PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (5) 815-821 May 2022

Bacteriological Profile of Palm Oil Sale Sites in Selected Markets in Kaduna State, Nigeria

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ABSTRACT: The bacteriological profile of palm oil sale sites in selected markets in Kaduna state, Nigeria were evaluated using standard techniques by collecting a (60) soil samples. Aside from bacteriological profile, pH, moisture content, temperature and lipase activity analyzed. The soil samples had pH that ranged from acidic to neutral (3.0-8.00) which indicates the presence of hydrolytic enzymes. Microorganisms isolated from soil samples palm oil sale sites were screened from their lipase producing ability. A total of (10) ten bacteria belonging to the genera Bacillus, *Streptococcus, Lactobacillus, Streptobacillus, Micrococcus, Staphylococcus* and *Pseudomonas.* The lipolytic activity of the bacteria when screened on solid agar ranged for 1.1 to 3.2cm, 2.3-3.7cm, 2.3-6.7cm at 24, 48 and 72hours respectively while that of fungal species ranged from 0.6 to12cm, 0.3-1.5cm and 1.1-1. 7cm at 3,7,14 days of incubation respectively. Exo-enzyme was produced from the various species screened for lipolytic activities. The study shows that *Bacillus subtilis* and *Trichoderma* spp. had high potential.

DOI:https://dx.doi.org/10.4314/jasem.v26i5.6

Impact factor: http://sjifactor.com/passport.php?id=21082

Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

Dates: Received: 15 February 2022; Revised: 13 April 2022; Accepted: 11 May 2022

Keywords: Markets, Palm Oil, Bacteria, Lipases, Soil

Palm oil is an edible vegetable oil derived from the mescoarp of the fruit of the oil palms, primarily the African oil palm *Elaeis guineensis* (Harrigan and McClance, 2006), and to a lesser extent from the American oil palm *Elaeis Oleifera* and the maripa palm *Attalea maripa*. It is naturally reddish in color because of its high beta-carotene content. Palm mesocarp oil is 41% saturated, while palm kernel oil and coconut oil are 81% and 86% saturated respectively (Cheesbrough, 2000). Palm oil, along with coconut oil is one of the few highly saturated vegetable fats. It is semi-solid at room temperature and contains several saturated and unsaturated fats in the form of glycerylarate (0.1%, Saturated), myristate (1%, saturated), Palmitate (44%, saturated), slearate (5% saturated), oleate (39% mono-unsaturated), linoleate (10%, poly-unsaturated), and alpha-linolenate (0.3%, poly-unsaturated) (Siew, 2000). Like all vegetable oils, palm oil does not contain cholesterol (WHO, 2010), although saturated fat intake increases both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Mamilov and Dilly, 2002). Palm oil is GMO-free (Larone, 1995).An oil palm fruit is a monocotyledon belonging to the genus *Elaeis.* Thekanel oil is obtained from the endocarp while palm oil is obtained from the fleshy mesocarp (Larone, 1995). It is rich in vitamin (tocopherols and tocotrienols), corotenoids and phytosterol (Sundram, 2003). Palm oil rich content of saturated and monounsaturated fatty acid has actually been turned into an asset in view of current dietary recommendations. The use of palm oil in combination with other oil facilitates development of a new generation of fat product that can be tailored to meet most current dietary recommendation (Sundram, 2003). The vitamin contained in palm oil (30% tocophnols and 70%tocotrienols) has been extensively studied for it nutritional and health properties including antioxidant activities cholesterol lowering anticancer effect and protection against sclerosis. A relatively new output from the oil palm fruit is the water soluble phenol-falconoid rich antioxidant

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complex which is effective against skin, breast and other cancer. Its water solubility is being currently tested for use as nutraceutical and cosmetic with potential benefit against skin aging (Sundram, 2003). Palm oil processing is carried out using large quantities of water in mills where oil is extracted from the palm fruit. About 50% of the water result in palm oil mill effluent (POME), it is estimated that for one tone of crude palm oil produced, 5.7 tons of water end up as poem (Ahmad *et al.,* 2003). The solid waste product that result from the milling operation is empty fruit bunches of palm fiber and palm kernel. In both traditional and modern milling setting, these waste products are all put to economically useful purpose such as fuel material and mulch in agriculture. It is the POME that is usually discharged in to the environment, either raw or treated (Paul and Clark, 2009). Raw POME consisting of complex vegetative matter is thick, brownish colloidal slurry of water, oil and solid including about 2% suspended soil originating mainly from cellulose fruit debris (Bek-Nielson *et al.,* 1999). This highly polluting waste water can therefore cause pollution of water ways due to oxygen depletion and other related effect as reported by Ahmed *et al.,* (2003). Thus while enjoying a most profitable commodity, palm oil, the adverse environment impact from palm oil industry cannot be ignored (Ahmed *et al.,*2003). Palm oil mill effluent application to soil can result to some beneficial soil chemical and physical characteristics such as increase in organic matter, water holding capacity and porosity (Marten *et al*., 1992). However it brings about undesirable changes such as decrease in pH and increase in salinity (Kittikun *et al.,* 2000). Soil microbiological and biochemical properties have been considered early and sensitive indicators in soil used to predict long-term effect in the quality of soil (Ross *et al.,* 2003). Thirukkumaran and Parkinson, (2000), reported that high rate of inorganic fertilizer application suppresses microbial respiration and dehydrogenate activity. Other factors as increase in salinity or decrease in water availability may also reduce biological activity in the soil (Parades *et al.,* 2005).Microorganisms are known to cause chemical changes in palm oil that lead to deterioration in quality and their chemical composition (Okopokwasili and Molokwu, 1996). Traditional methods of production are employed for the extraction of palm oil by individual who have little or no knowledge neither of modern aseptic production techniques nor of the microbiological implication of poor sanitation and storage methods. Therefore palm oil is prone to contamination by microorganisms found in the environment, raw materials and equipments used for the processing as well as used for storage and distribution (Larry, 1997).Therefore the objective of this study is to evaluate the bacteriological profile of soil samples from Palm Oil Sale Sites in Central, Kasuwa Barchi and Kakuri markets in Kaduna State, Nigeria.

MATERIALS AND METHODS

Soil Sample Collection: Soil samples from palm oil selling sites were collected from three different markets in Kaduna metropolis. The markets are Central Markets, Kasuwa Barchi and Kakuri Market. A total number of sixty (60) samples, twenty (20) for each market were collected and merge to form a composite soil sample. Visual inspection of the sampling sites was conducted and the difference between the site in terms of presence of constitution, soil colour, odour were observed and noted. A total of sixty (60) samples of palm oil selling site were collected at a depth of 0-30cm from palm oil selling sites in Kaduna. The samples from palm oil selling sites were collected in a sterile bag. Care was taken not to contaminate the bag before and during collection of sample the sample was then transported to the laboratory for microbiological analysis.

Determination of Total Aerobic Count: Pour plate method was used for the enumeration of bacterial in the soil sample. A stock solution of each of the sample was made by dissolving 1g of each of the soil sample into a test tube containing 9ml sterile distilled water. Serial dilution was carried out for 10^{-2} 10^{-3} 10^{-4} 10^{-5} and 10^{-6} dilution. Nutrient agar plates were inoculated and incubated at 37^{o}C for 24hours (Prescott *et al.,* 2008; Doughari *et al.,* 2008 and Udochukwu *et al*., 2014a). Colonies that developed were counted using the direct plate count method and the result expressed as colony forming unit per gram (cfu/g).

Isolations and Identification of Bacterial Isolates: The representative bacterial colonies that developed on the culture plates were sub cultured on nutrient agar until a pure culture were obtained using the streak plate technique. The various isolates were subjected to Gram's staining procedure and standard biochemical test as described by Cheesbrough (2002). References were also made to stock cultures and different microbiological monographs in addition to colonial morphology in order to make proper identification of the microbial isolate.

Physicochemical Analysis: The pH of the soil samples was determined using Jenway pH meter model 1305, The moisture content of the soil sample was determined according to Association of Official and Analytical Chemists (AOAC). The soil temperature was determined using a temperature probe; the identified lipase producing bacteria was cultivated in

nutrient broth for 24 hours at 37°c with shaking as pre-culture. One milliliter of pre-culture was transferred to 99ml NB supplemented with 1% castor oil and incubated at 37°c under the shaking condition for hours. The culture was then centrifuged at 10,000 rpm at 40°c for 10min. The supernatant was used as a crude enzyme for lipase activity measurement (Omoghie *et al.,* 2014b).

RESULTS AND DISCUSSION

The result of the microbial profile of palm oil selling sites as revealed in Table 1, shows that the soil samples from palm oil selling sites ranged from dark-brown with white patches to black with humus; having a sticky appearance and odour due to the presence of the enzyme lipase which aid in the hydrolysis of triglycerides to free fatty acid and glycerol thereby impacting odour on the soil. This trend was also recorded by (Larone, 1995; Zakaria, 2002), It was also reported that chronic exposure of soil to palm oil and effluent would brings changes in soil conditions such as the pH, hypoxia as well as reduction in the number and activities of soil micro-organisms (Maila and Cloete, 2005).

Table 1: Visual characteristic of soil samples collected from central market

Samples	Colour	Moisture	Odour	Constitution
Ss_1	Dark brown with white patches	Dry	Odourous	Powered with rope-like debris
Ss_2	Dark ash with white patches	Dry	Odourous	Free for debris
Ss_3	Black with humus damp	Damp	Odourous	Solid form and debris free
Ss_4	Black	Damp	Odourous	Solid form with rope-like debris
Ss_5	Brown with green patches	Damp	Odourous	Solid from with cotton-like debris
Ss_6	Dark brown mixed wigfth white	Dry	Slightly odourous	Powered and debris free.
Ss_7	Dark brown	Damp	Odourous	Solid and debris free.
Ss_8	Dark brown	Damp and sticky	Slightly odourous	Solid with rope like debris
Ss_9	Black with humus	Damp	Odourous	Solid with rope like debris
Ss_{10}	Dark ash with white patches	Dry	Odourous	Solid with debris
Ss_{11}	Black with humus	Damp and sticky	Odourous	Solid with hiread like debris
Ss_{12}	Dark brown with humus	Damp	Odourous	Solid with cotton like debris
Ss_{13}	Brown with humus	Dampand sticky	Odourous	Solid without debris
Ss_{14}	Black with humus	Dry	Odourous	Solid with thread like debris
Ss_{15}	Dark ash	Damp	Odourous	Solid and free from debris
Ss_{16}	Dark ash	Dry	Slightly Odourous	Solid and debris free
Ss_{17}	Black with humus	Damp	Odourous	Solid with debris from oil processing
Ss_{18}	Dark brown	Damp and sticky	Odourous	Solid with thread like debris
Ss_{19}	Black	Damp and sticky	Odourous	Solid and de debris free
Ss_{20}	Ash	Dry	Slightly odourous	Solid with whitish debris

Key: Ss= Sample name, 1 – 20=Sample number

Table 2: Visual characteristics of soil samples collected from Kakuri Market.

Samples	Colour	Moisture	Odour	Constitution
Lv_1	Dark brown with humus	Damp	Slightly odourous	Solid with thread like
Lv_2	Brown	Slightly damp	Free from odour	Sandy solid
Lv_3	Dark ash with green patches	Dry	Odourous	Powered and cotton-like debris
Lv_4	Black with humus	Damp and sticky	Odourous	Solid with thread like debris
Lv_5	Dark ash	Dry	Slightly odourous	Solid and debris free
Lv_6	Black with humus	Damp	Odourous	Solid with rope like debris
Lv_7	Dark ash with white patches	Dry	Odourous	Solid with debris
Lv_8	Brown	Dry	Slightly Odourous	Sandy solid
Lv_9	Dark brown with white patches	Dry	Odourous	Powered with rope like debris
Lv_{10}	Black with humus	Damp	Odourous	Solid with rope like debris
Lv_{11}	Brown	Dry	Free from odour	Sandy solid
Lv_{12}	Dark brown with humus	Damp and sticky	Odourous	Solid with debris
Lv_{13}	Dark ash with white patches	Dry	Odourous	Powered with cotton – like debris
Lv_{14}	Dark brown with white patches	Dry	Odourous	Powered with rope –like debris
Lv_{15}	Dark ash with write patches	Dry	Odourous	Powered and free from debris
Lv_{16}	Black with humus	Damp	Odourous	Solid from and free from debris
Lv_{17}	Black	Damp	Odourous	Solid with cotton like debris
Lv_{18}	Brown with green patches	Damp and sticky	Odourous	Solid with cotton like debris
Lv_{19}	Dark brown mixed with white patches	Dry	Slightly odourous	Powered and debris free.
Lv_{20}	Dark brown	Damp	Odourous	Solid and free from debris

Key: *$LV_{1+}LV_{20}$ = Sample name and number*

The result also revealed that the pH of the soil sample ranged from neutral (7.12) to acidic pH (3.00), and temperature of various samples of palm oil selling sites ranges from 30ºC to 20ºC. The temperature of the site is not as optimum as the temperature for good production of lipase as reported by (Musa and Adebayo-Tayo, 2012). The result observed in Table 2 showed that temperature of the samples increase as the pH value increases, although there were some exceptional cases which did not agree with the trend as reported that increase in temperature has an effect on the pH value of soil samples. This contrast agreed with Maila and Cloete, (2005) who reported that chronic exposure of soil to palm oil effluent would bring changes in the soil conditions such as pH, hypoxia as well as reduction in the number and activities of soil micro-organisms. This result agrees with the report of Garcia-Gil *et al.,* (2000), who worked on long term effect of municipal solid waste compost. The result obtained in Table 2 revealed that the soil samples have low moisture content due to the presence of oil, and increase in water holding capacity. This trend was also recorded by Logan *et al.*,(1997); Navast *et al.,* (1998); Mantzavinos and Kalogerakis, (2005) who reported that palm oil or palm oil effluent application to soil can result to some beneficial soil chemical and physical characteristics such as increase in organic matter, organic carbon, major nutrient water holding capacity and porosity. However, it brings about undesirable changes such as decreases in pH, and increase in salinity as reported by Kittikun *et al.,* (2000).

Table 3: Visual characteristics of soil samples collected from kasuwa Barchi.

Samples	Colour	Moisture	Odour	Constitution
TH_1	Ash	Dry	Slightly odourous	Powered with whitish debris
TH_2	Dark brown	Damp and	Slightly odourous	Solid with rope-like debris
TH_3	Black with humus	Damp	Odourous	Solid with rope-like debris
TH_4	Dark ash with white patches	Dry	Odourous	Solid with debris
TH_5	Black with humus	Damp and sticky	Odourous	Solid with debris
TH_6	Dark brown with humus	Damp	Odourous	Solid white cotton-like debris
TH_7	Brown with humus	Damp and sticky	Odourous	Solid without debris
TH_8	Black with humus	Dry	odourous	Solid with thread like debris
TH_9	Dark ash	Damp	Odourous	Solid without debris
TH_{10}	Dark ash	Dry	slightly	Solid and debris
TH_{11}	Black with humus	Damp	Free from odour	Solid without debris
TH_{12}	Dark brown	Damp and sticky	Odourous	Solid with debris from oil processing
TH_{13}	Black	Damp and sticky	Odourous	Solid that is free from debris
TH_{14}	Ash	Damp	Slightly odourous	Solid with whitish debris
TH_{15}	Brown	Slightly	Free from odour	Sandy without debris
TH_{16}	Dark brown	Dry	Odourous	Powered with rope –like debris
TH_{17}	Dark ash with white patches	Dry	Odourous	Debris free
TH_{18}	Black with humus	Damp	Slightly Odourous	Debris free an in solid form
TH_{19}	Black	Damp	Odourous	Solid with rope like debris
TH_{20}	Dark brown	Damp and sticky	Slightly odourous	Solid form and debris free.

Key: *TH_1 . TH_{20} = Sample name and number*

Table 4: Physicochemical properties of soil samples collected from the three markets

Sample	pH	Temperature (ºC)	Moisture content (%)	Water holding capacity
CM	3.30, 5.73±1.04	5.70, 23.65±1.71	7.30, 2.97±1.68	6.00, 3.98±1.47
KM	4.10, 5.53±1.28	11.40, 25.25±3.30	6.29, 4.34±1.54	5.00, 2.86±1.33
KBM	4.50, 5.53±1.35	9.20, 24.27±2.51	6.40, 3.81±1.83	3.15, 2.85±0.90

Key: CM= Central Market, KM= Kakuri Market, KBM= Kasuwa Barachi Market

The result of the total bacterial count were obtained after 24 hours of incubation in almost all the samples, but subsequent decrease or no growth at all into the 48 to 72 hours. Total aerobic plate counts of the various soil samples collected from different market in Kaduna metropolis is shown in (table 5). A decrease in growth was observed as incubation time increases; this could be associated with nutrient depletion and pooled effects of byproducts of metabolism. Similar findings have been reported by Ogiehor *et al.,* (2004). There was no growth recorded fot Central and Kasuwa barchi markets while kakuri had a very low count.

This work corresponds with (Omoghie *et al*., 2014a; Udochukwu *et al.,* 2014b). The various strains of microbial isolates have been extensively studied by Sharma *et al* (2001); Svendsen, (2000); Kamimura *et al.,* (2001) for their lipolytic activities. The microorganisms isolated from samples of palm oil selling site oils were further screened for their lipase producing ability. A total of 10 bacteria (*Staphylococcus* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Streptobacillus* sp., *Bacillus substilis, Bacillus cereus, Micrococcus* sp., *Bacillus alvei, and Streptococcus* sp.).

Table 5: Total aerobic plate counts of the various soil samples collected from different market in Kaduna metropolis

Time (hrs)	CM (cfu/g)	KM (cfu/g)	KBM (cfu/g)
24	$2.50 \times 10^4 \pm 2.60$	$3.12 \times 10^4 \pm 1.81$	$1.78 \times 10^4 \pm 1.11$
48	$1.85 \times 10^4 \pm 1.80$	$2.65 \times 10^3 \pm 1.02$	$3.03 \times 10 \pm 1.34$
72	No growth	$7.30 \times 10 \pm 0.64$	No growth

Key: CM= Central market; KM = Kakuri market; KBM = Kasuwa barchi market

Table 6: Bacterial isolates from the various soil samples

Sample No.	Probable Species
Ss_1	*B subtilis*
Ss_2	*B cereus*
Ss_3	*Staphylococus aureus*
Ss_4	*Lactobacillus* sp.
Ss_5	*Streptobacillus* spp.
Ss_6	*Streptococcus* spp.
Ss_7	*Pseudomonas* spp.
Ss_8	*Micrococcus* spp.
Ss_9	*B cereus*
Ss_{10}	*B alvei*
Ss_{11}	*Staphylococcus* spp.
Ss_{12}	*B alvei*
Ss_{13}	*B. cereus*
Ss_{14}	*B. cereus*
Ss_{15}	*B. substilis*
Ss_{16}	*Lactobacillus* spp.
Ss_{17}	*Lactobacillus* spp.
Ss_{18}	*Pseudomonas* spp.
Ss_{19}	*B. substilis*
Ss_{20}	*B. cereus*

Table 7: Lipolytic activities of various bacterial strains at 30°C for 24, 48 and 72 hours.

Strains	24hrs/cm	48hrs/cm	72hrs/cm
Lactobacillus spp.	1.7	3.0	3.0
Bacillus substilis	2.4	3.5	6.7
Streptobacillus spp.	1.1	5.2	5.2
Pseudomonas spp.	2.0	2.5	4.2
Streptococcus spp.	1.8	2.4	3.8
Staphylococcus spp.	1.7	3.0	3.1
Micrococcus spp.	1.3	2.3	2.3
Bacillus cereus	3.2	3.7	3.9

The screening of bacterial isolates for lipase production on solid agar is shown in table 4.The lipolytic activity ranged within 1.1-3.2cm, 2.3-3.7, and 2.3-6.7cm at 24,48 and 72hours of incubation respectively. *Bacillus cereus* had the highest activity at 24 and 48hours of incubation while *Bacillus substilis* had the highest activity at 72hours of incubation. One of the isolate did not show any activity throughout the incubation period. It could be inferred from the results obtained that maximum lipolytic activity was attained at the late period of incubation. This, however is in contrast with the report of Singh and Makhopaghyay, (2012) who reported that maximum activity are observed during the early period of incubation. Microbial lipase represents the most widely used class of enzymes in biotechnological application and organic chemistry (Jaeger *et al.*, 1994). However in few of the isolates the lipase activity and production remains constant as incubation period increases. This however is in contrast to the work of Williams (1998) who stated that lipase production and activity increase as incubation period increase but later falls with increase in incubation time. Reduction in lipase production could be due to proteolytic degradation of the enzyme system.

*Conclusion***:** From the study conducted, it was observed that *Bacillus substilis* gave the best lipase production and activities amongst the bacterial isolates screened for lipase production on solid agar. Other isolates such as *Streptobacillus* sp, *Pseudomonas* sp, *Streptobacillus* sp, *Bacillus cereus*, *Saccharomyces* sp. are also high potential lipase producers. Research on production, characterization and purification of their enzyme through optimization parameters such as pH, temperature and various substrate utilizations would reveal those species with higher lipase production potential.

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