at 105°C for 3 h in a smart oven until constant weight. After drying, they were cooled in a desiccator and re-weighed (W_2). The % moisture content was calculated using the formula;

$$\% \text{ Moisture} = \frac{(W_1 - W_2)\ g}{W_1} \times 100 \quad (1)$$

Where W_1 = weight of crucible + weight of palm oil before drying (1a)

W_2 = weight of crucible + weight of palm oil after drying (1b)

Specific Gravity

This was determined by the method described by Oji et al. (2015). A clean dry 50 ml capacity specific gravity bottle was weighed (W_1). The bottle was filled with already boiled and cooled distilled water to the brim to prevent trapping of air bubbles, and the stopper was inserted. The bottle was kept in a water bath at 300°C for 30 min. The bottle was removed from the water bath and all spillage from the capillary opening were wiped dry. It was weighed again (W_2), and to determine the weight of the water this formula below was used;

Weight of water in the bottle = $W_2 - W_1$ (2)

Where: W_2= Weight of bottle + water (2a)

W_1= Weight of bottle.

The palm oil sample was boiled and filtered with filter paper to remove all impurities and moisture that may be present. The sample upon cooling was used to fill the specific gravity bottle to the brim, holding the bottle on its side. The stopper was inserted and immersed in a water bath at 300°C for 30 min. The bottle was removed and oil spills from the capillary opening were wiped dry. It was weighed (W_3), and specific gravity determined using the formula: Specific gravity = weight volume of sample/ weight volume of water.

$$\text{Specific gravity} = \frac{W_3 - W_1}{W_2 - W_1} \quad (3)$$

Where W_3= weight in g of the specific gravity bottle + oil (3a)

Peroxide Value (PV)

The PV values were determined using methods previously described by Tagoe et al. (2012). Five (5g) grams of the palm oil sample was weighed into 250 ml capacity Erlenmeyer flask and 30 ml mixture of acetic acid (480 ml) and chloroform (320 ml) in a ratio of 3:2 was added. It was swirled on a hot plate to dissolve completely, and 0.5 ml of saturated potassium iodide solution was added using a graduated pipette and swirled for 1 min. Thirty (30 ml) milliliters of water was added with vigorous shaking to liberate iodine from the chloroform layer. It was titrated with 0.1N sodium thiosulphate slowly while mixing until the yellow colour lightened. One milliliter (1ml) of starch solution used as an indicator was added which gave it a blue coloration. It was homogenized by shaking vigorously until the blue colour disappeared. Using the same procedure, a blank sample that had no palm oil was analyzed. The peroxide value was determined using the formula below.

$$\text{Peroxide value (Meq Peroxide/kg)} = \frac{(S - B) \times N \times 1000}{\text{Sample weight}} \quad (4)$$

Where S = Sample titer
B = Blank
N = Normality of sodium thiosulphate.

Iodine Value

The iodine value was determined using Wijs' method as described by Ohimain et al. (2013). First, Wijs' solution was prepared by dissolving 8 g of iodine trichloride (ICl_3) in 200 ml glacial acetic acid, and 9 g of iodine in 300 ml carbon tetrachloride (CCL_4). The solutions were mixed together in a conical flask after dissolving, and glacial acetic acid was used to dilute it to 1L. The palm oil sample (0.3 g) was weighed into 250 ml conical flask and carbon tetrachloride was used to dissolve it before adding 25 ml of Wijs' solution. The mixture was mixed thoroughly and allowed to stand for 30 min in the dark. Twenty (20 ml) milliliters of 10% potassium iodide solution was added together with 100 ml of distilled

water. Using starch as an indicator, the solution was titrated with sodium thiosulphate to endpoint. The endpoint was when the blue colour changed to green. A blank was used to determine the amount of iodine equivalent to the halogen contained in 25 ml of Wijs' solution which enabled calculation of the amount of iodine absorbed by the oil under the reaction condition. The iodine value of oil was determined by the formula below.

$$\text{Iodine value} = \frac{(V_1 - V_2) \times N \times 1.269}{\text{Sample weight (g)}} \quad (5)$$

Where V_1 volume (cm^3) of thiosulphate used to titrate blank.
V_2 is volume (cm^3) of thiosulphate used to titrate sample.

Free Fatty Acid (FFA)

The FFA of the oils content was determined by methods of Oji et al. (2015). Five (5g) grams of the sample was weighed into a conical flask and 50 ml of ethanol was added to the oil to completely free the fatty acids. The mixture was homogenized on a hot plate stirrer at 40°C with gentle stirring.
Using phenolphthalein as an indicator, the ethanol-oil mixture was titrated with 0.1M NaOH. The volume of NaOH used to achieve a fairly pink endpoint was recorded. The free fatty acid of the palm oil was calculated using the formula below.

$$\%\ \text{FFA as palmitic acid} = \frac{\text{Vol. (ml) of NaOH used} \times \text{Molarity} \times 28.2\text{mg}}{\text{Weight of sample}} \quad (6)$$

Saponification Value

This was determined using the method described by Olorunfemi et al. (2014). Two (2g) grams of the sample was weighed into round bottom flask and 20 ml of 0.5N alcoholic potassium hydroxide (KOH) was added. The same procedure was carried out for the blank without adding oil. The flasks were both heated for 1h and left to cool. Both samples were titrated with 0.5N Hydrochloric acid (HCL) and 0.5 ml of 1% phenolphthalein was used as an indicator. A pink coloration indicated the endpoint of the titration.

$$\text{Saponification value} = \frac{(S - B) \times N \times 56.1}{\text{Sample weight (g)}} \quad (7)$$

Where S = Sample titration
B =Blank titration
N = Normal titration of the HCl, 56.1 = The Molar weight of KOH.

Impurity Content

Impurity content of the oils was determined using a modification of the method of Tagoe et al. (2012). A clean dry 250 ml conical flask was weighed (W_1) and 5g of oil was added and reweighed (W_2).
Twenty (20 ml) milliliters of hexane was added and was swirled in a hot plate to warm and homogenize the mixture. Filter paper was used to line a Gooch crucible, washed with hexane and dried at 105°C for 30 min. The crucible was cooled and weighed (W_3).
The hexane-oil mixture was poured into the crucible and was allowed to drain.
The conical flask that contained the hexane-oil mixture was rinsed with hexane to remove all the particles present in the oil and poured into the crucible and allowed to drain. After all the mixture had drained, the crucible was dried at 105°C for 30 min, cooled and reweighed (W_4). The non-oil solids obtained were the impurities. These were calculated with the formula below.

$$\text{Impurity} = \frac{W_4 - W_3\ (\text{Weight of impurities}) \times 100}{W_2 - W_1\ (\text{Weight of oil})} \quad (8)$$

Where:
W_1 = Weight of conical flask.
W_2 = Weight of flask + oil (8a)
W_3 = Crucible + filter paper (8b)
W_4 = Dry weight of crucible + impurity.

Total Aerobic Heterotrophic Count

A stock solution of each of the palm oil samples was prepared in 150 ml conical flask by emulsifying 10 ml each of the samples in 90 ml of sterile Tween 20 and vortexed. Serial dilution was carried out to 10^{-4} by dispensing 1ml of the solution into 9 ml of diluent. An aliquot (0.1ml) of the last dilution was inoculated in duplicates onto Nutrient Agar (NA) for Total Heterotrophic Bacteria (THB) count and Potato Dextrose agar (PDA) for Yeast and Mold Count (YMC). The inoculated NA plates were incubated at 37°C for 48 h. The PDA plates were incubated at ambient room temperature (30°C to 32°C) for 96 h (Cheesbrough, 2000). After incubation, the colonies that developed on both media (NA and PDA) were counted using Gallenkamp Electronic Colony Counter and expressed as Colony Forming Units (CFU) per ml of samples.

Isolation and Identification of Bacterial and Fungal Isolates

Discrete bacterial and fungal colonies that developed after incubation were purified by sub-culturing repeatedly onto freshly prepared NA and PDA plates, respectively.
The isolates were identified on the basis of

Table 1. Physicochemical quality parameters of the palm oil sample.

States	Analysis / Markets	Moisture Content (%)	Specific Gravity	Peroxide Value (meq/kg)	Iodine Value (Wij's)	Free Fatty Acid (%)	Saponification Value (mgKOH/g)	Impurity Content (%)
Abia	Aba Market	0.13 ± 0.02	0.82 ± 0.24	6.56 ± 0.28	46.42 ± 2.8	2.70 ± 0.08	194.10 ± 0.6	0.09. ± .001
	Ubakala market	0.13 ± 0.02	0.83 ± 0.24	6.58 ± 0.33	46.42 ± 2.8	2.60 ± 0.15	194.30 ± 0.7	0.08 ± 0.01
	Ubani market	0.14 ± 0.01	0.83 ± 0.05	6.53 ± 0.30	46.42 ± 2.8	2.60 ± 0.15	194.50 ± 0.3	0.08 ± 0.01
Imo	Orlu market	0.18 ± 0.03	0.85 ± 0.05	7.40 ± 0.45	47.51 ± 3.2	2.70 ± 0.26	196.80 ± 0.3	1.00 ± 0.01
	Ekeonunwa	0.17 ± 0.02	0.85 ± 0.05	7.32 ± .05	47.56 ± 3.7	2.70 ± 0.26	197.00 ± 0.6	1.05 ± 0.01
	Afo ogbe	0.19 ± 0.03	0.86 ± 0.05	7.32 ± 0.05	47.51 ±3.2	2.80 ± 0.15	196.80 ± 0.3	1.05 ± 0.01
Anambra	Onitsha int'l market	0.14 ± 0.01	0.82 ± 0.05	6.56 ± 0.33	46.56 ± 3.7	2.60 ± 0.15	194.00 ± 0.6	0.09 ± 0.01
	Nkwo nnewi	0.13 ± 0.02	0.83 ± 0.24	6.53 ± 0.30	46.15 ± 3.0	2.60 ± 0.15	194.30 ± 0.7	0.08 ± 0.01
	Nkwo ogbe	0.13 ± 0.02	0.83 ± 0.24	6.53 ± 0.30	46.56 ± 3.7	2.60 ± 0.26	194.30 ± 0.7	0.08 ± 0.01
Ebonyi	Abakpa market	0.18 ± 0.01	0.86 ± 0.24	6.53 ± 0.30	47.42 ± 2.8	2.70 ± 0.15	195.00 ± 0.6	1.05 ± 0.01
	Kpirikpiri market	0.200 ± 0.03	0.87 ± 0.07	7.40 ± 0.45	48.15 ± 3.0	2.80 ± 0.05	199.00 ± 0.6	1.05 ± 0.01
	Ishieke market	0.17 ± 0.02	0.85 ± 0.05	7.40 ± 0.45	47.56. ± 3.7	2.70 ± 0.15	195.00 ± 0.6	1.040 ± 0.01
Enugu	Akpugo market	0.20 ± 0.04	0.87 ± 0.07	8.30 ± 0.42	53.63 ± 3.3	2.80 ± 0.05	202.02 ± 0.3	1.05 ± 0.01
	Eke emene	0.18 ± 0.03	0.92 ± 0.13	7.32 ± 0.05	53.63 ± 3.3	2.80 ± 0.05	199.00 ± 0.6	1.05 ± 0.01
	Ngwo market	0.19 ± 0.04	0.91 ± 0.14	8.30 ± 0.42	48.15 ± 3.0	2.90 ± 0.23	204.02 ± 0.3	1.00 ± 0.01
Crude palm oil		0.14	0.84	6.55	47.11	2.6	194.5	1.00
Finished palm oil		0.13	0.82	6.53	46.45	2.6	194.0	0.08
SON/NIS standards		0.29	0.897-0.907	10	45-53	3.5	195-205	

Each value is expressed as mean of the triplicate ± Standard Error.

morphological and biochemical characteristics as described by Cheesbrough (2000).

RESULTS AND DISCUSSION

Results of the physicochemical properties of the palm oil samples are as shown on Table 1. The mean values of the moisture content recorded in all the oil samples were lower (0.130 to 0.2%) than the recommended standard of 0.29% Standards Organization of Nigeria (SON, 2000); Nigeria Industrial Standards (NIS, 1992) and National Agency for Foods and Drugs Administration and Control (NAFDAC). However, comparative analysis showed that there was no significant difference ($p>0.05$) between the recommended standard and the results obtained. The results of this investigation are in consonance with the reports of Madubuike et al. (2015), Udensi and Iroegbu (2007), Agbaire (2012), in which the moisture contents of palm oil sold in major markets of Delta, Abia and Enugu States were 0.140.17, 0.140.16 and 0.130.20%, respectively. The low moisture content of these products is an indication of high stability in storage and longer shelf-life. The moisture content is one of the parameters used in assessing the quality of the oil. It can be lowered during the processing stage by boiling at elevated temperatures and during clarification. High moisture encourages growth of microorganisms and causes an increase in FFA while low moisture, however, promotes the storage stability of the palm oil (Olorunfemi et al., 2014). The values of the specific gravity obtained in this study ranged from 0.82 to 0.92. These were within the standards recommended by SON (2000) and NIS (1992) (0.897 to 0.907). The results are similar to those of Oji (2015), (0.91), Madubuike et al. (2015), (0.820

Table 2. Total heterotrophic bacterial and fungal counts of palm oil samples collected in Imo State.

Sample Codes	Markets	Village/ Town	THB (CFU/ml) x10^4	YMC (CFU/ml) x10^4.
1A	Imo Int'l market	Orlu	NG	3.0
1B	Imo Int'l market	Orlu	NG	1.0
1C	Imo Int'l market	Orlu	NG	10.0
2A	Ekeonunwa	Owerri	NG	NG
2B	Ekeonunwa	Owerri	NG	1.0
2C	Ekeonunwa	Owerri	NG	3.0
3A	Afo Ogbe	Mbaise	NG	3.6
3B	Afo Ogbe	Mbaise	2.0	NG
3C	Afo Ogbe	Mbaise	NG	NG

Legend: THB = Total Heterotrophic Bacter**ia.** YMC = Yeast and Mold Count. NG = No Growth.

Table 3. Total heterotrophic bacterial and fungal counts of palm oil samples collected in Ebonyi and Enugu States.

Sample Codes	Markets	Village/ Town	THB (CFU/ml) x10^4	YMC(CFU/ml) x10^4
4A	Abakpa market	Abakiliki	NG	NG
4B	Abakpa market	Abakiliki	NG	NG
4C	Abakpa market	Abakiliki	NG	NG
5A	Kpirikpiri market	Kpirikpiri	NG	2.0
5B	Kpirikpiri market	Kpirikpiri	NG	3.0
5C	Kpirikpiri market	Kpirikpiri	NG	1.0
6A	Isieke market	Ishieke	NG	1.0
6B	Ishieke market	Ishieke	NG	1.0
6C	Ishieke market	Ishieke	NG	NG
7A	Akpugo	Akpugo	3.6	1.3
7B	Akpugo	Akpugo	2.4	10.0
7C	Akpugo	Akpugo	4.0	2.0
8A	Eke Emene	Emene	NG	1.2
8B	Eke Emene	Emene	NG	2.0
8C	Eke Emene	Emene	9.0	4.6
9A	Nkwo Market	Nkwo	NG	2.0
9B	Nkwo Market	Nkwo	2.0	5.0
9C	Nkwo Market	Nkwo	2.0	1.1

Legend: THB = Total Heterotrophic Bacter**ia.** YMC = Yeast and Mold Count. NG = No Growth.

to 0.896) and Udensi and Iroegbu (2007), (0.832 to 0.880). The Specific gravity of liquids is very closely related to density. It is a measure of the weight or density of a liquid relative to an equal volume of water at a given temperature. It aids in the determination of how pure a liquid or compound is. A value that is significantly different from the values stated recommended by SON (2000), NIS (1992) indicates adulteration. The Peroxide values (PV) recorded ranged from 6.5 to 8.30 meq/Kg. The results were in conformity with the standards recommended by SON (2000), NIS (1992) of 10 meq/kg. The method of processing palm oil remarkably affects the PV. This can be observed in the report of Aletor et al. (1990), who reported that mechanically processed palm oil recorded a peroxide value of 5.6 meq/kg and 2.70 to 7.40 meq/kg for traditionally processed palm oil. Peroxide values of 0.70 meq/kg for industrially processed palm oil and 7.4 meq/kg for traditionally processed palm oil have been reported by Onwuka and Akaerue (2006). Agbaire (2012) argued that the PV determines the degree of oil oxidation; it is a measure of oxidation during storage and the freshness of the lipid matrix. It is a useful indicator of the early stages of rancidity occurring under mild conditions thus it is used as an indication of quality and stability of fats and oils. The mean iodine values obtained in this study ranged from 46.42 to 50.31Wij's, and are within standard range of 45 to 53 Wij's recommended by SON (2000) and NIS (1992). Like other quality parameters, iodine value provides an indication of the stability and shelf life of oil. Iodine value is an indication of the quantity of iodine required

Table 4. Total heterotrophic bacterial and fungal counts of palm oil samples collected in Abia and Anambra States.

Sample Codes	Markets	Village/ Town	THB (CFU/ml) x10^4	YMC (CFU/ml) x10^4
10A	Ahiaohuru market	Aba	NG	NG
10B	Ahiaohuru market	Aba	NG	NG
10C	Ahiaohuru market	Aba	NG	NG
11A	Ubakala market	Ubakala	NG	NG
11B	Ubakala market	Ubakala	NG	NG
11C	Ubakala market	Ubakala	NG	NG
12A	Ubani market	Umuahia	NG	NG
12B	Ubani market	Umuahia	NG	NG
12C	Ubani market	Umuahia	NG	NG
13A	Onitsha Int market	Onitsha	NG	NG
13B	Onitsha Int market	Onitsha	NG	NG
13C	Onitsha Int market	Onitsha	NG	NG
14A	Nkwo market	Nnewi	NG	NG
14B	Nkwo market	Nnewi	NG	NG
14C	Nkwo market	Nnewi	NG	NG
15A	Nkwo Ogbe Market	Ihiala	NG	NG
15B	Nkwo Ogbe Market	Ihiala	NG	NG
15C	Nkwo Ogbe Market	Ihiala	NG	NG

Legend: THB = Total Heterotrophic Bacteria**.** YMC = Yeast and Mold Count. NG = No Growth.

by one gram of oil to saturate its sigma bond. It shows the level of unsaturation and consequent susceptibility of oil to oxidation and rancidity (Norizzah et al., 2014; Agbaire, 2012). This result agrees with a number of previous reports. Okechalu et al. (2011) reported a value range of 46.74 to 63.59 Wij's in palm oil sold in Plateau State, Okonkwo and Ogbuneke (2010) recorded 46.47 to 51.54Wij's in palm oil sample from Enugu.

The free fatty acid (FFA) values of the palm oil samples range from 2.60 to 2.90mgKOH. Comparative analysis showed non-significant difference between the results obtained and the maximum permissible limit (3.5 mgKOH/g) set by SON (2000) and NIS (1992) ($p>0.05$). These low values imply lower hydrolysis of triglycerides and indicate that the oil could have a long shelf life, which allows it to be consumed as virgin edible oil (Quilly et al., 2017). The results of this investigation correlates values of 2.86 to 2.97 mgKOH/g, 2.68 to 2.96 mgKOH/g and 2.86 to 2.97 mgKOH/g recorded in the study on palm oil samples from Delta State, Enugu metropolis and Ihiala Local Government of Anambra State (Agbaire, 2012; Madubuike et al., 2015; Okonkwo and Ogbuneke, 2010). The higher the FFA the lower the quality of the palm oil, and vice versa (Oji et al., 2015). The saponification values (SV) obtained (194.3 to 204 mgKOH/g) were within the recommended range of 195 to 205 mgKOH/g for palm oil (SON, 2000; NIS, 1992). The amount of potassium hydroxide (in milligram) required to neutralize the fatty acid resulting from the complete hydrolysis of an oil sample is the saponification value (SV). SV is a measure of average molecular mass of fatty acid present in an oil sample. The lower value of saponification suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This may imply that the fat molecules did not interact with each other (Zahir et al., 2014). Muhammad et al. (2011) opined that 'High SV indicates high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or length of fatty acids. The Impurity Content (IC) of the palm oil samples from Imo, Anambra, Ebonyi and Enugu States exceeded the recommended values set by SON (2000) and NIS (1992), however, the values were not significantly different ($p>0.05$). The level of impurity in the samples from Abia State (0.08 to 0.09%) was however, within the recommended range. Other researchers have made similar findings (Akubor and Ogu, 2012; Frank et al., 2011), while some have reported higher levels of impurity (Poku, 2002; Ohimain et al., 2012). The high impurity level may be attributed to the methods of processing and extraction of the palm oil.

The results of the total aerobic heterotrophic bacterial and fungal counts are shown in Tables 2,3 and 4. The total bacterial counts in the samples ranged from 0.0 to 9.0 x104cfu/ml, with the sample from Eke Emene in Enugu State having the highest bacterial load. Similarly, the mold counts ranged from 1.0 x10^4cfu/ml to 10.0 x10^4cfu/ml, with the highest mold count recorded in samples from Imo International market, Orlu and Akpugo market in Imo State and Enugu State,

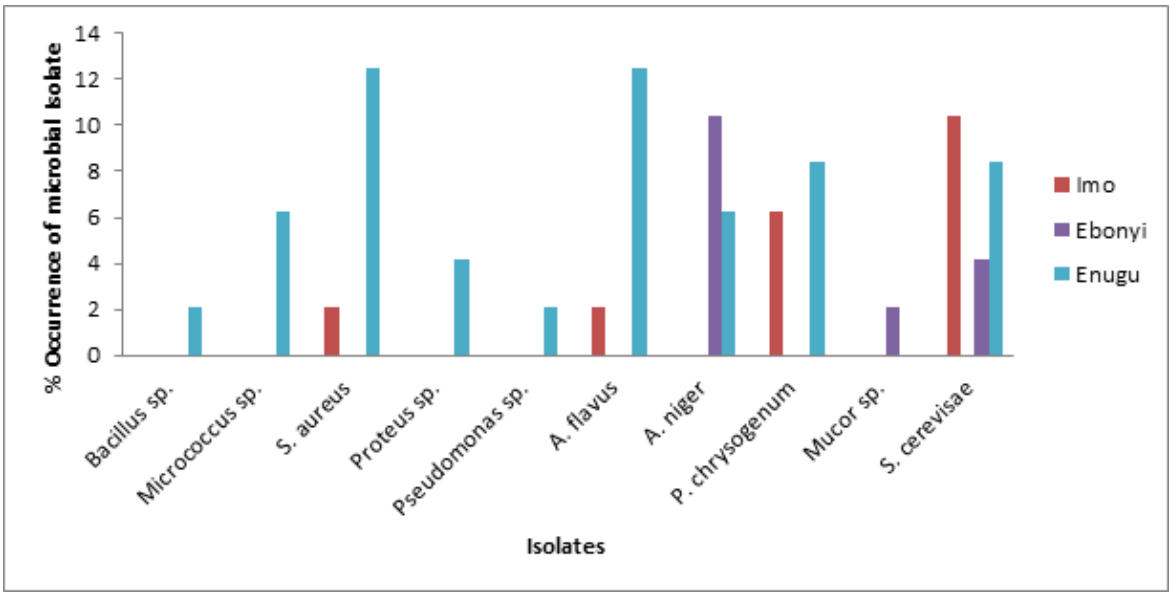

Figure 1. Microbial isolates from the palm oil samples and their percentage occurrence.

respectively. The total viable bacterial and mold counts of the palm oil samples were within the minimum acceptable range stipulated by NAFDAC. Different microorganisms isolated from the palm oil samples and their percentage occurrences are shown in Figure 1. *Bacillus and Pseudomonas sp* had the lowest frequency of occurrence, while *S. aureus and A. flavus* had the highest frequency of occurrence. Similar observations and reports have been made by a number of authors (Onifade and Bolarinwa, 2016; Ezediokpu et al., 2015) *Proteus* sp, *S. aureus* and some *Bacillus* sp. are pathogenic to humans. The presence of *Bacillus sp* in some of the samples may be due to exposure to the spores of the organism which are usually dormant under unfavorable conditions and are highly resistant to heat. *A. flavus* is known for its ability to produce aflatoxin which is capable of inducing toxic syndromes especially cancer, and it is therefore of health significance (Okechalu et al., 2011).

CONCLUSION

The present investigation shows that the microbial and physicochemical properties of palm oil from major markets in the five South-Eastern States of Nigeria met the stipulated standards set by SON (2000) and NIS (1992) thus the samples prove to be of good quality. The low moisture content recorded enhances the storage stability of palm oil samples. The low free fatty acid implies lower hydrolysis of triglycerides which indicate that the oil samples may have capacity for long shelf life. The results demonstrate a strong indication of good quality and suitability of the palm oils obtained from South-Eastern Nigeria markets for domestic and industrial uses. Though the microbial load falls within the permissible range, some of the isolates are known to be of human health significance and can result in health challenges if not properly heated before consumption. Some others for example, *Mucor sp* may enhance deterioration and spoilage of the palm oil during storage.

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