

Microbial Quality and Internal Spoilage Assessment of *Persea americana* (Avocado pear) Sold at Oba, and Ochanja Markets, in Anambra State, Nigeria.

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Abstract: *Persea americana* (Avocado pear) fruits are often lost postharvest due to microbial spoilage, with internal decay frequently remaining undetected until the fruit is cut. There is limited recent data, especially under local market conditions on the microbial contamination and internal spoilage status of avocados postharvest. This study evaluated the microbial quality and internal spoilage characteristics of *Persea americana* (avocado) fruits obtained from Oba and Ochanja markets in Anambra, Nigeria. A total of four (4) spoiled fruits were purchased from Oba and Ochanja markets and were transported in a sterile container, to the laboratory for microbial analysis. Standard microbiological methods were used to enumerate total viable counts (TVC), yeasts and molds. TVC were carried out using pour-plate technique. Detection of yeasts and moulds were carried using microscopic examination. Biochemical characteristics of the isolated organisms were determined using catalase, coagulase, oxidase, motility, sugar fermentation, indole, methyl red(MR), Voges proskuar and citrate tests. Results showed that total bacterial count ranged between 1.1×10^3 and 4.7×10^4 , with OBM 2 having the highest count, while OBM 1 had the least. *Escherichia coli* (*E. coli*) spp and *Salmonella* spp were not detected in any of the samples. The total yeast count ranged between 1.1×10^3 and 3.5×10^2 . The total mould count ranged between 3.2×10^2 and 4.7×10^4 . Microorganisms isolated in this study include: *Staphylococcus* sp., *Streptococci* sp. *Mucor* sp., *Saccharomyces* sp. and *Geotichum* sp. Internal browning was the predominant defect. Firmness decreased significantly with increasing spoilage. Overall, the study shows that poor postharvest handling and sanitation in open markets contribute to elevated microbial contamination and high spoilage incidence in avocados. The results highlight the need for improved storage, reduced handling, and better hygienic practices along the supply chain to enhance fruit quality, extend shelf life, and reduce economic losses.

Keywords: *Persea americana*, spoilage, assessment, quality, Anambra State.

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Introduction

Avocado fruits are often lost postharvest due to microbial spoilage, with internal decay frequently remaining undetected until the fruit is cut. Similar postharvest losses attributable to microbial deterioration, particularly fungal spoilage have been widely reported in tropical root and fruit crops under Nigerian market conditions (Agu et al., 2011; Agu et al., 2014; Agu et al., 2015; Frank & Kingsley, 2014a). There is limited recent data, especially under local market conditions, on the microbial contamination and internal spoilage status of avocados postharvest. Without up-to-date, context-specific data, efforts to reduce postharvest losses and ensure food safety remain suboptimal. Current postharvest handling, storage, and marketing practices may not adequately minimize spoilage or ensure microbial safety, as demonstrated in studies on yams, cassava, cocoyam, and other tropical produce (Agu et al., 2016a; Agu et al., 2016b; Ogbo & Agu, 2015).

While existing research has expanded understanding of avocado postharvest spoilage, including microbial communities, pathogen identity, and control options significant gaps remain. Many studies focus on surface microbiomes or controlled postharvest treatments; however, data on microbial contamination and internal spoilage under local market conditions remain limited. Comparable studies on tropical crops in southeastern Nigeria have shown that internal tissues often harbor spoilage fungi that are not externally visible, contributing substantially to hidden postharvest losses (Agu et al., 2011; Agu et al., 2014; Okigbo et al., 2015). There is also a lack of context-specific studies, particularly in African and tropical market settings that examine both bacterial and fungal contamination within edible fruit pulp.

The relationship between microbial load (quantitative assessment) and internal spoilage symptoms (qualitative pulp assessment) remains underexplored. Previous pathogenicity studies on yam, cocoyam, cassava, and sweet potato have demonstrated strong

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associations between microbial population density and tissue deterioration severity (Agu et al., 2015; Agu et al., 2016a; Ogbo & Agu, 2015). However, such integrated assessments are scarce for avocado fruits marketed under ambient tropical conditions. In addition, the application of biocontrol strategies or alternative storage and packaging methods under local market conditions remains poorly documented.

The avocado (*Persea americana*) is a fruit of growing global importance, prized for its nutritional value especially its healthy lipids, vitamins, minerals, and antioxidants which has driven increasing consumption worldwide (Correa-Pacheco et al., 2022; Donoso et al., 2024). However, despite these benefits, avocado fruits remain highly perishable and vulnerable to rapid deterioration after harvest. Similar perishability challenges have been documented for other high-moisture tropical crops in Nigeria, where fungal spoilage significantly reduces shelf life and market value (Frank & Kingsley, 2014a; Frank & Kingsley, 2014b).

A major contributor to postharvest losses in avocado is microbial spoilage caused by bacteria and fungi. Microorganisms can invade fruits through natural openings, wounds, or latent infections and subsequently cause internal decay that may not be apparent from external observation (Korsten, Coetzee, & Duvenage, 2023; Koffi et al., 2024). Studies on cassava, cocoyam, and yam have similarly shown that spoilage fungi can establish internally and express pathogenicity during storage and marketing stages (Agu et al., 2014; Agu et al., 2016b; Okigbo et al., 2015).

Recent advances in microbial ecology have deepened understanding of the microbial communities associated with avocado fruits. High-throughput sequencing studies indicate that both potentially pathogenic and beneficial fungi inhabit the avocado carposphere, and that postharvest treatments can significantly alter these communities (Korsten et al., 2023). Comparable microbiological evaluations of fruits and tubers in Nigeria have revealed diverse spoilage-associated fungi and bacteria with varying pathogenic potentials (Obianom et al., 2023; Agu et al., 2015). Moreover, biocontrol using antagonistic microorganisms has proven effective in suppressing spoilage fungi and extending shelf life in several crops (Koffi et al., 2024; Okigbo et al., 2015).

Given the perishable nature of avocado, its economic value, and emerging evidence of microbial community dynamics, there is a strong need to assess microbial quality and internal spoilage status of avocados—particularly in local tropical markets where environmental conditions favor microbial proliferation. Such assessments have proven valuable for informing postharvest handling and spoilage mitigation strategies in other Nigerian crops (Agu et al., 2011; Agu et al., 2016a).

Therefore, this study seeks to investigate the microbial load (bacteria and fungi) and internal spoilage status of *P. americana* fruits, with the aim of identifying spoilage-associated microorganisms and relating microbial contamination levels to internal spoilage symptoms under local market conditions.

Materials and methods

Study Design

This study employed a laboratory-based experimental design to assess microbial contamination and internal spoilage in avocado (*Persea americana*) fruits. Fruits were collected from selected local markets and farms. The study combined visual assessment of

internal spoilage with microbial isolation, identification, and quantification.

Sample Collection and Handling

Total of four spoiled fruit samples, two each, were purchased from two different markets, Oba market in Idemili South local government area, and Ochanja market in Onitsha south local government area, both in Anambra State Nigeria.

The samples were transported in sterile containers to the laboratory for microbial analysis.

Cleaning: Fruits were washed under running potable water; external surfaces were blotted with sterile paper.

Microbial Isolation and Identification

Sample Preparation: 1 g of pulp from the boundary of healthy and spoiled tissue was aseptically collected. Samples were homogenized in 9 mL sterile distilled water, and serially diluted (10^{-1} to 10^{-3}).

Enumeration of Bacterial

Total aerobic bacterial count was carried out using pour-plate technique. 0.1ml each, of the serially diluted samples was inoculated onto labelled sterile petri dish in duplicate, about 20mL sterile nutrient agar medium was poured on each of the samples, and incubated at 37° C for 24 hours. The colonies were counted using direct count method, and the results were recorded in colony forming unit per milliliter (CFU/mL).

Enumeration and Identification of Fungi

Total fungal count was carried out using pour-plate technique. 0.1mL of each, of the serially diluted samples was inoculated onto labelled sterile petri dish in duplicate, and about 20mL of Sabouraud Dextrose agar (SDA) medium was poured on each of the samples, and incubated at 25° C for five (5) to seven (7) days. The colonies were counted using direct count method, and the results were recorded in colony forming unit per milliliter (cfu/mL). Fungal isolation and characterization were performed based on the visual assessment of fungal colony morphology on SDA culture medium. Microscopic evaluation was conducted using the modified slide culture technique with lactophenol cotton blue stain, viewed under X10 and X40 magnification (Agu and Chidozie, 2021). Identification was further guided by reference to various Manuals of Fungal Atlases (Barnett and Hunter, 2000; Ellis et al., 2007).

Isolation of Microorganisms

The distinct colonies on nutrient agar and SDA, were carefully examined using microscope for their morphological characteristics. The bacterial colonies were subcultured on nutrient agar using streak plate technique, and were incubated at 37°C for 24 hours

Identification of Isolates

Gram staining, and other biochemical tests were carried out following the method of Cheesbrough (2010). The biochemical tests performed in this study include: catalase test, indole test, coagulase test, citrate test, oxidase test, sugar fermentation test, methyl red test, voges proskaur test.

Catalase Test

A drop of 3% hydrogen peroxide was placed on a clean slide. A colony of the test organism was added. Immediate bubbling

indicates a positive result, showing the presence of the catalase enzyme.

Coagulase Test

A loopful of the bacterial isolate was mixed with a drop of rabbit plasma on a clean slide. Clotting or clumping of the plasma indicates a positive coagulase result, typical of *Staphylococcus aureus*.

Indole Test

The test organism was inoculated into sterile peptone water and incubated at 37°C for 24–48 hours. After incubation, a few drops of Kovac’s reagent were added. The appearance of a red or pink layer on the surface indicates a positive result, showing the organism can produce indole from tryptophan.

Citrate Utilization Test

The test organism was streaked on Simmon’s citrate agar slant and incubated at 37°C for 24–48 hours. A color change from green to blue indicates a positive result, showing citrate utilization.

Oxidase

Oxidase test was performed to determine the presence of cytochrome c oxidase enzyme in the bacterial isolates. A colony of each isolate was exposed to oxidase reagent, and the appearance of a purple coloration within a few seconds was recorded as a positive result, while no color change indicated a negative reaction.

Sugar Fermentation

To assess the ability of the isolates to ferment sugars, peptone water broth containing either glucose or lactose, along with phenol red as a pH indicator and an inverted Durham tube to detect gas production. After inoculation and incubation, a color change from red to yellow indicated acid production (positive fermentation), while the presence of gas bubbles in the Durham tube confirmed gas production. No color change indicated a negative fermentation result.

Table 1a: Cultural Characteristics of Bacterial Isolates spoiled *Pearsia american* Sold in Oba and Ochanja markets in Anambra State.

Organism	Colony Appearance on Nutrient Agar	Colony Appearance on Selective/Differential Media	Growth Pattern	Pigmentation	Other Notes
<i>Staphylococcus spp.</i>	Smooth, round, convex colonies; opaque; 1–3 mm diameter	MSA : Yellow colonies (<i>S. aureus</i>) or pink (non-pathogenic species); Blood agar : β-hemolysis (<i>S. aureus</i>) or γ-hemolysis	Facultative anaerobe; grows rapidly	Cream, white, or golden yellow	Buttery consistency; non-motile
<i>Bacillus spp.</i>	Large, flat, irregular, spreading colonies; rough/dry surface	Blood agar : β-hemolysis common (e.g., <i>B. cereus</i>)	Aerobic/facultative; fast-growing	Cream, white, or gray	Often shows rhizoid edges; some strains have ground-glass appearance
<i>Streptococcus spp.</i>	Small, pinpoint colonies; translucent	Blood agar : α-hemolysis (e.g., <i>S. pneumoniae</i>), β-hemolysis (<i>S. pyogenes</i>), or γ-hemolysis	Microaerophilic; slow to moderate growth	Usually non-pigmented	Non-motile; often requires enriched media

Table 1b: Cultural Characteristics of Fungal Isolates from spoiled *Pearsia american* Sold in Oba and Ochanja markets in Anambra State.

Organism	Morphology on Sabouraud Dextrose Agar (SDA)	Texture	Color (Obverse/Reverse)	Growth Rate	Other Notes
<i>Mucor spp.</i>	Cottony, fluffy, rapidly spreading colonies	Very fluffy, woolly	White → gray to brown with age	Very fast	Produces tall sporangiophores; non-septate hyphae
<i>Saccharomyces spp.</i>	Creamy, smooth, yeast-like colonies	Moist, butyrous	Cream to off-white	Moderate	Ovoid budding yeast; no mycelium; sweet fermentative odor
<i>Geotrichum spp.</i>	White, dry, powdery colonies (“ground-glass” appearance)	Chalky	White to cream	Moderate to fast	Produces arthroconidia; colonies may appear dull

Table 2: Total Bacterial counts, Yeast and Mould Counts of Isolates from Spoiled *Persea americana* sold in Oba and Ochanja Markets In Anambra State.

Sample	Total Bacterial Count (CFU/g)	Yeast Count (CFU/g)	Mould Count (CFU/g)
.OBM 1	4.2×10^5	1.1×10^3	4.2×10^2
OBM 2	3.8×10^5	3.5×10^2	4.7×10^4
OBM 3	2.4×10^5	3.4×10^2	3.9×10^2
OCM 4	3.6×10^3	2.5×10^3	3.2×10^2

Key: CFU/g= colony forming unit per gramm, += positive, spp = specie, OBM 1= Oba sample 1, OBM 2= Oba sample 2, OCM 3 = Oba sample 3; OCM 4= Ochanja sample 4

Table 3: Gram-reaction, and Biochemical Characteristics of Bacterial Isolates from Spoiled *Persea americana* sold in Oba and Ochanja Markets in Anambra state. Nigeria.

Biochemical Test	<i>Staphylococcus spp.</i>	<i>Bacillus spp.</i>	<i>Streptococcus spp.</i>
Gram reaction	Gram-positive cocci	Gram-positive rods	Gram-positive cocci in chains
Catalase test	Positive	Positive	Negative
Oxidase test	Negative	Variable (commonly negative)	Negative
Sugar fermentation	Ferments glucose, mannitol	Ferments glucose (acid)	Ferments glucose, lactose, sucrose
Indole test	Negative	Negative	Negative
Methyl Red (MR)	Variable	Variable	Negative
Voges-Proskauer (VP)	Positive	Positive	Negative
Citrate utilization	Negative	Positive	Negative

Discussion

Avocado fruits are often lost postharvest due to microbial spoilage, with internal decay frequently remaining undetected until the fruit is cut. Bacteria and fungi are the common contaminants of our fruits and they could be easily transferred from the vendors to the processed fruits through mishandling. Microorganisms are ubiquitous and they have been found to colonize *Persea americana* fruit due to its high nutritional content that can support their growth and cause spoilage. Availability of nutrient is crucial to increase or decrease of microbial numbers in fruits during spoilage. The result from this research identified six isolates namely, *streptococcus sp*, *staphylococcus sp* and fungal isolates : *Geotichum sp*, *mucor sp*, and *Saccharomyces sp*.

These organisms have been reported as the causative agent for bacteria soft rots. Like fungi they can hydrolyse pectin giving rise to soft mushy appearance or consistency (Liao *et al.* 1993). Their presence is suggestive of contamination from soil, harvesting equipment, handling and storage facilities, and on food contact surface throughout the distributing chain.

The colonization process involves the ability of the microorganisms to establish them within the produce. This is initiated when the microorganism following adhesion and release of enzymes degrade certain specific cell wall polymers such as protopectin, the magnitude of the symptoms of the induced disease is a reflection of the extent of colonization, whereas both fruits and vegetables are highly susceptibility to microbial spoilage ,there are variation in the susceptibility which is due largely to the

differential chemical composition such as pH and moisture contents of the fruits make them more prone to fungal spoilage

.The fungal isolates in this study *saccharomyces spp* and *Mucor* probably grew due to the amount of oil found in Avocado pear suggested from the report of previous investigation. These organisms present a formidable challenge to commercial fresh fruits product operations from the farm to retail and wholesale outlets (Liao *et al* 1993,1997) .

Damage inflicted on produce at the time of harvest is a major cause of infection since most of the spoilage microorganism invade the produce through such damaged tissues, similarly the extent of deterioration is influenced by the depth of the wound. Furthermore the incidence of infection is worsened by poor sanitary practices such as cross-contamination, contact infection during the transporting of product. During harvest operation. The process of the fungi which may result in post-harvest contamination and spoilage of these fruits and vegetables originated from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoilt fruits.

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