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Comparative Analysis of Acetobacter aceti Prevalence in Ripe Fruits Sold at Ozuoba Market, Port Harcourt, Rivers State

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Abstract
The study screened for *Acetobacter aceti* in ripped fruits marketed in Ozuoba due to the alleged use of Calcium Carbide, a chemical for fruit ripening. Twenty-five (25) samples each of banana, orange, watermelon, and pineapple were purchased and taken to the labouratory for microbiological investigation. The fruit samples were cultured on Glucose Yeast-extract Calcium Carbonate (GYC) media, Gram-stained, and characterized; biochemically and morphologically. Results showed that watermelon had the highest *A. aceti* load of 5.9×10^3 CFU/ml and a heterotrophic fungi load of 4.0×10^3 CFU/ml. Banana had the lowest *A. aceti* load of 1.0×10^3CFU/ml and 1.6×10^3CFU/ml load of heterotrophic fungi. Morphological characterization revealed non-pigmented, convex, shiny, rod-shaped colonies of *A. aceti*, consistent with previous literature. Biochemical tests indicated catalase positivity and glucose fermentation capability, while reactions for oxidase, indole, gelatinase, and nitrate reduction were negative. The microbial loads in all fruits investigated exceeded WHO's recommended threshold of 10^2 CFU/ml. Frequency data showed *A. aceti* was most frequently recovered from watermelon (20%) and least from pineapple (4%). However, the difference between fruit type and microbial presence was not statistically significant (χ^2=3.196, P>0.05). The high *A. aceti* load recovered, specifically, in watermelon, suggests a significant health risk particularly, due to the opportunistic nature of *A. aceti*. In addition, the high prevalence of *A. aceti* in watermelon over other fruits, indicated ripening may have been induced. *Acetobacter aceti* in watermelon is of health risk to consumers. The study therefore discourages ripening of fruits through inducement and calls for further investigation of *A. aceti* in other ripened fruits not investigated in this study.

Keywords: Acetobacter Aceti, Calcium Carbide, Heterotrophic Fungi, Microbial Load, GYC Medium, Food Safety

Introduction

The use of chemicals to ripen fruits for sale have continued to increase proportionately, and welcomed in the agroindustry (Fattah & Ali, 2010). Fattah and Ali (2010) noted that the act has been embraced following increased demand for fruits by consumers as diverse fruits flood the market. Following this senerio, fruit consumers are allegedly exposed to chemicals, however, safe levels are expected. Fruit ripening is the initiation of an unripe fruit to a ripe state for edibility (Saddigui & Dhua, 2010). Ripening is a biochemical, physiological and organoleptic changes that take place in fruit (Saddigui & Dhua, 2010). According to Maduwanthi and Marapana (2019) changes in texture, color, flavor, aroma and sugar contents are some of the observable features noted in ripe fruit. Although, fruits are alleged to have been induced with chemicals such as; ethylene gas, acetylene gas, calcium carbide, ethephon lauryl alcohol amongst many others (Zahoor et al., 2016). Zahoor et al. (2016) noted that most of these chemicals contain high proportions of inorganic compounds that might be harmful to the health of consumers, and exposure to these chemicals are dependent on the amount through ingestion (Gomes et al., 2018). Gomes et al. (2018) noted that low leveled exposure would not result to any adverse health defects. However, high leveled exposure definitely is bad for the body (Gomes et al., 2018; Proctor et al.,1988). Basically, acetic acid is naturally, present in some fruit and humans naturally, produce it for the metabolism of fats and carbohydrates (Gomes et al., 2018). Acetic acid which is converted to ethanol by the action of *Acetobacter aceti* is of immerse importance in the agroindustry (Pokhrel, 2014; Emaga et al., 2007). The bacterium is also used for production of vinegar, soup, salad dressings, preservatives, insecticides, and other useful products (Gomes et al., 2018). Besides, conversion of ethanol to acetic acid by the bacteria, *Acetobacter aceti*

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is noted to causes rot in apples, pears and pink disease in pineapple (Zahoor et al., 2016). Hence, implicated in fruit spoilage (Klaw & Bovonsombut, 2017). Fruits are desired for their taste and nutrient composition as they provide consumers with nutrients such as minerals and vitamins that boost overall well-being (Brookie et al., 2018). According to Brookie et al. (2018), fruits are of various varieties such as orange, cucumber, watermelon, pineapple etc. Each type brings its own benefit to consumers. As a result of the essential benefits of fruits on the human health, fruits are reported to have an increased market value (Brookie et al., 2018) hence, the use of chemicals to ripen fruits which in other words presents a challenge. Sequel to this there is need to evaluate *Acetobacter aceti* in the fruits put for sale in Ozuoba community following that *Acetobacter aceti* is an indicator organism for the presence or production of acetic acid that predisposes consumers to health issues. The study therefore evaluated *Acetobacter aceti* in some commonly sold ripened fruits sold in Ozuoba market of Port Harcourt, Rivers State.

Materials and Methods

Study Area

Ozuoba market, the study area for the study is notably a local market located within the Obio/Akpor Local Government Area of Rivers State, Nigeria. The area serves as a key commercial hub for the residents of Ozuoba, a semi-urban community that is part of the greater Port Harcourt metropolis. The market plays a vital role in the socio-economic activities of the area, providing a space for trade, livelihoods, and interaction among diverse groups. Ozuoba community lies along the East-West Road, serving as a gateway between central Port Harcourt and the neighboring communities of Choba, Rumuosi, and Rumuokoro. The market is strategically situated in a semi-urban environment, reflecting both rural and urban characteristics. This location makes it an important node for studying the interplay between rural supply chains and urban consumption. Ozuoba Market is a medium-sized, traditional open-air market that caters to daily needs such as foodstuffs, vegetables, livestock, clothing, and household items. It supports a wide range of informal economic activities and provides livelihoods for hundreds of traders, artisans, and transport operators. The market faces typical challenges of urban markets in Nigeria, such as poor waste management, overcrowding, inadequate sanitation facilities, and traffic congestion. These issues make it a relevant case for research in urban planning, public health, and environmental management. Ozuoba Market reflects the cultural diversity of Port Harcourt. Thus, the study area is densely populated with high fruit market turnover, making it suitable for the study.

Study Design and Sample Selection/Collection Procedure

The study adopted a cross-sectional, observational, and microbiological investigation which involved stratified random sample collection and selection of ripe banana, orange, watermelon and pineapple from various vendors across four market sections, North, South, East, and West sections. Twenty-five (25) fruits were purchased from each market section, making a total of one hundred (100) samples (25 per fruit x 5 fruits). Sterile gloves were aseptically used to collect the fruit samples in a sterile polythene bags and then transported in cool box to the laboratory within 2 hours for microbiological investigation. Samples were microbiologically isolated using The Glucose Yeast-Extract Calcium Carbonate (GYC) agar medium and identified using standard biochemical techniques. Basically, quantitative (CFU/ml) and qualitative data were recovered and analyzed accordingly.

Isolation and Enumeration of *Acetobacter aceti*

The collected fruits (banana, orange, watermelon and pineapple**)** were taken to the Biology Laboratory, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt for microbiological analyses. In the laboratory, the fruit samples were cut open with a sterile knife, crushed and put into several sterile bottles. The bottles and the crushed contents were then incubated for 7 days at 30ºC. Following the incubation, the content from the fruit samples were then scoped and introduced into test tubes containing freshly prepared sterile peptone water for enrichment of *A. aceti*. The medium now served as the stock as recommended by Kohinka et al. (2012). The GYC agar was adopted as a media for the isolation of *Acetobacter* sp. as employed by Raham et al. (2024). The composition of the broth: glucose (100g), calcium carbonate (20g), yeast extract (10g) and distilled water (1000ml) were compounded with 500 milligram of penicillin antibiotics to inhibit fungal growth. The media thereafter were sterilized.

Determination of *A. aceti* load in the various prepared fruit samples were carried out as reported by Gonzalez et al. (2006). Gonzalez et al. (2006) adopted the spread plate technique where-by a sterile 1ml pipette was used to transfer 0.1 ml of the inoculum of the stocked into the freshly prepared GYC medium. Thereafter, with the use of a sterile jockey stick, the inoculum was evenly spread on the media. The media was then incubated at 30˚C for 3-4 days under aerobic condition. Colonies that developed were noted, counted and reported as colony forming unit per ml.

Morphological and Biochemical Identification of *Acetobacter aceti*

Morphological features such as the colony appearance, size, and shape were presumptively used to identify *Acetobacter* spp. isolates on GYC agar media plates, while relevant biochemical tests as described by Raham et al. (2024) were used to confirm the presence of *A. aceti* isolates.

Gram's Staining

The colonies recovered from the GYC medium were heat-fixed on a glass slides, were-in a drop of normal saline was introduced onto the slide, followed by introduction of the test bacterium onto the slide using a sterile wire loop. The slide was then passed over a burning flame three times to heat-fix the bacterium onto the slide. Following these, crystal violet, iodine solution, alcohol and safranin were applied accordingly and, in each application, clean water was used to rinse the slide, air dried and viewed under a microscope.

Catalase Test

The test bacteria were examined for catalase activity were-in a sterile wire loop was used to transfer a small amount of the test bacterial culture onto a clean glass slide. Thereafter, few drops of hydrogen peroxide solution was added and observed immediately for the appearance of gas bubbles, which indicated a positive catalase bacterium, while the absence of bubbles signified a negative result.

Oxidase Test

The bacterial isolate was tested for oxidase activity, were-in the test bacteria ability to facilitate electron transfer between electron donors and acceptors was assessed. The procedure involved the use of Whatman No. 1 filter paper, which was soaked in a freshly prepared 1% solution of tetramethyl-p-phenylenediamine dihydrochloride reagent and the test bacterium sterilely impregnated on it. A color change on the paper indicated the presence of oxidase activity while an absence indicated no color change.

Indole Test

The indole test was conducted to determine the ability of the test bacteria to break down the amino acid tryptophan into indole. A freshly prepared indole broth was inoculated with the bacterial isolate and incubated at 37°C for 48 hours. After incubation, 0.5 mL of Kovac's reagent was added to the broth, and the medium was observed for the presence of a red layer, indicating a positive indole reaction while an absence indicated indole negative.

Glucose Fermentation Test

This test was performed to assess the ability of the test bacteria to ferment glucose. The glucose broth was prepared, sterilized, and inoculated with a loopful of the bacterial isolate. The inoculated broth was then incubated at 37°C for 18 to 24 hours. A color change in the broth from red to yellow indicated a positive result for glucose fermentation, while no color change indicated a negative result.

Gelatin Hydrolysis Test

The gelatinase test was conducted to determine whether the test organism could produce the enzyme gelatinase. A nutrient agar medium containing 1% gelatin was prepared. The medium was then inoculated with the test organism using a sterile wire loop by streaking across the surface. The inoculated plate was incubated at 37°C for 24 hours. After incubation, the plate was flooded with a mercuric chloride ($HgCl_2$) solution and observed after five minutes. The presence of clear zones around the streaked area indicated positive gelatin hydrolysis, whereas no clear zone indicated a negative result.

Nitrate Reduction Test

This test was carried out to evaluate the ability of the test bacteria to reduce nitrate. Nitrate broth was dispensed into a clean test tube and sterilized by autoclaving for 15 minutes. After cooling to room temperature, the broth was inoculated with the bacterial isolate and incubated at 35°C for 2 hours. Following incubation, two drops each of sulfanilic acid and N,N-dimethyl-1-naphthylamine were added to the broth and mixed thoroughly. The development of a red color within 2 minutes indicated a positive result for nitrate reduction, while the absence of color change indicated a negative result.

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Data Analysis

The data obtained were analyzed using descriptive and inferential statistical methods. The primary variables of interest are the **colony-forming unit per milliliter (CFU/ml)** of *Acetobacter aceti* and its **morphological, biochemical characteristics**, and also the **frequency of occurrence** across the fruits. **Percentages** were used to express the **frequency of microbial occurrence** in each fruit type, providing insight into the proportion of samples contaminated by *A. aceti*. The **mean bacterial loads** (in CFU/ml) were calculated to summarize the central tendency of contamination levels. A **t-test** was applied to compare the loads of *A. aceti* across the fruit types. A **Chi-square (χ^2) test of independence** was performed to examine the relationship between fruit type and the **frequency of occurrence**. All statistical analyses were conducted at a **95% confidence level**, and results were interpreted with a significance threshold of $\mathbf{p < 0.05}$.

Results

Bacterial Load Analysis of Fruit Samples: *Acetobacter aceti*

Table 1 presents the mean bacterial load of *A. aceti* isolated from four types of fruit samples (banana, orange, watermelon and pineapple). The *A. aceti* count ranged from 5.0×10^2 CFU/ml in pineapple to 5.9×10^3 CFU/ml in watermelon. Orange and banana recorded intermediate counts of 3.5×10^3 and 1.0×10^3 CFU/ml, respectively. Notably, all fruit samples exceeded the World Health Organization (WHO) standard limit of 1.0×10^2 CFU/ml for safe microbial presence in fresh produce, suggesting significant microbial contamination. However, the t-test result ($p > 0.05$) indicates that the variation in *A. aceti* load among the fruit types is not statistically significant. The results suggest that all tested fruits harbored *Acetobacter aceti* loads that are significantly above international safety standards, raising potential concerns regarding food hygiene, handling, and post-harvest storage practices. Although no statistically significant differences were observed among fruit types, the high overall counts indicate a systemic issue rather than fruit-specific contamination.

Table 1: Mean *Acetobacter aceti* Load in the Various Fruit Samples

Microbes	Banana (n=25) (CFU/ml)	Orange (n=25) (CFU/ml)	Water Melon (n=25) (CFU/ml)	Pineapple (n =25) (CFU/ml)	T-test	WHO Standard
Acetobacter aceti	1×10^3	3.5×10^3	5.9×10^3	5×10^2	p>0.05	10^2

Morphological Characteristics of *Acetobacter aceti*

Table 2 outlines the morphological traits observed in the *A. aceti* isolates recovered from various fruit samples. These characteristics are important for preliminary identification and differentiation of bacterial species in microbiological studies. The colonies of *A. aceti* were described as non-pigmented, a typical feature of this species, reflecting their translucent to whitish appearance on nutrient media. The absence of pigmentation distinguishes *A. aceti* from pigmented bacterial species and can be used as a key diagnostic trait. The isolates exhibited large, circular colonies, and microscopically, the cells were rod-shaped. This is consistent with the known morphology of *Acetobacter* species, which are Gram-negative, aerobic rods. The large colony size may indicate a relatively expansive spread on the culture medium despite a slow growth rate. *A. aceti* demonstrated a very slow growth rate, which is characteristic of many acetic ethanol oxidations and its sensitivity to environmental conditions such as pH and oxygen availability. The colonies were described as convex and shiny. This smooth and glistening texture is typical for *A. aceti*, often linked to the production of surface polysaccharides or biofilms, which aid in environmental survival and adherence to substrates. The observed morphological features align well with documented descriptions of *A. aceti*, supporting accurate identification. These traits also highlight the organism's adaptation to acidic and sugar-rich environments, such as fruit surfaces. The combination of non-pigmentation, slow growth, and shiny, convex colonies further reinforces its role in the spoilage of sugar-rich foods under aerobic conditions.

Table 2: Morphological Characteristics of *Acetobacter aceti*

Isolates	Colour	Size/Shape	Growth Rate	Texture
Acetobacter aceti	Non pigmented	Large/Circular and rod shaped	Very Slow	Convexed /shinny

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Biochemical Characterization of *Acetobacter aceti*

Table 3 presents the results of standard biochemical tests conducted to characterize *Acetobacter aceti* isolates. These tests are essential for confirming *Acetobacter* identity based on metabolic and enzymatic activities. The isolate tested positive for catalase activity, indicating its ability to decompose hydrogen peroxide into water and oxygen. This is a common trait among aerobic bacteria, including *A. aceti*, and supports its classification as a strict aerobe. A negative result was observed for the oxidase test, suggesting the absence of cytochrome c oxidase enzyme. This is consistent with the metabolic profile of *Acetobacter* species, which utilize alternative electron transport pathways. The isolate was indole-negative, indicating it does not produce the enzyme tryptophanase necessary for breaking down tryptophan into indole. A positive glucose test indicated the bacteria ability to utilize glucose, likely through oxidative fermentation pathways. *A. aceti* is known for oxidizing sugars and alcohols to produce acetic acid, and this supports its functional classification as an acetic acid bacterium. The isolate was negative for gelatinase activity, meaning it lacks the ability to hydrolyze gelatin. This result helps differentiate it from proteolytic bacteria capable of degrading protein substrates. A negative nitrate test suggests the organism does not reduce nitrate to nitrite or other nitrogenous compounds. This is in line with the metabolic behaviour of many *Acetobacter* species, which do not typically engage in anaerobic respiration. The isolate was Gram-negative, consistent with the known cell wall structure of *A. aceti*. This further confirms its classification within the Proteobacteria phylum. The biochemical profile observed; catalase-positive, oxidase-negative, glucose-fermenting, and indole-, gelatinase-, and nitrate-negative, corresponds well with standard descriptions of *A. aceti*. These results, combined with morphological features, provide strong evidence for accurate identification and can support further ecological or applied studies involving this organism.

Table 3: Biochemical Characterization of the *Acetobacter aceti* Isolates

Catalase	Oxidase	Indole	Glucose	Gelatinase	Nitrate	Gram Reaction	Bacteria
+	-	-	+	-	-	-	*Acetobacter aceti*

Frequency of Occurrence of Microbial Isolates from Fruit Samples

Table 4 summarizes the frequency of occurrence of *A. aceti* and heterotrophic fungi isolated from four different fruit types: banana, orange, watermelon, and pineapple, each with 25 samples (n=25). *A. aceti* was most frequently isolated from watermelon samples, with a recovery rate in 5 out of 25 samples (20%). Orange followed with 3 isolates (12%), while banana and pineapple had lower frequencies, 2 (8%) and 1 (4%) respectively. However, the differences in association between frequency of isolates and each fruit type was not statistically significant (χ^2=3.196; P>0.05).

Table 4: Frequency of Occurrence of the *A. aceti* Recovered from the Fruit Samples

Microbes	Banana n(f%)	Orange n(f%)	Watermelon n(f%)	Pineapple n(f%)
Acetobacter aceti	2(8%)	3(12%)	5(20%)	1(4%)

Key: n = number of isolates, f= frequency of occurrence

Discussion

Heavy loads of *Acetobacter aceti* in watermelon as reported in this study indicates the fruit contains acetic acid, although the loads are below the reports of Rahman et al. (2024). Rahman et al. (2024) pointed out that pineapple fruit, is the most ideal for acetic acid bacteria proliferation. Although, Oladipupo et al. (2022) noted that Calcium Carbide showed promising results when induced in pineapple plant. The permissible limit of acid producing bacteria in all fruit samples under-investigation have shown not to have exceed 100 colony forming unit per ml (Oladipupo et al., 2022). Hence, the study adhered to the permissible limit. The counts of *Acetobacter aceti* revealed, a likely public health risk, more to consumers of the watermelon, following reports by Gouby et al. (2007). Gouby et al. (2007) noted, *Acetobacter aceti* presence should alert clinicians to the risk of opportunistic infections. However, their presence according to Hata et al. (2022) is useful for food production as it provides several health benefits where it has the ability to oxidize sugar to organic acid. The least *Acetobacter aceti* counts in banana as noted in this study may have resulted from the protective barrier offered the banana against microbial invasion even after allegedly use of chemicals (Mostafa, 2021). Mostafa (2021) reported that banana possess a barrier against bacteria contamination following its slight acidic content property. Banana plant has a source of valuable antimicrobial compounds and hence is applied in

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most food sector to ward of microbes (Oladipo et al., 2020). The morphological identification and recovery of *Acetobacter aceti* quite agrees with study carried out by Zahoor et al. (2016) where *Acetobacter aceti* was purified and identified: off-white in color, circular in shape and small in size. The noted high prevalence of *Acetobacter aceti* in watermelon may be associated with some unique features possessed by the watermelon. These features include their acidic nature that encourage their growth and its soil inhabitant nature of the watermelon. According to Annon (2008) watermelons have some characteristics that make them more susceptible to *Acetobacter aceti*. This is as a result of the fruit's high-water content, which is ideal for growth and the rough/textured skin surface for the bacterium colonization (Annon, 2008). Watermelon has high water content and also rich in sugar, all of which provides the proliferation of *Acetobacter aceti* when compared to other fruits under this investigation. Consequently, banana has low water content and may not have the surface area to accommodate *Acetobacter aceti* proliferation (Chukwuegbo et al., 2023). As alleged, the use of chemical to ripen the fruit cannot be over emphasized as the result so obtained is a pointer to *Acetobacter aceti* proliferation. Higher level exposure and consumption of acetic acid can result to potential damage of the gastrointestinal tract. However, Safe level can reduce blood sugar levels and support weight loss (Gomes et al., 2018).

Conclusion

The study identified significant loads of *Acetobacter aceti* proliferation in watermelon more compared to orange, banana and pineapple fruits. Hence, the alleged use of chemical (Calcium Carbide) cannot be over emphasized giving that *Acetobacter aceti* proliferate in such circumstance. Basically, the presence of *Acetobacter aceti* in watermelon should alert clinicians on the risk of opportunistic infection on consumers health.

Recommendation

Because of the potential of acetic acid generation from the watermelon fruits, the Ozuoba community and her visitors are hereby put on notice and advised to be mindful of watermelon consumption.

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