THOMAS ADEWUMI UNIVERSITY, OKO, KWARA STATE
Science | Technology | Medicine

Thomas Adewumi University

Journal of Innovation,

Science and Technology (TAU-JIST)

ISSN:3043-503X

RESEARCH ARTICLE

EVALUATION OF MICROBIAL DIVERSITY ASSOCIATED WITH PALM OIL PROCESSING FLOORS AND BARRELS FROM PALM OIL FACTORIES IN SELECTED GEOGRAPHICAL ZONES IN AKOKO LAND, ONDO STATE

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ARTICLE DETAILS

Article History:
Received 02 July 2024
Accepted 05 October 2024
Available online 10 December 2024

ABSTRACT

The surging global palm oil demand, has led to the transformation of oil-palm cultivation to be a source of livelihood for numerous rural families, fundamentally shaping the agricultural practices across Nigeria. This study aimed at identifying and evaluating the microbial diversity associated with palm oil floors and barrels used for the production of palm oil in Akoko land, Ondo State. Samples were collected from various processing floors and barrels of palm oil in Akoko. Using standard methods, microbial isolates were cultured, characterized and identified. Additionally, the resistance profile against commonly used antibiotics and antifungal drugs were assessed. Results revealed the presence of a diverse range of microorganisms including bacteria and fungi, with notable bacteria strains such as Salmonella sp., Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis. The fungi isolated from the samples include Aspergillus carbonarius, Penicilium aethiopicum, Fusarium graminearum, Alterneria alternata and Mucor plumbeus. Nalidixic acid had the highest zone of inhibition against Staphylococcus aureus (28.0mm) followed by ofloxacin (26.0mm) and levofloxacin (26.0mm) against Enterococcus faecalis, Salmonella sp. and Staphylococcus aureus respectively while cefotaxime showed no inhibition zone. Nystatin had the highest zone of inhibition of 28.0mm against Penicillium aethiopecum and Aspergillus flavus while griseofulvin and ketoconazole had no inhibition zone (0.0mm) against Mucor plumbeus. The findings underscore the importance of stringent quality control measures to mitigate microbial contamination risks and ensure the safety and quality of palm oil products in the study location.

KEYWORDS

Microbiological assessment, Palm oil processing floors**,** palm oil barrels, *Elaeis guineensis,* Palm oil factories, Palm Oil.

INTRODUCTION

One of the few vegetable oils that is comparatively high in saturated fats (e.g coconut oil) is palm oil, which turns semi-solid at room temperature. One of the main ingredients used to make animal feed is palm kernel cake. The bunch spikes/spikelets that remain after the fruits are cut from the palm bunch are high in potassium (K), which is utilized locally to make soap (Okechalu et al., 2011) and to soften dishes like breadfruit and other vegetables. In Sub-Saharan Africa, the palm tree is arguably the most beneficial tree to date. Among Nigeria's most important commercial tree crop is the palm oil plant (*Elaeis guineensis*). The increase in demand for palm oil around the world has made oil-palm farming a

Quick Response Code	Access this article online	
	Website: https://journals.tau.edu.ng/index.php/tau-jist	**DOI:** https://doi.org/10.5281/zenodo.15464977

Cite the Article: Oludare T. Osuntokun, Stephen D. Olorundare, Oluwakemi A. Thonda, Ilesanmi A. Elizabeth & Ayodele O. Tosin. (2024). Evaluation of Microbial Diversity Associated with Palm Oil Processing Floors and Barrels from Palm Oil Factories in Selected Geographical Zones in Akoko Land, Ondo State

source of health for people in rural settlement, radically altering farming methods of Nigerians. The oil palm tree is valued for its many uses and is significant in both economic and cultural spheres in many facets of life (Alabi *et al*., 2020).

Palm oil proves to be an exceedingly versatile tree crop, with nearly all its components possessing economic value, contributing significantly to daily sustenance. Fronds, leaves, trunk, and roots are various parts of the oil palm that yield a diverse array of products, thereby enhancing the overall well-being of humanity (Ibitoye, *et al*., 2011). Palm oil is gotten from the fleshy mesocarp which is abundant in Vitamin-E (30% tocopherol and 70% tocotrienol), carotenoids and phytosterols. Crude palm oil is widely utilized in its unrefined state for culinary purposes due to its cost-effectiveness and longstanding dietary tradition (Ngando *et al*., 2013). Not only is it economically advantageous, but it also offers nutritional benefits such as high source of fatty-acids which are essential, vitamin-E, and carotenoid. The well-established nutritional advantages of tocopherol encompass its cholesterol-lowering properties, anticancer/antioxidant effects, and protective attributes against a disease known as atherosclerosis (Imoisi *et al*., 2015; Dongho *et al*., 2017). Additionally, owing to its high content of provitamin A carotenoids, Crude Palm Oil emerges as a significant food source in the prevention of deficiencies in tocopherol (Dongho *et al*., 2017).

The significance of oil-palm to Nigeria's economy is immeasurable, including the production of food, generation of employment, provision of raw materials, source of income for farmers and the nation. In Western Africa, palm oil is known to be the most essential component in most receipe, and it is mostly produced by small-scale farmers who employ traditional method of oil production rather than modern and advanced methods that produce palm oil that is safe (Hinson et al., 2024; Dongho et al., 2017). In South East Nigeria, oil palm plays a pivotal role in contributing to the country's foreign exchange earnings and a crucial revenue source for a significant sum of the rural community (Onoh and Peter-Onoh, 2012). Palm fruit is the primary product derived from oil palm, from which three key commercial products are obtained: palm-oil, palm-kernel oil, and palm-kernel cake. The products derieved from palm fruits are with distinctive properties which hold significant importance in global trade (Barcelos *et al*., 2015). This crop has multiple values and stands out for its economic importance, as highlighted in the literature (Akangbe *et al*., 2011). Notably, in Nigeria, the domestic consumption of palm-oil between the year 2017 - 2018 reached approximately 1.29 million metric tons, emphasizing its widespread utilization in various industries, including the production of margarine, soap, candles, lipstick bases, waxes, polish bases, and confectionery (Conway, 2018; Embrandiri *et al*., 2011).

There are three distinct outlets from which palm-oil is sourced from: the harvesting of fresh palm fruit bunches from natural, uncultivated areas, private plantations under the ownership or management of individual farmers, and extensive corporate or government-owned plantations (Ngando *et al*., 2013). This expansive industry involves numerous smallholders scattered across an estimated land area spanning between 1.65 - 2.4 million hectares, with a maximum potential of 3 million hectares. The estimated expanse of oil-palm plantations in Nigeria varies, encompassing a range from 169,000 hectares (comprising 72,000 hectares of estate plantations and 97,000 hectares of smallholder plantations) to 360,000 hectares of plantations (Agriculture Nigeria, 2019). Crude palm oil boasts considerable benefits for human consumption, existing studies highlight persistent issues regarding safety and quality (Ngando *et al*., 2013). Primarily, the traditional methods employed by individuals with limited knowledge on modern aseptic production techniques and understanding of the microbiological quality leads to poor sanitation and storage methods (Madhusudhan *et al*., 2015). Additionally, concerns persist over changes in the quality of oil, oil due to inappropriate conditions of storage, thereby, posing a substantial risk to public health, given the varied containers used for packaging under different storage conditions that could contribute to spoilage of oil (Viana *et al*., 2019). The quality of oil can further deteriorate through microorganism contamination, stemming from the environment, raw materials, processing equipment, storage and distribution practices (Madhusudhan *et al*., 2015). Recognizing the pivotal role of crude palm oil in the production of food, animal feed, and traditional medicine, where it serves as a major ingredient, underscores the critical importance of assessing and maintaining its microbial quality (Dongho *et al*., 2017). As reported by Ngangjoh *et al*., 2020, that while frying and cooking can minimize microbial load, ensuring optimal microbial quality at earlier stages remains paramount. It is well recognized that the presence of microbes in palm oil can result in chemical alterations that degrade the compositions' quality and endanger human health. Rancidity, acidity, bitterness, soapiness, and other bad flavors might result from fungi's lipolytic action on the triglycerides of oils and fats used in baking recipes. Seeds and other plant parts that are used to make oils may engage in these and other activities. As a result, microorganisms from the environment, raw materials, processing equipment, storage, and distribution can all contaminate palm oil and may ultimately lead to health problems. Hence this study.

MATERIALS AND METHODS

Study Area

This study was carried out in Akoko area, Ondo State, Southwestern, Nigeria, which shares a border with Owo and other Akoko communities in Ondo State. As shown in Figure 1 (GPS), It is the host community to Adekunle Ajasin University, with location coordination 7.5714° N, 5.7063° E

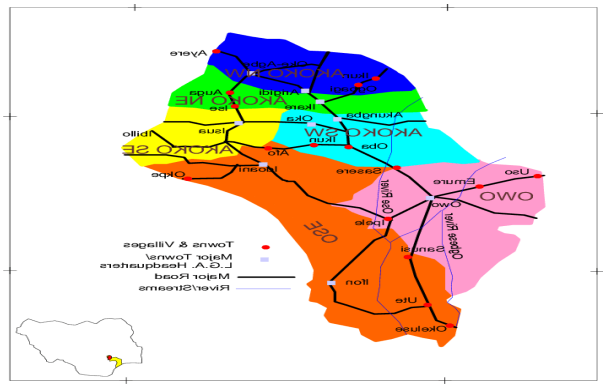


Figure 1: Map showing the location where sample were collection

Collection of Samples and Analysis

Palm oil samples from the processing floors and barrels were collected from Ayegunle, Ikare and Akungba towns in Akoko region. Palm oil samples from the processing floors and barrels during palm oil production were collected using a sterile cotton swab stick from various sources, ensuring representation from different production areas. Samples were aseptically collected and transported immediately to Microbiology laboratory for analysis. One millimeter of each sample was dispensed into nine millimeters of sterile broth solution to make the stock solution. Then from the stock solution, six–fold serial dilution was made. Inoculation on Plate Count Agar (PCA) in duplicates of aliquots of each dilution sample was done using the pour-plate method. All the plates were subjected to incubation at 35°C for 24 hrs. The observed colonies were counted after 24 hours and results represented as cfu/ml (Okechalu *et al*., 2011). The isolates were sub-cultured to obtain pure cultures. One milliliter of third dilution (10^{-3}) of each samples was inoculated unto potato dextrose agar which support growth of fungi, using pour-plate method and incubation was done at room temperature for three to seven days. All fungi isolated were identified using the macroscopic appearance regarding mycology online (Seiyaboh *et al.,* 2018).

Isolation and Identification of Isolated organisms

Identification of all isolates on plates was based on their macroscopic, microscopic and biochemical characteristics, as stated by Bergy's manual. The following biochemical characterization were carried out; catalase, oxidase, indole, citrate, and Gram staining tests to further identify the isolates and predict their fermentative profiles (MacArthur *et al*., 2021; Thonda et al 2021).

Identification of Fungal Isolates (Macroscopic and Microscopic)

Macroscopic identification involved assessing the cultural features and appearances of the isolates on PDA. Standard mycological identification processes, as outlined by Seiyaboh et al. (2018), were followed to identify general features of molds. Additionally, stringent microscopic identification procedures, as explained by Enemuor, (2012), were adhered to. Briefly, a little portion of the culture was transferred onto a clean sterile slide, a drop of lactophenol blue was added and then covered with a cover-slip and examined under a light microscope. The microscopic attributes of hyphae were observed and compared with the reference manual of Barnett and Hunter for accurate identification (Seiyaboh *et al.,* 2018).

Antimicrobial Susceptibility Testing of Isolates

The pure culture (18-24 hours old cultures) of each microorganism was inoculated in sterile normal saline solution, incubated for 24 hours at 37°C, then the cultures were adjusted to a turbidity equivalent of 0.5 McFarland standard. This standardized suspension ensures consistent inoculum density (CLSI, 2020). Kirby-Bauer disk diffusion method was adopted to test the antibiotic susceptibility profile (Thonda et al., 2020).

Antifungal Susceptibility Testing of Fungal Isolates

The antifungal agent (Nystatin (NYS), Fluconazole (FLU), Ketoconazole (KET) and Griseofulvin (GRI)) were procured from commercial sources and were used in their commercial concentration. Stock solutions of NYS, FLU, KET and GRI were made in sterile distilled water. The fresh inoculum of a filamentous fungi was prepared, introduced and suspended in 4 ml of sterile normal saline. The mixture was mixed thoroughly. Potato dextrose agar plates supplemented with chloramphenicol was prepared and left for 1 hour to solidify (Khan *et al.,* 2016). A sterile cotton swab was dipped into the prepared culture solution, pressed firmly against the inside wall of the tube to remove excess liquid, and then used to streak the solidified agar plate, with clockwise and anti-clockwise rotation for even distribution of the inoculum across the plates. After the inoculum has diffused into the agar, a flamed 6mm cork-borer was used to bore four holes adjacent to each other on each plate. Then drops of each of the antifungal agents (Fluconazole, Ketoconazole, Nystatin and Griseofulvin) was placed into the holes (Magaldi *et al*., 2004). Incubation of the plates was done at 28°C for 3 days. After the inoculation period, the zone inhibition for each antifungal agent was measured (CLSI, 2022). The test was done in triplicates.

Growth Dynamic and Death Rate of Isolates

Growth dynamic of the isolates was investigated to determine the rate of growth and the killing time. Briefly, a loopful of 24 hours old of test isolates was transferred from the broth culture into three sets of nutrient broth labeled set A, B, and C respectively. Ultraviolet spectrophotometer was set at 480λ wavelength to determine the turbidity (Osuntokun *et al*., 2019).

RESULTS

Table 1 showed the sample location and collection site of palm oil from the processing floors of the selected palm oil factories in selected geographical zones in Akoko land, Ondo State. A sumof 20 samples were collected from different processing floors (10) and barrels (10) sites of palm oil production Ayegunle, Ikare and Akungba towns in Akoko land. The town comprises of four farms in which two samples were collected each from Alheri farm in Ayegunle, three samples each from Agolo farm and Okela farm in Ikare and two from Oroke farm in Akungba.

Table 2 shows the number of bacterial colonies isolated in palm oil samples from the processing floors and barrels. The total number of colonies isolated from all the processing floor samples is fourty-six, with seven distinct bacterial isolates. For the samples from the floor, FIKOK1 and FAKg1 had the highest number of colonies ($8x10^3$cfu/ml), followed by FIKAg3 with $7x\ 10^3$cfu/ml. However, the total number of bacterial colonies in palm oil samples from the processing barrels is fifty-four (54), with nine (9) distinct bacterial isolates and the colony forming unit ranges between 8×10^3 and 3×10^5 cfu/ml.

Table 3 depicted the biochemical profile and identification of the bacterial isolates obtained from the processing floors and barrels using Bergey's manual. Reconciliation of the macroscopic, microscopic morphology and biochemical characteristics with a standard database (Bergey's manual) on bacteria identification indexed eight unique species of bacteria in the samples which include *Salmonella* spp., *E. coli, B. subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus*, *Lactobacillus* sp. and *Staphylococcus epidermidis.*

Antimicrobial susceptibility profile of the bacterial isolates from processing palm oil floors and barrels is depicted in Figure 2a and 2b.

The bacteria isolates were tested against various antibiotics including cefixime, amoxicillin, cefotaxime, imipenem, ofloxacin, nalidixic acid, ceftriaxone, amoxicillin-clavulanic acid, gentamicin, levofloxacin and augmentin. All the bacteria isolates were susceptible to ofloxacin, nalidixic acid and levofloxacin while they were resistant to the other antibiotics. From the palm oil floors, nalidixic acid had the highest zone of inhibition (28.0mm) against *Staphylococcus aureus* followed by ofloxacin (26.0mm) and levofloxacin (26.0mm) against *Enterococcus faecalis, Salmonella* spp. and *Staphylococcus aureus* respectively. Similarly, nalidixic acid had the highest zone of inhibition (30.0mm) against *Staphylococcus aureus* and levofloxacin (25.0mm) against *Staphylococcus aureus*, *Enterococcus faecalis* while cefotaxime and ceftriaxone had no zones of inhibition.

Table 1: Samples Location and Collection from Palm Oil Processing Floors and Barrels

S/N	Place	Location	Number of samples	Sample source	Time of collection
1	Ayegunle	Alheri farm	2	Floor	4.00AM
			2	Barrel	6.00 AM
2	Ikare	Agolo farm	3	Floor	6.00AM
			3	Barrel	6.00AM
3	Ikare	Okela farm	3	Floor	3.00PM
			3	Barrel	3.00PM
4.	Akungba	Oroke farm	2	Floor	2.00PM
			2	Barrel	2.00PM
		Total	20		

Table 2: Colony forming units of bacterial isolated from the processing floors and barrels of palm oil factories

Sample code (Floors)	Number of colonies (cfu/ml)	Sample code (Barrels)	Number of colonies (cfu/ml)
FAAL1	3 x 10^5	BAAL1	3 x 10^{-5}
FAAL2	6 x 10^3	BAAL2	6 x 10^{-3}
FIKAg1	2 x 10^6	BIKAg1	4 x 10^{-6}
FIKAg2	4 x 10^4	BIKAg2	4 x 10^{-4}
FIKAg3	7 x 10^3	BIKAg3	7 x 10^{-3}
FIKOK1	8 x 10^3	BIKOK1	8 x 10^{-3}
FIKOK2	4 x 10^4	BIKOK2	4 x 10^{-4}
FIKOK3	2 x 10^6	BIKOK3	5 x 10^{-6}
FAKg2	2 x 10^6	BAKg2	5 x 10^{-6}
FAKg1	8 x 10^3	BAKg1	8 x 10^{-3}

Table 3: Biochemical Profile of Bacteria Isolates from the Processing Floors and Barrels of Palm Oil

Isolate code	Cat	Oxi	Cit	Coag	Mot	Ind	Ure	MR	VP	Glu	Lac	Suc	Probable identity
FAAL1	+	-	+	+	-	-	-	+	-	+	+	+	*Staphylococcus aureus*
FAAL2	+	-	+	-	+	-	-	-	-	+	-	-	*Salmonella* sp.
FIKAg1	+	+	+	-	+	+	-	+	-	+	+	+	*Escherichia coli*
FIKAg2	+	-	+	-	+	-	-	-	-	+	+	+	*Pseudomonas aeruginosa*
FIKAg3	+	-	+	-	-	-	-	-	-	+	+	+	*Staphylococcus epidermidis*
FIKOK1	+	-	+	-	+	-	-	+	-	+	+	+	*Bacillus subtilis*
FIKOK2	-	-	-	-	-	-	-	-	-	+	+	+	*Enterococcus faecalis*
FIKOK3	+	+	+	-	+	+	-	+	-	+	+	+	*Escherichia coli*
FAKg2	+	-	+	-	-	-	-	-	-	+	+	+	*Pseudomonas aeruginosa*
FAKg1	+	-	+	+	-	-	-	+	-	+	+	+	*Staphylococcus aureus*
BAAL1	+	-	+	+	-	-	-	+	-	+	+	+	*Staphylococcus aureus*
BAAL2	-	-	-	-	-	-	-	-	-	+	+	+	*Lactobacillus* sp.
BIKAg1	+	+	+	-	+	+	-	+	-	+	+	+	*Escherichia coli*
BIKAg2	+	-	+	-	+	-	-	-	-	+	+	-	*Pseudomonas aeruginosa*
BIKAg3	+	-	+	-	-	-	-	-	-	+	+	+	*Staphylococcus epidermidis*
BIKOK1	+	-	+	-	+	-	-	+	-	+	+	+	*Bacillus subtilis*
BIKOK2	-	-	-	-	-	-	-	-	-	+	+	+	*Enterococcus faecalis*
BIKOK3	+	+	+	-	+	+	-	+	-	+	+	+	*Escherichia coli*
BAKg2	+	-	+	-	-	-	-	-	-	+	+	+	*Pseudomonas aeruginosa*
BAKg1	+	-	+	+	-	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*

KEY: Cat- Catalase; Oxi- Oxidase; Cit- Citrate; Coag- Coagulase; Mot- Motility; Ind- Indole; Ure-Urease; MR- Methyl-red; VP-Voges-Proskauer; Glu- Glucose; Lac- Lactose; Suc- Sucrose

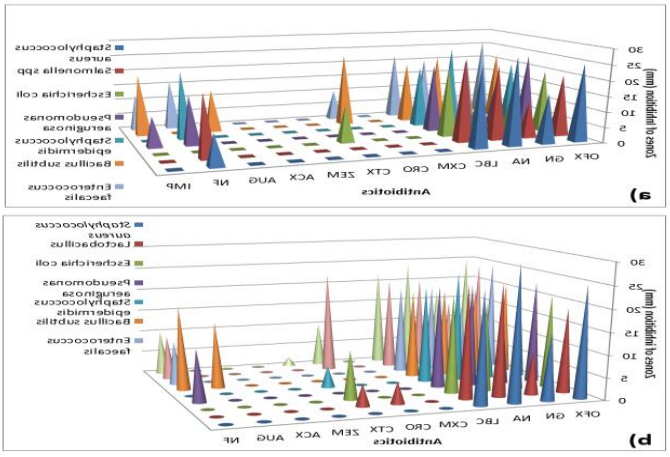


Figure 2: Antimicrobial Susceptibility profile of Bacterial Isolates Obtained from Processing Palm Oil (a) Floors and (b) Barrels of Selected Geographical Zones

KEY: CXM- Cefixime, ACX- Amoxicillin, CTX- Cefotaxime, IMP- Imipenem, OFX- Ofloxacin, NA- Nalidixic acid, CRO- Ceftriaxone, AUG- Amoxicillin-clavulanic acid, GN- Gentamicin, LBC- Levofloxacin, AUG- Augmentin

Table 4a depicts the macroscopic characteristics of the fungal isolated from processing palm oil floors. All the fungal isolates obtained from the floor were observed to have conidiophores except *Mucor plumbeus* that was observed to have sporangiophores. The fungal isolates were identified to be *Aspergillus niger*, *Penicillium aethiopicum*, *Mucor plumbeus*, *Aspergillus carbonarius*, *Aspergillus flavus*, *Fusarium graminearum* and *Alterneria alternate*. The morphological characteristics of the fungal isolated from palm oil processing barrels is depicted in Table 4b. The fungal isolates were identified as *Geotrichum candidum*, *Eurotiumam stelodami*, *Aspergillus flavus*, *Aspergillus niger* and *Fusarium graminearium*. All the fungal isolated from both palm oil floors and barrels had septate and hyaline hyphae. Figure 3a showed the antifungal susceptibility profile obtained from the processing floors of selected geographical zones. The fungi isolates

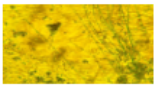
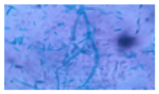
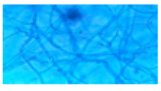
were tested against nystatin, fluconazole, griseofulvin and ketoconazole. All the fungi isolates were susceptible to nystatin. *Aspergillus niger, Fusarium graminearum* and *Aspergillus flavus* were susceptible to fluconazole and ketoconazole while all the fungi isolates showed resistant to griseofulvin. Nystatin had the highest zone of inhibition (28 mm) against *Penicillium aethiopecum* and *Aspergillus flavus* while griseofulvin and ketoconazole had no zone of inhibition against *Mucor plumbeus*. The antifungal susceptibility profile of fungi spp. obtained from the processing barrels from selected geographical zones is shown in Figure 3b. All the fungi isolates were susceptible to nystatin. *Geotrichum candidum* and *Aspergillus flavus* were susceptible to fluconazole while all the fungi isolates were resistant to griseofulvin. Nystatin showed the highest zone of inhibition value of 29 mm against *Fusarium graminearium.*

The growth dynamics and killing time of bacteria and fungi isolated from oil palm processing floors and barrels at 0-96th hour using ultraviolet spectrophotometer (480λ) are depicted in Figure 4. From the oil palm processing floor, it was deduced that at 0 hour, *Enterococcus faecalis* showed a growth rate of 0.273λ which was the highest, followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (0.271λ), while *Salmonella* spp. had 0.266λ. At 24 hours, *Staphylococcus aureus* had the highest growth rate of 0.213λ while *Pseudomonas aeruginosa* has the lowest at 0.161λ. At 48 hours, *Staphylococcus aureus* had the highest growth rate of 0.165λ while *Bacillus subtilis* had the lowest at 0.158λ. At 96 hours, the growth rate of *Staphylococcus aureus* was 0.021λ while *Enterococcus faecalis* was 0.00λ. Figure 4c and 4d showed the growth dynamic and killing time of fungal isolates between 0-96th hours using ultraviolet spectrophotometer at a wavelength of 480λ. At 0 hour, *Alterneria alternata* showed growth rate of 0.813λ which was the highest while *Aspergillus niger* possessed the lowest rate of 0.805λ. At 24 hours, *Aspergillus niger* had highest growth rate of 0.778λ while *Alterneria alternate* had a value 0.704λ which was low as compared to *A. niger*. At 48 hours, a 0.376λ growth rate was observed to be the highest which was expressed by *Aspergillus flavus* with *Aspergillus carbonarius* expressing a low growth rate of 0.300λ. The growth rate of *Aspergillus niger* was 0.036λ while *Aspergillus flavus* and *Alterneria alternate* had a low growth rate of 0.001λ at 96 hrs.

Table 4a: Microscopic & Morphological Characteristics of Fungal Isolates obtained from the Palm Oil Processing Floors

Isolate code	No of Colonies	Colony appearance on PDA	Nature of Hyphae	Spore bearing Structure and spore producing structure	Microscopy	Probable Identity
FAAL1	3 x10^5	Powdery, Brown, Velvety	Septate (Hyaline)	Conidiophores are long, each ending in a bulbous head (vesicle) and bear globular conidia		*Aspergillus* spp
FAAL2	4 x10^4	Velvety, Green	Septate (Hyaline)	Conidiophores are branched bearing conidia that are spherical and in chains		*Penicillium* spp
FAKg1	3 x10^7	Cottony, Light gray	Septate (Hyaline)	Sporangiophores are erect (unbranched), and bear Sporangiospores that are ellipsoidal or nearly spherical.		*Mucor plumbeus*
FAKg2	3 x10^5	Powdery, Green brown	Septate (Hyaline)	Conidiophores are unbranched and bear conidia that are spherical and in chains		*Aspergillus carbonarius*
FAKg1	2 x10^4	Powdery, Green	Septate (Hyaline)	Conidiophore are sparsely unbranched and bear conidia usually borne in chains at the tips		*Aspergillus flavus*
FAAL1	3 x10^5	Floccose, Greyish rose	Septate (Hyaline)	Conidiophores bear conidia that is fusiform and multicelled by transverse septa		*Fusarium graminearum*
FIKAg2	3 x10^3	Wooly, Brown	Septate (Hyaline)	Conidiophores bears conidia that are darkly pigmented and have distinctive elongated, beak-like shape		*Alternaria alternata*

Table 4b: Microscopic & Morphological characteristics of fungal isolates obtained from Palm oil processing barre

Isolate code	No of colonies	Colony appearance on PDA	Nature of Hyphae	Spore bearing Structure and spore producing structure	Microscopy	Probable identity
BAAL1	3 x10^5	Powdery, White, dry	Septate (Hyaline)	Conidiophores are long, each ending in a bulbous head (vesicle) and bear globular conidia		*Geotrichum candidum*
BAAL2	4 x10^4	Velvety, Wolly, Cotton in texture, Filamentous	Septate (Hyaline)	Conidiophores are branched bearing conidia that are spherical and in chains		*Eucatinuum setosum*
BIKAg1	4 x10^6	Cotton, Light gray	Septate (Hyaline)	Sporangiophores are erect (unbranched), and bear Sporangiospores that are ellipsoidal or nearly spherical.		*Aspergillus flavus*
BAKg2	3 x10^6	Powdery, Green brown	Septate (Hyaline)	Conidiophores are unbranched and bear conidia that are spherical and in chains		*Aspergillus niger*
BAKg1	3 x10^6	Powdery, Green	Septate (Hyaline)	Conidiophore are sparsely unbranched and bear conidia usually borne in chains at the tips		*Fusarium graminearum*
BAAL1	3 x10^6	Floccose, Greyish rose	Septate (Hyaline)	Conidiophores bear conidia that is fusiform and multicelled by transverse septa		*Aspergillus niger*
BAAL2	3 x10^6	Wooly, Brown	Septate (Hyaline)	Conidiophores bears conidia that are darkly pigmented and have distinctive elongated, beak-like shape		*Aspergillus flavus*

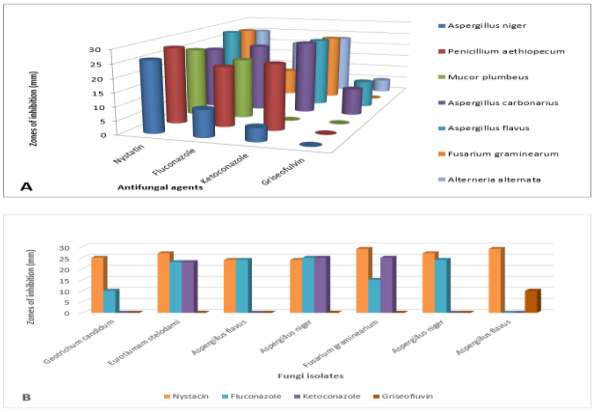


Figure 3: Antifungal Susceptibility Profiles of Fungi spp. obtained from (A) Processing Palm Oil Floors (B) Processing Palm Oil Barrels

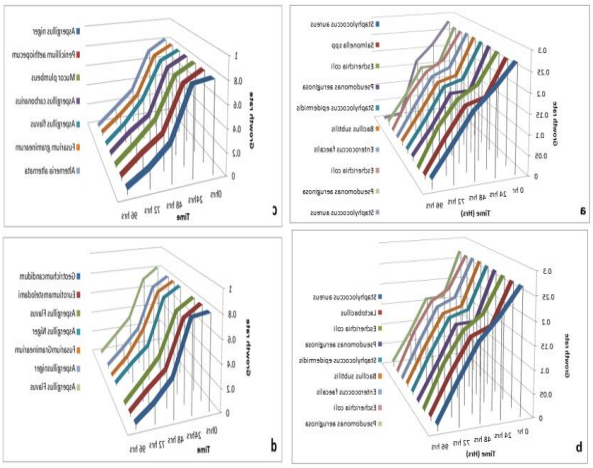


Figure 4: Growth Dynamic/Killing Time of Bacteria and Fungi isolated from Oil Palm Processing Floors and Barrels at 0-96th hour using Ultraviolet Spectrophotometer (a) Bacterial Isolates from Floors (b) Bacterial isolates from barrels (c) Fungal isolates from floors and (d) Fungal isolates from Barrels

DISCUSSION

The microbiological assessment of isolates from the processing floors and barrels of palm oil in Akungba Akoko, Ondo State, provides valuable insights into the microbial ecology of palm oil processing environments and its potential implications for food safety and quality. The findings of this study revealed a diverse microbial community inhabiting the processing floors of palm oil facilities. Bacterial isolates such as *Salmonella* sp., *E. coli, S. aureus, B. subtilis, Enterococcus faecalis, P. aeruginosa* and *S. epidermidis* were among the prominent species identified which are similar with the organisms isolated by MacArthur *et al.* (2021), who conducted experimental research on the microbial assessment of palm oil. Assessment the microbes contaminating palm-oil sold in major Ghana cities oil-producing regions was focused on the research authored by MacArthur *et al*. (2021). *Salmonella* species, *S. aureus, S. epidermidis, E. coli*, and *P. aeruginosa* were documented and reported by MacArthur *et al*. (2021), as prevalent coliforms recovered in the oil sample after the experiments. *Staphylococcus aureus* is ones of the common bacteria identified to produce enterotoxins that can cause food illnesses and intoxication as well as gastroenteritis when the oil is uncooked and eaten raw without further adequate heat treatment and processing, and *S. aureus* was one of the organisms identified in this study (Tesfaye et al., 2015). The existence of these pathogens in the palm oil confirmed the poor making and unhygienic practices of the palm oil. Having known that microorganisms are ubiquitous, it is worth mentioning that palm oil because of its composition, It provides a suitable medium for the growth of microorganisms. Coliforms have been associated with pathogenicity and toxicity, and thus their consumption and accumulation in the system could be harmful and may trigger some physiological responses (Hinson et al., 2024).

Fungal isolates including *Aspergillus flavus, Aspergillus niger, Aspergillus carbonarius, Penicilium aethiopicum, Fusarium graminearum, Alterneria alternata* and *Mucor plumbeus* were also prevalent in this study which corresponds to the study of Okechalu *et al.* (2011), who carried out a research work in Plateau State, Nigeria on the microbiological qualities and chemical characteristics of palm-oil sold within Jos Metropolis. In his result fungi species such as *Aspergillus niger, A. flavus, A. fumigatus, Candida sp., Mucor sp.*, and *Penicillium sp.* were identified (Okechalu *et al*., 2011). *Penicillium sp* had the highest frequency of occurrence (26.67%), followed by *Aspergillus niger* with a percentage frequency of 16.67. Furthermore, the identification of fungal species like *Aspergillus* sp. and *Penicillium* sp. is significant due to their ability to produce mycotoxins, which can contaminate palm oil and pose health hazards to consumers (Delbes *et al*., 2016). The presence of possible toxin producing bacteria such as *Staphylococcus aureus* and pathogenic bacteria like *Escherichia coli,* and *Pseudomonas aeruginosa* raises concerns regarding food safety (Okechalu *et al*., 2011), as *Staphylococcus aureus* can produce heat-stable toxins causing foodborne illnesses, while *Escherichia coli* and *Pseudomonas aeruginosa* are indicators of fecal contamination and can pose health risks if ingested (Aryee *et al*., 2020). The presence of this pathogens in processing environment and barrels can contaminate the palm oil product and can pose a serious health risks to consumers. Asides this, some of the organisms are the causative agent of food spoilage and may increase the spoilage of palm oil. *Aspergillus flavus* with the ability to produce aflatoxin can induce toxic syndromes especially cancer, is of health significance. From similar studies and scientific reviews, other microbes that has been isolated include species of Aspergillus, *Salmonella*, *Penicillium*, *Mucor, Candida, Enterobacter, Bacillus, Proteus,* and *Micrococcus, Trichphyton scohoenleinii*, and *Microsporum canis* (Enyoh et al., 2019; Ngangjoh et al., 2020). The antifungal susceptibility testing provided crucial insights into the response of these bacterial isolates to various antibiotics such as cefixime, amoxicillin, cefotaxime, imipenem, ofloxacin, nalidixic acid, ceftriaxone, amoxicillin-clavulanic acid, gentamicin, levofloxacin and augmentin and antifungal agents such as nystatin, ketoconazole, griseofulvin and fluconazole. The result revealed that all the bacteria isolates were susceptible to ofloxacin, nalidixic acid and levofloxacin and resistant to other used antibiotics such as cefixime, amoxicillin, cefotaxime, imipenem, ceftriaxone, amoxicillin-clavulanic acid, gentamicin and augmentin. Most of fungal isolates were susceptible to the antifungal agents such as nystatin and ketoconazole. Generally, resistance to certain antifungal agents

(ketoconazole, fluconazole and griseofulvin) could raise concerns about the long-term sustainability of these treatment options (Sanguinetti and Posteraro, 2018). Several factors contribute to the development of reduced susceptibility and very little susceptibility to antifungal agents in fungal populations associated with palm oil. The indiscriminate use of antifungal agents, suboptimal dosing practices, and environmental factors all play roles in shaping resistance patterns (Arora *et al*., 2017). The presence of these microorganisms on the processing floors and barrels highlights the importance of implementing stringent hygiene practices and sanitation protocols in palm oil facilities. Regular cleaning and disinfection of equipment and surfaces, proper waste management, and monitoring of water sources can help minimize microbial contamination. Moreover, establishing hazard analysis and critical control points (HACCP) systems tailored to processing of palm oil can enhance food safety by identifying and controlling potential microbial hazards at critical stages of production. By implementing effective control strategies, palm oil producers can mitigate microbial contamination risks and safeguard public health.

CONCLUSION

The microbiological analysis of isolates from the processing floors and barrels of palm oil in study location revealed a diverse array of microorganisms, including bacteria and fungi which indicated the complex nature of microbial communities within the processing environment. The presence of these potentially pathogenic bacteria underscores the importance of implementing rigorous sanitation protocols to ensure the safety and quality of palm oil products. Continued monitoring and adherence to strict hygiene practices are essential to uphold food safety standards and safeguard public health in palm oil production.

RECOMMENDATIONS

Implementing strict hygiene protocols and regular sanitation procedures is crucial to minimize microbial contamination throughout the processing chain. This includes thorough cleaning of equipment and surfaces, as well as proper waste management practices. Continuous monitoring of microbial populations, particularly coliform bacteria and fungi, should be conducted to identify potential sources of contamination and implement timely corrective actions. Additionally, providing training on the use of technologies and education for workers on proper hygiene practices and food safety measures is essential to maintain a sanitary processing environment. Furthermore, further research can be done on microbial control methods tailored to palm oil processing facilities in the region.

FUNDING

Self-funded research: Financial assistance was not provided by any Research Foundation.

COMPETING INTERESTS

The authors declared no competing interests

REFERENCES

Akangbe, J. A., Adesiji, G. B., Fakayode, S. B. and Aderibigbe, Y. O. (2011). Towards Palm Oil Self-sufficiency in Nigeria: Constraints and Training needs Nexus of Palm Oil Extractors. *Journal of Human Ecology*. 33(2): 139-145.

Alabi, D. L., Famakinwa, M., and Akinnawonu, O. E. (2020). Involvement of rural households in oil palm (*Elaeis guineensis*) fruits postharvest activities in Ondo State, Nigeria. *Journal of Agricultural Extension*, 24(1): 42–53. https://doi.org/10.4314/jae.v24i1.5.

Arora, S., Dhuria, N. Jindal, N. and Galhotra, S. (2017). Speciation, biofilm formation and antifungal susceptibility of *Candida* isolates. *International Journal of Research and Development in Pharmacy and Life Sciences*, 6: 2517-21. https://doi.org/10.21276/IJRDPL.2278-0238.2017.6(2).2517-2521

Aryee, A., Rockenschaub, P., Gill, M. J., Hayward, A. and Shallcross, L. (2020). The relationship between clinical outcomes and empirical antibiotic therapy in patients with community-onset Gram-negative bloodstream infections: a cohort study from a large teaching hospital. *Epidemiology and infection*, 148, e225. https://doi.org/10.1017/S0950268820002083

Barcelos, E, de Almeida Rios, S., Cunha, R. N. V, Lopes, R., Motoike, S. Y., Babiychuk, E., Aleksandra, S. and Kushnir, S. (2015). Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science* (6):190. https://doi.org/10.3389/fpls.2015.00190

Clinical and Laboratory Standards Institute (CLSI) (2020). Performance standards for antimicrobial disk susceptibility tests. *Approved standard M2-A10, 2009Wayne*.

Clinical and Laboratory Standards Institute (2022). Method for Antifungal Disk Difusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline. *CLSI document M51-A. Wayne.*

Conway, J. (2018). Nigeria's palm oil consumption 2011/12-2018/2019. Available online at https://www.statista.com/statistics/489451/palm-oil-consumption-nigeria/

Delbes, C., Alomar, J., Chougui, N., Martin, J. F. and Montel, M. C. (2016). *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows' raw milk. *Journal of Food Protection,* 69: 2161–2167. https://doi.org/10.4315/0362-028x-69.9.2161

Dongho, F. F., Gouado, I., Sameza, L. M., Mouokeu, R. S., Demasse, A. M., Schweigert F. J., and Ngono A. R. (2017). Some Factors affecting quality of crude palm oil sold in Douala,

Cameroon. *Journal of Food Research.* 6(1): 50–58. https://doi.org/10.5539/jfr.v6n1p50

Embrandiri, A., Singh, R.P., Ibrahim, H. M. and Ramli, A. A. (2011). Land application of biomass residue generated from palm oil processing: its potential benefits and threats. *Springer Science. Environmentalist,* https://doi.org/10.1007/s 10669-011-9367-0

Enemuor, S. (2012). Environmental and fungal contamination of palm oil sold in Anyigba Market, Nigeria. *African Journal of Microbiology Research.* 6: 2744-2747. https://doi.org/10.5897/AJMR11.1287

Enyoh, C. E., Verla, A.W., Enyoh, E. C. and Verla, E. N. (2019). A review on the quality of palm oil (*Elaeis guineensis*) produced locally in Imo State, Nigeria. *Sustainable Food Production.* 4: 40–50. https://doi.org/10.18052/www.scipress.com/SFP.4.40

Hinson, T. D. C., Houssou, A. P. F., Chabi, W. N., Tchobo, P. F., Dansou, V., Aboudou, K., Mensah, A. G. (2024). Assessment of Good Hygiene and Manufacturing practices in red palm oil production process in Benin. *Journal of Food Stability,* 7 (1), 1-12 https://doi.org/10.36400/J.Food.Stab.7.1.2024-001

Ibitoye, O.O., Akinsorotan, A.O., Meludu, N.T. and Ibitoye, B.O. (2011). Factors affecting oil palm production in Ondo State of Nigeria. *Journal of Agriculture and Social Research.* 11(2): Pp. 97 – 105. https://doi.org/10.4314/jasr.v11i1

Imoisi, O. B., Ilori, G. E., Agho, I and Ekhator, J. O. (2015). Palm oil, its nutritional and health Implications. *Journal of Applied Science and Environmental Management*, 19(1): 127-133 https://doi.org/10.4314/jasem.v19i1.17

Izah, S. C. and Ohimain, E. I. (2013). Microbiological quality of crude palm oil produced by smallholder processors in the Niger Delta. *Nigeria.* 3(2): 30–36.

Khan, S., Singhal, S., Mathur, T., Upadhyay, D. and Rattan, A. (2016). Antifungal Susceptibility Testing Method For Resource Constrained Laboratories. *Indian Journal of Medical Microbiology*, 24(3): 171–176. https://doi.org/10.1016/S0255-0857(21)02345-8

MacArthur, R. L., Teye, E., and Darkwa, S. (2021). Microbial contamination in palm oil selected from markets in major cities of Ghana. *Heliyon*, 7(7), e07681. https://doi.org/10.1016/j.heliyon.2021.e07681

Madhusudhan, A., Tesfaye, L. and Sahile, S. (2015). Microbial quality and chemical characteristics evaluation of edible oils sold at Gondar town markets, North West Ethiopia. *International Journal of Modern Chemistry and Applied Science,* 2(4): 238-247.

Mukherjee S. and Mitra A. (2009).Health Effects of Palm Oil. *Journal Human Ecology*, 26(3): 197-203. https://doi.org/10.1080/09709274.2009.11906182

Ngando, G., Mpondo-Mpondo, A. E. and Ewane, M. A. (2013). Algunsparâmetros de qualidade de óleo de palm abrutonos principais mercados de Douala, Camarões. *Jornal Africano de Ciência Alimentar* 7(2):473- 478.

Ngangjoh, A. S., Tatanja, N. A., and Ejoh, A. R. (2020). Spoilage and microbial quality of crude palm oil from the North-west Region of Cameroon. *African Journal of Food Science,* 14*(*9), 304–312. https://doi.org/10.5897/ajfs2020.1993.

Okechalu, J. N., Dashen, M. M., Lar, P. M., Okechalu, B. and Gushop, T. (2011). Microbiological quality and chemical characteristics of palm oil sold within Jos Metropolis, Plateau State, Nigeria. *Journal of Microbiology and Biotechnology Research* 1(2): 107-112

Onoh, P. A .and Peter-Onoh .C .A. (2012). Adoption of improved oil palm production technology among farmers in Aboh Mbaise local government area of Imo State. *International Journal of Agriculture and Rural Development*. 15 (2); Pp. 966 – 971.

Osuntokun, O. T., Ibukun, A. F., Yusuf-Babatunde, A. M., Abiodun, S. (2019). Pre/post-plasmid profile analysis, killing- kinetics and secondary metabolites screening of *Adenopus breviflorus* (Benth) fruit extract against Multiple Drug Resistant Isolates Using *Staphylococcus aureus* (MDRSA) as a case study. *Journal of Advanced Research and Biotechnology,* 4:1-17. https://doi.org/10.15226/2475-4714/4/1/00144

Sanguinetti, M., and Posteraro, B. (2018). Susceptibility Testing of Fungi to Antifungal Drugs. *Journal of Fungi,* 4(3): 110-115. https://doi.org/10.3390/jof4030110

Seiyaboh, E. I., Kigigha, L. T., Alagoa, C. T. and Izah, S. C. (2018). Microbial Quality of Palm Oil Sold in Amassoma, Bayelsa State, Nigeria. *International Journal of Public Health and Safety.* 3:2

Tesfaye, L., Sahile, S., Madhusudhan, A. (2015). Microbial quality and chemical characteristics evaluation of edible oil sold at gondar town markets. North West Ethiopia. *International Journal of Modern Chemistry and Applied Science.* 2(4): 238–247.

Thonda O. A., Okorie D., Ogidi C. O., Aladejana O. M., Olowookere B. D., Olawoye A. A. (2020). Antibacterial Efficacy of *Vernonia amygdalina* against Bacteria Strains Recovered from Hospital Fomites, Nigeria. *Current Trends in Biotechnology and Microbiology,* 2(2): 381-388. https://doi.org/10.32474/CTBM.2020.02.000131

Thonda, O. A., Oluduro, A. O., Adewole, O. O. and Obiajunwa, P. O. (2021). Phenotypic and genotypic characterization of plasmid-mediated *AmpC* beta-lactamases in enteric Gram-negative bacteria from patients with lower respiratory tract infections in a tertiary hospital, Southwest Nigeria. *African Journal of Clinical and Experimental Microbiology.* 22 (4): 465–472. https://doi.org/10.4314/ajcem.v22i4.6

Viana, V. T., Almeida, D. T., Costa, M. M., Silva, C. and Feitosa, S. (2019). Effects of different storage conditions on the oxidative stabiity of crude and refined palm oil, oleina and steastore (*Elaeis guineensis*). *Food Science and Technology* 39(1): 211-217. https://doi.org/10.1590/fst.43317