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BVA designed and performed the experiments. BVA and SA wrote and AW revised the paper.

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Microbial Contamination in Traditional and Semi-Mechanized Palm Oil Processing in Nigeria

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Abstract:

Palm oil is widely used edible oil in Nigeria, processed through both traditional (manual) and semi-mechanized methods. However, microbial contamination during processing and storage raises significant concerns regarding food safety and industrial microbiology. This study evaluated the microbial quality of manually processed palm oil from Oju, Benue State, and semi-mechanized processed palm oil from Agbor, Delta State. A total of twenty-seven (27) palm oil samples were aseptically collected from 12 traditional processors and 15 semi-mechanized processors. Isolation and enumeration of microorganisms were performed using Nutrient Agar, MacConkey Agar, and Mannitol Salt Agar for bacteria, while Sabouraud Dextrose Agar was used for fungi. Microbial counts were reported in CFU/mL. Bacterial identification was carried out based on biochemical, cultural, and morphological characteristics, while fungal identification involved macroscopic and microscopic analysis. In total, 61 microbial isolates were identified, including *Bacillus* spp. (8), *Escherichia coli* (11), *Staphylococcus* spp. (8), *Pseudomonas* spp. (2), and *Enterobacter* spp. (2) among bacteria, as well as *Aspergillus* spp. (12), *Mucor* spp. (10), *Rhizopus* spp. (3), and *Penicillium* spp. (5) among fungi. *Aspergillus* spp. was the most prevalent fungal isolate (19.67%), while *E. coli* was the dominant bacterial isolate (18.03%), indicating fecal contamination. The total viable count (TVC) and total coliform count (TCC) for traditionally processed palm oil were significantly higher than those for semi-mechanized palm oil ($p < 0.05$), reflecting differences in hygiene practices and processing conditions. Although the microbial loads were within NAFDAC's permissible limit of 2.0×10^4 CFU/mL, the presence of multiple microbial species exceeded regulatory thresholds. These findings highlight the need for strict hygiene regulations, improved microbial monitoring, and enhanced processing practices to ensure food safety and maintain industrial quality standards in Nigeria's palm oil industry. Further research on microbial control strategies is recommended to reduce contamination risks and improve product stability.



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INTRODUCTION

Palm oil production is a significant economic activity in Nigeria, the largest producer of palm oil in Africa and one of the top global producers. Approximately 80% of palm oil in Nigeria is processed by smallholder farmers who rely on traditional or semi-mechanized processing techniques (Obayelu *et al.*, 2022). These processing methods play a crucial role in determining the free fatty acids (FFA) contents and microbiological quality of the final product (MacArthur *et al.*, 2021; Obayelu *et al.*, 2022). Microbial contamination during processing can influence oil stability, shelf-life, and safety, making it essential to understand the microbial communities associated with different processing methods (Ansari *et al.*, 2024; Iqbal, 2024).

In Nigeria, manually processed palm oil is prevalent in rural communities, where traditional extraction methods such as fermentation, pounding, and boiling are used (Bankole and Hammed, 2024). These methods often involve extensive human handling, increasing the likelihood of microbial contamination from environmental sources, equipment, and processing water (Uchenna *et al.*, 2024). Common microbial contaminants of palm oil during processing include: *Bacillus* spp., *Klebsiella* spp., *Serratia marcescens*, *Micrococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., various *Aspergillus* spp. and *Penicillium* spp., *Candida* spp. and other fungi like *Mucor* spp., *Geotrichum* spp., *Saccharomyces* spp., *Fusarium* spp., *Trichoderma* spp. and *Rhizopus* spp. (Abdullahi *et al.*, 2023; Ahiakwo, 2023; MacArthur *et al.*, 2021; Popoola *et al.*, 2022; Senkoh *et al.*, 2020). These microorganisms can contribute to the degradation of palm oil by increasing FFA content, leading to rancidity and reduced market value. Additionally, some fungal contaminants, particularly *Aspergillus* spp., produce mycotoxins, which pose significant food safety concerns (Ahiakwo, 2023).

It is reported that about 80% of the palm oil processed in Nigeria is produced by low-income farmers using traditional methods while semi-mechanized processors and mechanized processors account for 16% and 4%, respectively. Semi-mechanized processing, which involves the use of hydraulic or screw presses, is increasingly adopted in Nigeria as a means of improving the efficiency of oil yield and reducing contamination (Uchenna *et al.*, 2024). However, microbial contamination still occurs, primarily due to poor sanitation, inadequate maintenance of processing equipment, and contaminated water sources. The processing methods and storage periods have adverse effects on the palm oil quality (Enyi and Ojimelukwe, 2021).

The impact of microbial contamination on palm oil quality is not only a food safety concern but also an economic issue. Nigerian palm oil faces challenges meeting international quality standards due to high FFA levels, microbial load, and mycotoxin contamination (Ahiakwo, 2023; Nwachukwu *et al.*, 2019). As Nigeria strives to enhance its palm oil industry for local consumption and export, there is a growing need for improved processing hygiene, microbial monitoring, and the adoption of best practices to minimize contamination.

This study aims to compare the microbial populations in traditionally processed and semi-mechanized processed palm oil in Nigeria, highlighting the specific bacteria and fungi that influence oil quality. By understanding the microbiological profile of palm oil from different processing methods, this research seeks to provide insights that will contribute to improved quality control, enhanced shelf-life, and better compliance with global food safety standards.

MATERIALS AND METHODS

Sample collection

A total of twenty-seven (27) randomized palm oil (olein) samples were collected from two distinct

regions in Nigeria known for palm oil production: Oju in Benue State and Agbor in Delta State.

In Benue State, 250 mL samples of palm oil were collected from twelve different traditional processing mills located in four localities within the Oju Local Government Area: Ibailla, Oju Clan, Umoda, and Ainu. In Delta State, the samples were collected from fifteen different semi-mechanized processing mills in five localities, including Isibor Mills, Felix Mill, Friday Mill, Amayo Mill, and Usiamate Mill. All samples were aseptically collected in sterile universal bottles with screw caps and transported in ice packs for microbiological analysis.

Total viable count and total coliform count

The Total Viable Count (TVC) and Total Coliform Count (TCC) of each palm oil sample were determined using the method described previously with slight modifications (Iqbal *et al.*, 2015; Okechalu *et al.*, 2011). One millilitre of each sample was mixed with 9 mL of sterile distilled water emulsified with a 10% v/v Tween 80 solution to enhance microbial dispersion. The resulting mixture was serially diluted up to 10^{-3} , and 1 mL of the diluted sample was inoculated in duplicates using the pour plate method on various selective and differential media.

For bacterial counts, samples were inoculated on Nutrient Agar (NA) to determine total viable bacteria, Eosin Methylene Blue (EMB) Agar for the selective isolation of *Escherichia coli* and other coliforms, and Mannitol Salt Agar (MSA) for isolating *Staphylococcus* species. Bacterial plates were incubated at 37°C for 24 to 48 hours, while fungal plates were incubated at 25°C for 3 to 5 days before colony enumeration (Esmail *et al.*, 2020; Iqbal *et al.*, 2016; Saleem *et al.*, 2018; Saleem *et al.*, 2020).

For the total coliform count, 1 mL of the 10^{-3} dilution was inoculated onto MacConkey Agar (MCA) using the pour plate technique and incubated at 37°C for 24 to 48 hours. Coliform colonies were identified by their pink-to-red colouration, which indicates lactose fermentation. After incubation, colonies were

counted using a digital colony counter, and results were expressed as colony-forming units per millilitre (CFU/mL) (Esmail *et al.*, 2020; Iqbal *et al.*, 2015).

Isolation and identification of bacteria

Bacterial isolates were purified by streaking onto sterile Nutrient Agar and incubating at 37°C for 24 to 48 hours. Identification was based on colonial morphology, microscopic examination, and biochemical tests (Aernan *et al.*, 2024; Aernan *et al.*, 2023; Ebah *et al.*, 2024; Mohammad *et al.*, 2021; Paray *et al.*, 2023; Shahzad *et al.*, 2017).

Identification of fungi

Fungal counts were assessed using Sabouraud Dextrose Agar (SDA). Fungal isolates were characterized based on cultural and microscopic morphology. Macroscopic features, such as colony colour, texture, and growth patterns, were observed, while microscopic examination using Lactophenol Cotton Blue staining helped visualize spore arrangement and hyphal structure under the microscope (Echevarría, 2019; Echevarría and Iqbal, 2021; Larone, 2011). These characteristics were compared with standard identification keys for proper classification.

Statistical analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) to determine significant differences among microbial counts in the palm oil samples. Post hoc comparisons were conducted using Tukey's test to separate and compare means at a 95% confidence level ($p < 0.05$).

RESULTS

A total of 61 microbial isolates were identified in this study, consisting of 31 bacterial and 30 fungal isolates. The bacterial isolates included *Bacillus* spp., *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Enterobacter*

spp., while the fungal isolates comprised *Aspergillus spp.*, *Mucor spp.*, *Rhizopus spp.*, and *Penicillium spp.* Among these, *Aspergillus spp.* was the most dominant, accounting for 19.67%, followed by *E. coli* (18.03%), *Mucor spp.* (16.39%), and *Staphylococcus spp.* (13.11%), as shown in Table 1.

Figure 1 illustrates the diversity and distribution of bacterial isolates in traditionally processed palm oil samples. *E. coli* was dominant in Ainu, Ibillia, and Oju clan, while *Bacillus spp.* and

Enterobacter spp. were predominant in Ainu and Umoda, respectively. *Staphylococcus spp.* was present in all locations but in significantly lower numbers. For fungi, *Mucor spp.* was dominant in Umoda and Oju clan, whereas *Aspergillus spp.* was present in all locations but most abundant in Umoda, as shown in Figure 2. There was a significant difference in bacterial and fungal diversity and distribution across sample locations ($p < 0.05$).

Table 1. Frequency of occurrence of bacteria and fungi isolated from traditional and semi-mechanized processed palm oil samples.

S/No.	Isolate	Number (%)
1.	<i>Bacillus spp.</i>	8(13.11)
2.	<i>Escherichia coli</i>	11(18.03)
3.	<i>Staphylococcus spp.</i>	8(13.11)
4.	<i>Pseudomonas spp.</i>	2(3.28)
5.	<i>Enterobacter spp.</i>	2(3.28)
6.	<i>Aspergillus spp.</i>	12(19.67)
7.	<i>Mucor spp.</i>	10(16.39)
8.	<i>Rhizopus spp.</i>	3(4.92)
9.	<i>Penicillium spp.</i>	5(8.20)
Total		61(100)

■ *E. coli* ■ *Bacillus spp.* ■ *Staphylococcus spp.* ■ *Enterobacter spp.*

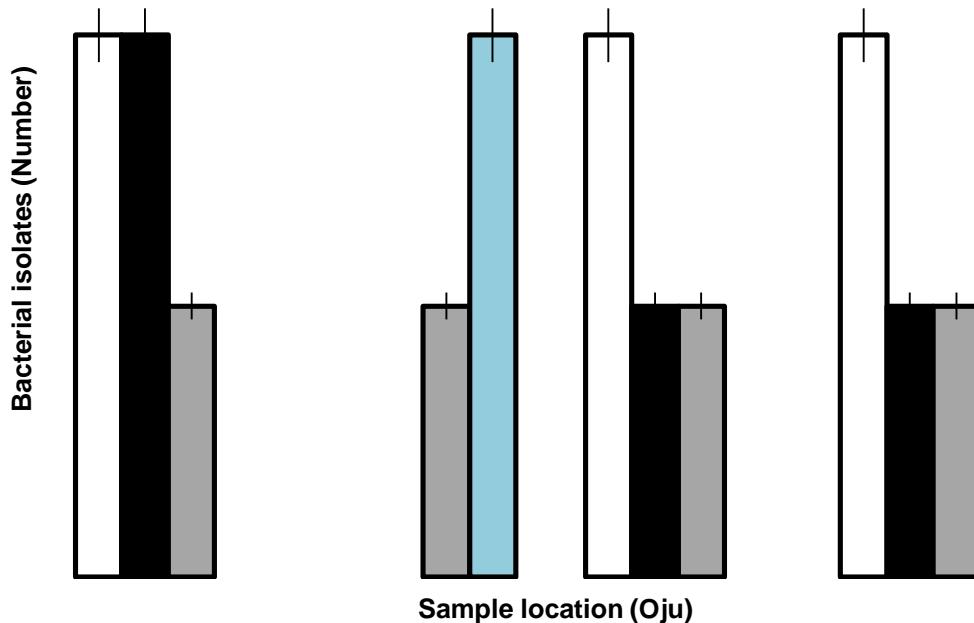


Fig. 1. Diversity and distribution of bacterial isolates in traditionally processed palm samples oil from Oju. The bar represents the standard error of duplicate determination.

■ *Aspergillus spp.* ■ *Aspergillus niger* ■ *Penicillium spp.* ■ *Mucor spp.*

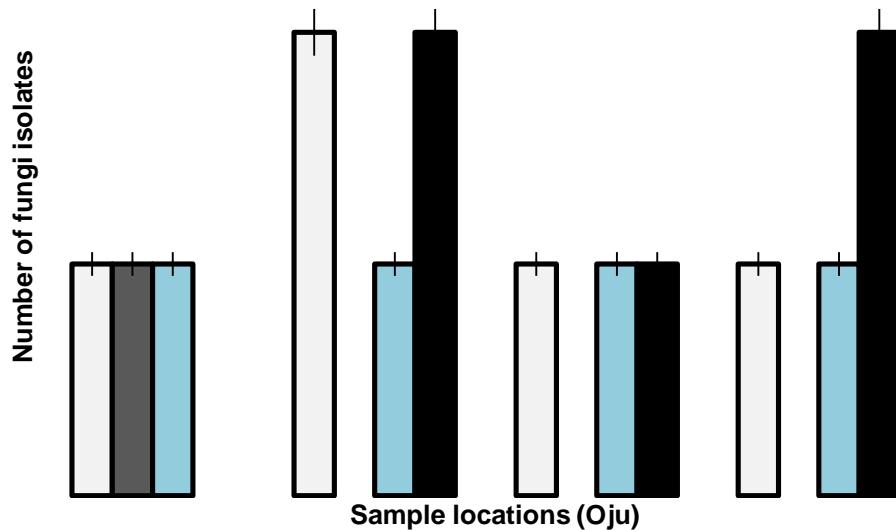


Fig. 2. Diversity and distribution of fungal isolates in traditionally processed palm samples oil from Oju. The bar represents the standard error of duplicate determination.

Figure 3 presents the diversity and distribution of bacterial isolates in semi-mechanized processed palm oil samples. *Bacillus spp.*, *Staphylococcus spp.*, and *Pseudomonas spp.* were dominant in samples from Amayo, Isibor, and Friday, respectively, while *E. coli* was predominant in samples from Usiamata and Felix. Fungal distribution (Figure 4) showed *Aspergillus spp.* as the dominant species in Amayo and Isibor, whereas *Mucor spp.* was most prevalent in Felix. The observed differences were statistically significant ($p < 0.05$).

Figure 5 presents the total viable count (TVC) and total coliform count (TCC) for traditionally processed palm oil samples. The highest TVC was recorded in Ainu (2.24×10^2 cfu/mL) and Umoda (1.56×10^2 cfu/mL), while Oju clan had the lowest TVC (4.6×10^1 cfu/mL). Similarly, the highest TCC values were observed in Ainu (1.09×10^2 cfu/mL) and Umoda (5.6×10^1 cfu/mL), with the lowest in Ibilla (3.0×10^0 cfu/mL) and Oju clan (5.0×10^0 cfu/mL).

Figure 6 presents the TVC and TCC results for semi-mechanized processed palm oil from Agbor. Samples from Felix (1.85×10^2 cfu/mL)

and Friday (1.11×10^2 cfu/mL) had the highest TVC, while Isibor (2.9×10^1 cfu/mL) recorded the highest TCC. A significant difference ($p < 0.05$) was observed between the TVC of traditionally processed and semi-mechanized processed palm oil. However, the TCC between the two processing methods was not significantly different ($p > 0.05$).

DISCUSSION

This study examines the microbial diversity in palm oil produced through traditional and semi-mechanized methods in two major Nigerian palm oil-producing regions—Oju in Benue State and Agbor in Delta State. Palm oil is a dietary staple and a key commercial product in Nigeria, yet concerns persist regarding contamination and safety, particularly for traditionally processed oils. This study highlights the microbial hazards associated with different processing techniques and emphasizes the need for enhanced food safety measures.

■ *E. coli* ■ *Bacillus* spp. ■ *Staphylococcus* spp. ■ *Pseudomonas* spp.

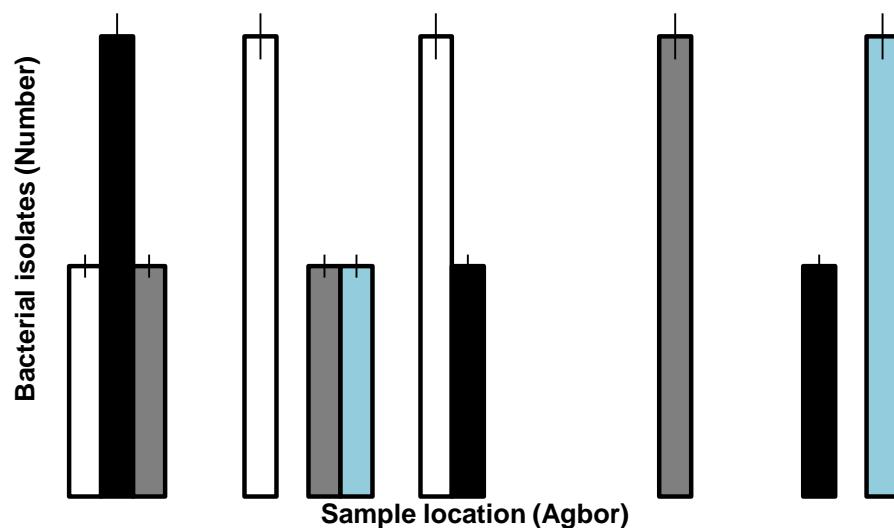


Fig. 3. Diversity and distribution of bacterial isolates in semi-mechanized processed palm samples oil from Agbor. The bar represents the standard error of duplicate determination.

■ *Aspergillus* spp. ■ *Penicillium* spp. ■ *Mucor* spp. ■ *Rhizopus* spp.

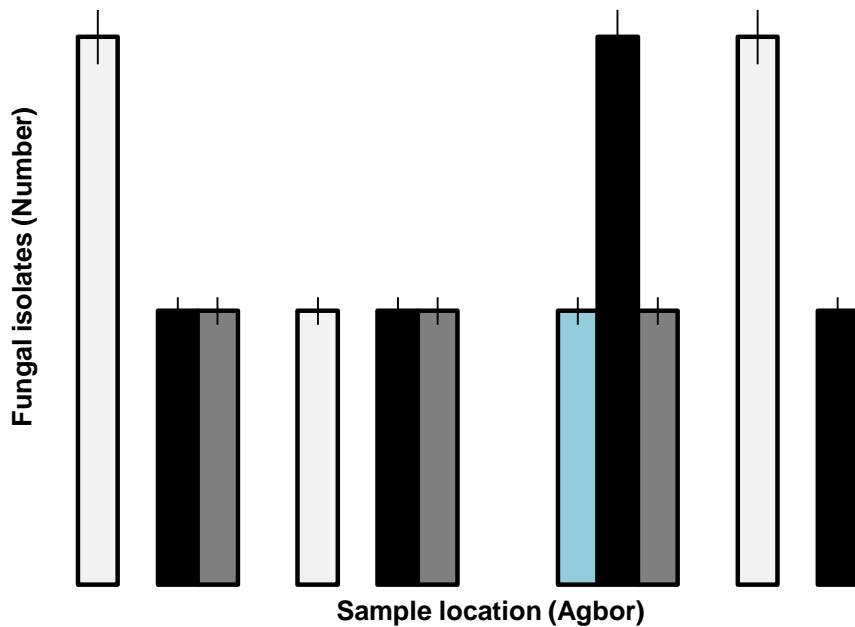


Fig. 4. Diversity and distribution of fungal isolates in semi-mechanized processed palm samples oil from Agbor. The bar represents the standard error of duplicate determination.

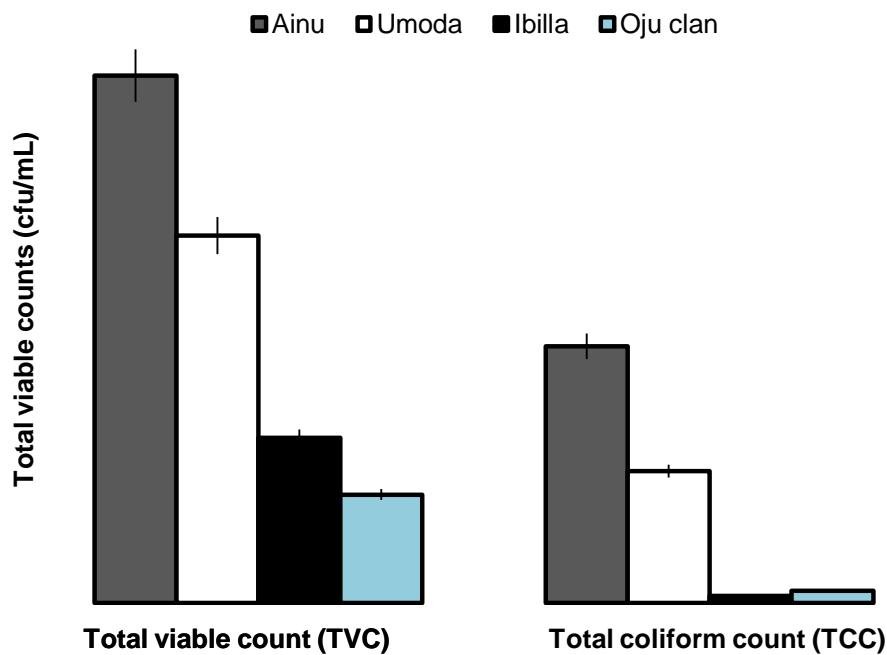


Fig. 5. Total viable counts and total coliform counts of traditionally processed palm oil samples from Oju. The bar represents the standard error of duplicate determination.

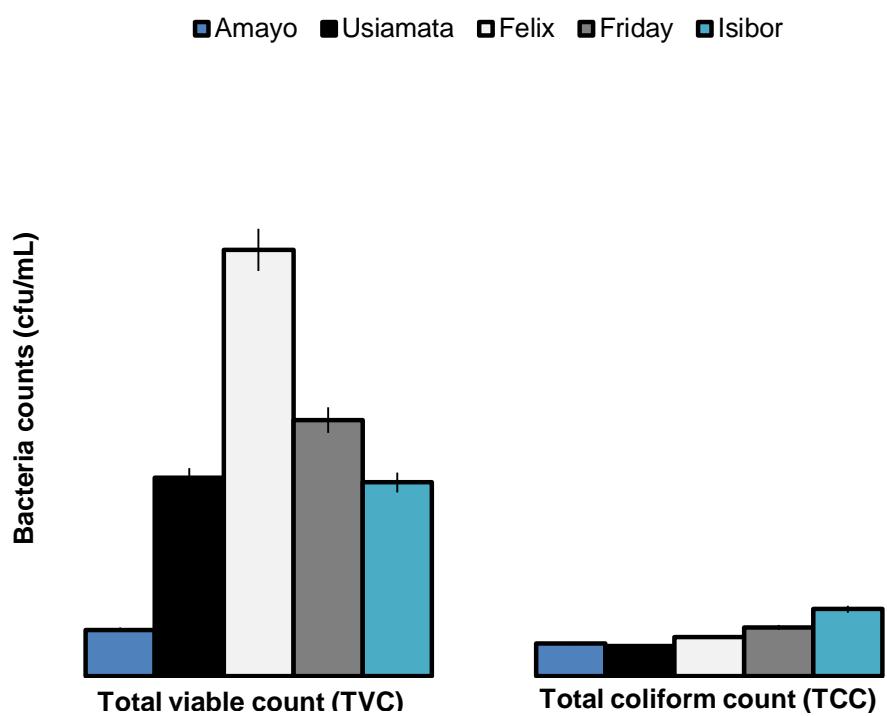


Fig. 6. Total Viable Counts and Total Coliform Counts of semi-mechanized processed palm oil samples from Agbor. The bar represents the standard error of duplicate determination.

The microbiological analysis revealed a substantial presence of bacterial and fungal isolates in the palm oil samples. The predominant bacterial contaminants were *Bacillus* spp., *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Enterobacter* spp., while fungal isolates included *Aspergillus* spp., *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp. These findings align with previous studies reporting similar microbial contaminants in crude palm oil consumed in Nigeria (Ahiakwo, 2023; Izah and Ohimain, 2016; Ohimain et al., 2013; Okechalu et al., 2011).

Notably, *E. coli* was predominant in traditionally processed samples, raising concerns about fecal contamination. As an indicator organism, the high prevalence of *E. coli* suggests poor sanitary conditions during processing and potential exposure to gastrointestinal pathogens, particularly in regions where palm oil is consumed raw (MacArthur et al., 2021). Traditional palm oil production in Nigeria often involves open-air extraction, with workers using rudimentary tools and unclean water sources, increasing the likelihood of coliform bacteria contamination. The presence of *E. coli* in these samples suggests contamination from unsanitary water, dirty equipment, or improper handling, all of which are common in rural food production (Okogbenin et al., 2014; Uchenna et al., 2024). This finding aligns with studies indicating that processing methods significantly impact the physicochemical and microbial quality of palm oil (Enyi and Ojimelukwe, 2021). Furthermore, the significantly lower *E. coli* and total coliform counts in semi-mechanized samples ($p < 0.05$) reinforce the role of improved processing techniques in reducing microbial contamination. The semi-mechanized method has also been reported to enhance oil yield and operational efficiency compared to traditional methods (Uchenna et al., 2024).

The presence of *Bacillus* spp. in palm oil is concerning, as its prevalence indicates high microbial contamination (Izah and Ohimain, 2016). The widespread occurrence of *Bacillus* spp. and *Staphylococcus* spp. is particularly

significant, as both bacteria produce lipases that hydrolyze triglycerides into free fatty acids, accelerating rancidity and negatively affecting the peroxide and iodine values of palm oil and storage (MacArthur et al., 2021).

Fungal contamination was also notable, with *Aspergillus* spp. being the most prevalent ($p < 0.05$), consistent with prior studies that isolated multiple *Aspergillus* species from palm oil (Ahiakwo, 2023; Senkoh et al., 2020). This is particularly concerning as certain *Aspergillus* species produce aflatoxins—potent mycotoxins linked to liver cancer and immune suppression (Iqbal et al., 2021). A previous study reported a 75% prevalence of *Aspergillus* spp. in palm oil samples from Port Harcourt markets, with fungal counts and aflatoxin concentrations exceeding permissible limits (2.0×10^4 CFU/mL and 20 µg/kg, respectively). The detection of *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp. further highlights the risk of fungal contamination, which can cause spoilage and pose health hazards (Ahiakwo, 2023). Given that many Nigerian households and food vendors rely on locally processed palm oil, these contaminants present significant public health risks.

A comparison between traditional and semi-mechanized processing revealed that while semi-mechanized methods significantly reduced coliform contamination ($p < 0.05$), they still harbored substantial bacterial and fungal loads. According to NAFDAC standards, palm oil samples with microbial loads between 10^3 and 10^4 CFU/mL are considered acceptable; however, the presence of more than two microbial species exceeds regulatory limits (Izah and Ohimain, 2016). A diverse microbial population in palm oil samples indicates contamination and poor handling practices. The lower bacterial load in semi-mechanized samples can be attributed to better-controlled processing conditions and reduced direct human contact. However, persistent microbial contamination in these samples emphasizes the need for stricter hygiene standards and improved equipment sanitation.

The transition to semi-mechanized processing in Nigeria has been slow due to high costs and limited access to modern technology, particularly in rural areas. Consequently, small-scale producers—who account for 80% of Nigeria's palm oil production—continue to rely on traditional methods, increasing the risk of microbial contamination (Uchenna *et al.*, 2024).

Given the widespread consumption of palm oil in Nigeria, both domestically and in commercial food production, ensuring its safety is paramount. The findings of this study emphasize the urgent need for improved hygiene practices, stricter regulatory oversight, and enhanced education for local producers. Encouraging the use of clean water, proper storage conditions, and routine microbiological testing can help mitigate contamination risks. Additionally, government agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and the Standards Organization of Nigeria (SON) should enforce stricter quality control measures for locally processed palm oil.

CONCLUSION

This study provides critical insights into the microbial contamination of palm oil produced in two major Nigerian palm oil-producing regions. The results indicate that traditionally processed palm oil is more susceptible to bacterial contamination, particularly from coliform bacteria, due to poor sanitary conditions. Fungal contamination, including potentially mycotoxin-producing *Aspergillus* spp., was present in both processing methods, emphasizing the need for improved storage and handling. Although semi-mechanized processing exhibited lower contamination levels, the persistence of bacterial and fungal contaminants highlights the necessity for further improvements in hygiene and equipment sterilization. Addressing these challenges through enhanced processing techniques, stricter regulations, and better education for local producers will improve the safety and quality of palm oil in Nigeria, ensuring

its continued role as a reliable and healthy food source for consumers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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