



Microbial Quality of Sliced and Packaged Fruits in Polyethylene Sold in Port Harcourt Rivers State, South-South Nigeria

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Abstract

Fresh fruits promote good health but harbor a wide range of microbial contaminants. This study assessed the microbial quality of fruits and safety of Polyethylene packed sliced fruits sold in Port-Harcourt markets. 36 samples of freshly cut fruit comprising of Pawpaw (*Carica papaya*), Pineapple (*Ananas comosus*) and Water Melon (*Citrullus lanatus*) were randomly collected from different sellers within Port Harcourt metropolis. Samples were analyzed using standard microbiological methods. Mean Total viable count ranged from $\text{Log}_{10}\text{cfu/g}$ 5.85 – 6.34, mean Total Fungi Count $\text{Log}_{10}\text{cfu/g}$ 3.23– 3.85. Nine bacteria were identified. *Staphylococcus aureus* (27.4%) was the most frequently isolated followed by *Shigella spp* (19.4%), *Escherichia coli* (16.1%), *Bacillus spp* (14.5%), *Aeromonas Sp* (8%), *Enterobacter sp* (4%), *Klebsiella spp* and *Salmonella spp* (4.8%) respectively. Samples of Pawpaw had high microbial contamination when compared to other fruit samples. The study comes to the conclusion that consumers' awareness of the risks of eating packed sliced fruits should be increased, and that they should once again insist on properly processed/packaged sliced fruits.

Keywords: Fruits, Microbial contamination, Pathogens, Polyethylene packed sliced fruits, Port Harcourt Metropolis

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I. INTRODUCTION

Fresh-cut raw fruits are defined as uncooked food that has been diced, shredded, peeled, abraded, or otherwise processed into manageable amounts that are prepared for consumption. Fruits are a vital component of a healthy diet because they are a fantastic source of vitamins, fiber, minerals, and other elements for humans Jolaoso *et al.*, (2010). A well-balanced diet full of fruits and vegetables is crucial for preventing vitamin C and vitamin A deficiency in addition to lowering the risk of a number of ailments (Kalia and Gupta, 2006). Fruits are frequently susceptible to microbial contamination through handling after harvest or during postharvest processing, contact with soils, dust, and water, among other things, as a result, they are habitat to a wide variety of microorganisms, including human and plant infections (Nguyen and Carlin, 1994; Dunn *et al.*, 1995; Carmo *et al.*, 2004). The addition of nonresident microflora via the use of animal manures, sewage, or irrigation water, as well as the handling and transportation techniques employed by retailers, are the main causes of alterations in the microbial profiles of various fruits (Ray and Bhunia, 2007; Ofor *et al.*, 2009). Pollution is significantly increased by the continuous

use of untreated waste water and manure as fertilizers for the development of fruits and vegetables in developing countries like Nigeria (Olayemi, 1997; Amoah *et al.*, 2009). Although eating freshly cut fruit provides health and nutritional benefits, current research has connected consuming fresh or minimally processed fruits and vegetables to an increase in human infection outbreaks (Hedberg *et al.*, 1994; Altekruse *et al.*, 1997; Beuchat, 2000); Odu and Adeniji, 2013). Enteric bacteria like *Escherichia coli* and *Salmonella* are among the most worrisome pathogen when food-related outbreaks happen (Buck *et al.*, 2003; Odu and Adeniji, 2013). Eating contaminated vegetables have been linked to typhoid fever outbreaks. These vegetables that were grown in contaminated soil or sewage or treated with it (Beuchat, 1995). Since most people spend a lot of time outside, it's probable that consumption of tainted fruits and vegetables has increased outside the home. For instance, street sellers are currently quite popular and commerce is thriving in Nigeria. They market easy, prepared-to-eat fruit and vegetable slices. Fruits continue to be a fantastic source of nutrients that are vital to human health and reduce the risk of serious diseases like cancer and heart disease. Fruits are consumed often in marketplaces and on the streets since they are good for human

health. Some of these fruits come in packaging that don't require washing or cutting the fruit before consumption. Additionally, the majority of these vendors practice poor personal cleanliness, which exposes the processed fruits to contaminated air, filthy equipment, and unsanitary surroundings.

Fruit slices are now more frequently consumed in Nigeria than in previous years. This is due to the fact that entire fruits and vegetables are often out of reach for customers, making sliced fruits more accessible, handy, and, most importantly, more affordable. The fruits are peeled, diced, and packaged in clear polythene bags before being sold to the public. These fruits are primarily sold by unregistered street vendors or hawkers who have no formal education, are untrained in food hygiene, and operate in squalid, unhygienic conditions. Because many consumers of these products consume them in order to fulfill their nutrient needs for a healthy lifestyle, the country's economic situation has also contributed to the strong demand for these fruits (Nwachukwu *et al.*, 2008). However, it's possible that these fruits' processing exposed them to harmful microbes, making them a potential source of food-borne illness. These sliced fruits may become polluted from sources such as irrigation or rinsing with contaminated water or coming into contact with sewage. Other sources include inappropriate handling, the wrapping materials, cross contamination with knives, trays, and staff. The convenience of these fruits is more important to the consumers who rely on them than their safety, quality, or hygiene. Packaging of fruits in polyethylene bags hinders microbial contamination during retailing after fruits have been washed appropriately. Sealed polyethylene bags however modify the atmosphere around fruits, it reduces oxygen and increases carbon dioxide as well as relative humidity levels (Mahajan *et al.*, 2014). The purpose of this study was to evaluate the microbiological quality of sliced fruits marketed in the vicinity of the University of Port Harcourt and the risk factors related to their intake.

II. MATERIALS AND METHODS

A. Sample Collection

A total of 36 samples of freshly cut fruit comprising of Pawpaw, Pineapple and Water Melon was randomly obtained from different sellers within Port Harcourt. Rivers State.

B. Preparation of Samples for Microbial Analysis

25g of each sample was put in a sterile stomacher bag and homogenized using a stomacher with 225ml of 0.1% peptone water of 2min. 10 fold Serial dilution were done;

- (a) Total viable counts - 0.1 ml of 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} in duplicate were dispersed on already prepared plate count Agar.
- (b) Enumeration of Yeast/mold - 0.1 ml of 10^{-4} , 10^{-5} , were dispersed onto already prepared Potato dextrose Agar incubated at 25°C for 3-7 days.
- (c) Staphylococcus Counts - 0.1 ml of 10^{-3} , 10^{-4} , and 10^{-5} were dispersed onto mannitol salt agar.

(d) Total Coliform Counts - 0.1 ml of 10^{-3} , 10^{-4} , and 10^{-5} were dispersed onto MacConkey Agar.

(e) Total Salmonella Counts - 0.1 ml of 10^{-3} , 10^{-4} , and 10^{-5} were dispersed onto Salmonella Shigella Agar and incubated at $25-37^{\circ}\text{C}$ for 24-48hrs.

Counts obtained, cfu/g calculated, representing colonies was picked, sub cultured, store in slants, perform series of biochemical test for identification according Cheesbrough, (2005).

C. Identification of Isolates

On several solid media agar plates, the bacterial organisms its colonial morphological traits were seen. According to Barrow *et al.*, (1993) and Cheesbrough, (2005) further identification and characterization of the suspect species was done using their microscopic, physiological, and biochemical characteristics. The present or absent of Salmonella species was evaluated based on APHA (1992).

D. Antibioqram Susceptibility Testing

The antimicrobial susceptibility tests of all isolates were performed using Kirby Bauer disc diffusion method using Ofloxacin (OFX) ($5\mu\text{g}$), Pefloxacin (PEF) ($5\mu\text{g}$), Ciprofloxacin (CPX) ($5\mu\text{g}$), Augmentin (AU) ($30\mu\text{g}$), Gentamicin (GEN) ($10\mu\text{g}$), Streptomycin (S) ($10\mu\text{g}$), Cefuroxime (CEF) ($30\mu\text{g}$), Nalidixic acid (NA) ($30\mu\text{g}$), Trimethoprim/sulfamethoxazole (SXT) ($25\mu\text{g}$), Ampicillin (AM) ($10\mu\text{g}$). Bacteria suspension was prepared using normal saline. A sterile swab stick was dipped into the suspension and excess fluid was removed by rotating the swab stick against the upper wall of the tube. The whole surface of Mueller Hinton Agar was streaked using the swab stick. Sterile forceps was used to place the antibiotic disc on the plate.

E. Statistical Analysis

Microbiological data of several fruit samples were obtained and entered into a Microsoft Excel spreadsheet before being evaluated with a one-way ANOVA using statistical software from the Statistical Package for Social Sciences (SPSS) version 16. Statistical significance was defined as a 95% confidence interval with a P value 0.05 or lower.

III. RESULTS

The mean total viable count of the fruit samples ranged from $\text{Log}_{10}\text{cfu/g}$, 5.85 to 6.34. The mean total fungi count of the fruit samples ranged from $\text{Log}_{10}\text{cfu/g}$, 3.23 to 3.85, the mean total Staphylococcus count of the fruit samples ranged from $\text{Log}_{10}\text{cfu/g}$, 3.34 to 5.13, the mean coliform count of the fruit samples ranged from $\text{Log}_{10}\text{cfu/g}$ 2.13 to 3.44. and the mean Salmonella-shigella count of the fruit samples ranged from $\text{Log}_{10}\text{cfu/g}$ 2.13 to 2.34 as shown in Fig1-5.

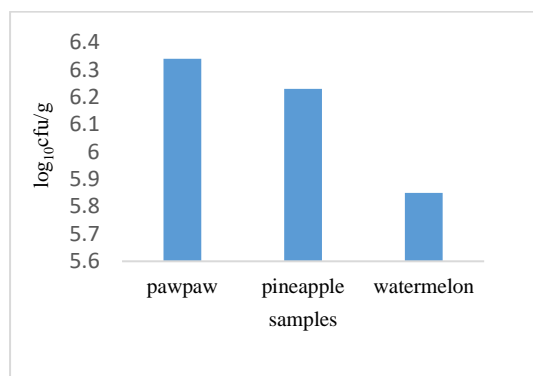


Figure 1. Mean total viable count of the different fruit samples.

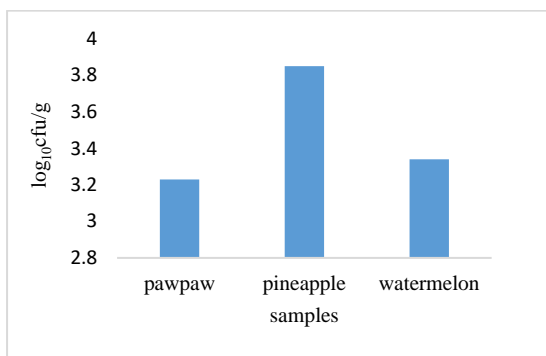


Figure 2. Mean Total Fungal count for fruit samples.

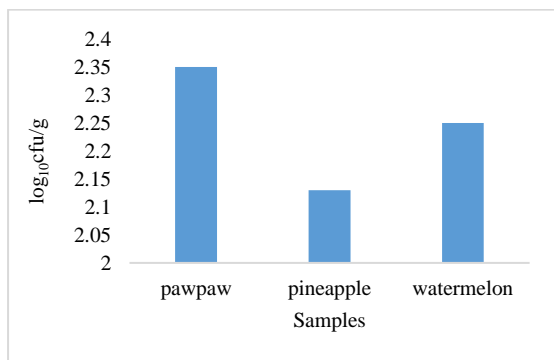


Figure 3. Mean Total Salmonella-Shigella count for fruit samples.

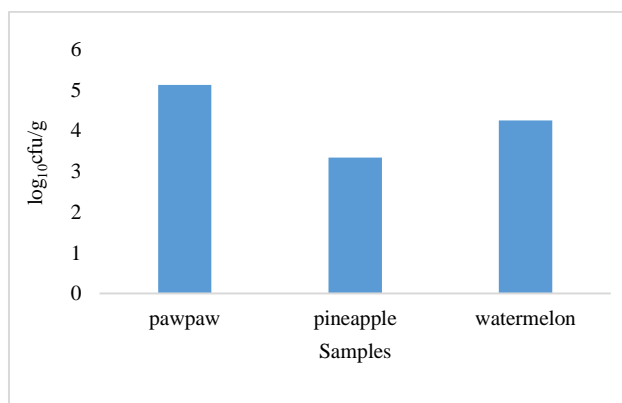


Figure 4. Mean Total Staphylococcal count for fruit samples.

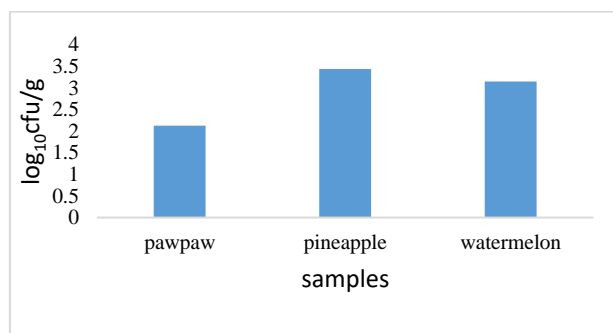


Figure 5. Mean Total Coliform count for fruit samples.

A total of 62 bacterial isolates were isolated from the 36 samples comprising of 9 bacteria species *Staphylococcus* sp (27.4%), *Aeromonas* sp (8.0%), *Shigella* sp (19.4%), *Salmonella* sp (4.8%), *Escherichia coli* (16.1%), *Bacillus* sp (14.5%), *Klebsiella* sp (4.8%) and *Serratia* sp (1.6%), *Enterobacter* sp (3.2%), as presented in tables 1. A total of 22 fungal isolates were isolated from the 36 fruit samples comprising of 4 fungal species. Fungal species identified were *Aspergillus* sp (27.3%), *Fusarium* sp (27.3%), *Penicillium* sp (18.2%) and *Saccharomyces* sp (27.3%).

Table 1. Distribution of bacterial organisms isolated from fruits samples investigated.

Bacterial organisms	Pawpaw (%)	Pineapple (%)	Watermelon (%)	Total (%)
<i>Escherichia coli</i>	6	2	2	10 (16.1)
<i>Staphylococcus</i> sp	10	3	4	17 (27.4)
<i>Salmonella</i> sp	-	-	3	3 (4.8)
<i>Shigella</i> sp	7	1	4	12 (19.4)
<i>Enterobacter</i> sp	2	-	-	2 (3.2)
<i>Klebsiella</i> sp	2	-	1	3 (4.8)
<i>Aeromonas</i> sp	1	2	2	5 (8.0)
<i>Serratia</i> sp	1	-	-	1 (1.6)
<i>Bacillus</i> sp	4	2	3	9 (14.5)
Total	33(53.3)	10(16.1)	19(30.6)	62 (100)

The antibiotic test revealed 100% was susceptible to Ciprofloxacin and Gentamicin. 90.9% of the isolates were seen to be susceptible to Ofloxacin, Pefloxacin, streptomycin, septrin, and 82% was seen to be susceptible to Augmentin and ampicillin, 72.7% was seen to be susceptible to Nalidixic acid and Cefuroxime.

Table 2. Percentage (%) of Sensitivity/ Resistance of Isolates to Antibiotics.

Isolates	OFX	PEF	CPX	AU	GN	S	CEF	NA	SXT	AM
Sensitive	90.9	90	100	82	100	90	72.7	72.7	90	82
Resistant	8.1	10	-	18	-	10	27.3	27.3	10	18

IV. DISCUSSION

Fresh fruit consumption has increased in Nigeria as people try to take advantage of these products, which are thought of as seasonal. Additionally, as a result of consumer demand, there are now more fresh cut fruits in the marketplaces. This is due to the fact that ready-to-eat fruits are more readily available, practical, and affordable than entire fruits. However, it is

challenging to verify the cleanliness of the fruit sellers or processors. According to the study, the majority of the sliced watermelon, pawpaw, and pineapple fruits packaged in polyethylene were deemed to have "unsatisfactory" bacterial quality as Total Bacteria Count values and total coliform counts of the samples were higher than the recommended standard for food ($< 2 \log_{10}$ cfu of coliform per g of food sample) and ($< 5 \log_{10}$ cfu of total bacterial counts per g of food sample) this in accordance with the findings of Thunberg *et al.*, (2002) and Afolabi *et al.*, (2015).

The result of the Total bacterial count obtained are significantly high ($p < 0.05$). This could result from improper handling, from washing all the fruits in the same pail of water (Khali and Mazhar, 1994), or from cross-contamination from cutting and displaying the fruits with the same utensils (Ugwu and Edeh, 2019). These findings agree with those of Nwachukwu *et al.*, (2008), Farzana *et al.*, (2011), Oranusi and Olorunfemi (2011), Edeghor *et al.*, (2019). This finding indicates that unwashed fruits, dirty utensils, and unhygienic street vendors are the likely sources of the microbial contamination of street vending fruits (Tambekar *et al.*, 2009).

This study's findings also showed that pineapple was linked to fewer bacteria than previously thought. The pineapple's acidic nature could be responsible for this (Eni *et al.*, 2010; Nwachukwu and Chukwu, 2013); Oranusi and Olorunfemi, 2011). The mean coliform count of pineapple had significantly high count ($p < 0.05$) followed by watermelon and Pawpaw. One of the primary sources of fecal coliform contamination in washing and processing water is the presence of coliforms in street vended fruits (Durgesh *et al.*, 2008).

The mean Salmonella-shigella count of Pawpaw had significantly high count ($p < 0.05$) followed by watermelon and pineapple. *Salmonella* was found in fresh cut fruit as a result of contaminated utensils and knives (Barro *et al.*, 2006). Additionally, flies have been known to attack knives used for slicing and dicing fruits (Mensah *et al.*, 2002). Freshly cut fruits may become contaminated with *Salmonella spp.* by contact with polluted water (Beuchat, 1995; Gayler *et al.*, 1995). This finding conforms with the study of Eni *et al.* (2010). The mean total Staphylococcus count of the fruit samples in with pawpaw had significantly high count ($p < 0.05$) followed by pineapple and watermelon. The main hosts of *S. aureus* are people and animals. 50 percent or more of healthy people have staphylococci in their nasal passages, throats, hair, and skin (Lund *et al.*, 2000). There are often few instances of *S. aureus* on healthy, undamaged, and disease-free skin since the bacterium is ephemeral and seldom integrates into the skin's natural flora. However, on persons who repeatedly dip their hands in water, The bacterium might establish itself as a part of the local flora and grow to large populations, causing discomfort and skin aging. It has been documented that workers in food processing industries can develop this ailment on their hands. *S. aureus* may be on the sliced fruits if the fruits are handled and cut with bare hands. Unacceptable levels of *S. aureus* suggest that poor food

handling with bare hands may have been followed by misuse of time and temperature.

Pineapple has the highest mean total fungi count followed by watermelon and Pawpaw. The existence of these fungi suggests that they might enter fruits through processes including picking, storing, packaging, and conveying them, which could result in physical damage and increase post-harvest losses as well as the risk of fungal contamination (Isa *et al.*, 2014). Inadequate vendor hygiene can render contaminated vending surroundings worse. In most cases, the processing is done with rudimentary equipment which encourages chance inoculation from the environment. The consequences of the problems could be increased by the spread of foodborne diseases associated with fruits. Bacterial and fungal diversity recorded in this study corroborated with other several previous reports by Amoah *et al.*, (2009); Isa., *et al.* (2014); Mathur *et al.*, (2014); Tango., *et al.* (2018); Mahfuza *et al.*, (2016); Eni, *et al.* (2010); Nwachukwu *et al.*, (2018); Tournas, (2005) had similar bacteria and fungi species on apple, mango, pawpaw, cucumber, carrot, onion, tomato, pineapple, lettuce etc.

The isolation of these organisms is supported by the work of Eni *et al.* (2010) and Jolaoso *et al.* (2010), who detected *S. aureus*, *Klebsiella sp.*, *Salmonella*, and *Escherichia coli* from fruits. Additionally recovered by Daniyan and Ajibo (2011) from sliced fruits sold in Minna city were *S. aureus*, *S. epidermidis*, *Bacillus sp.*, *E. coli*, and *Enterobacter aerogenes*. The work of Oranusi and Olorunfemi (2011), who isolated *Bacillus*, *S. aureus*, *E. coli*, *Enterobacter*, *Salmonella*, *Klebsiella*, *P. aeruginosa*, *Proteus*, *Micrococcus*, and *Lactobacillus sp.* from marketed ready-to-eat fruits sold in Ota, Ogun State, lends more credence to Tambekar *et al.* (2009) isolated similar organisms from fruit juices sold on the side of the road in Amravati, India. Nwachukwu *et al.*, (2008) also isolated similar organisms from sliced water melon.

S. aureus (27.4%) and *Escherichia coli* (16.1%) are the bacteria commonly isolated from the fruits samples. Nwachukwu *et al.* (2008) and Jolaoso *et al.* (2010) found low levels of *Escherichia coli* in watermelon slices. Majority of the bacteria found in these cut fruits may have been introduced via the environment, the soil, or during handling or shipping. The majority of the organisms found in this study's isolates may have entered the fruits through wrapping materials, low-temperature exposure, and feces-polluted water used to wash tools (such as knives, trays, and pans). *S. aureus* may be present due to the fact that people who manufacture or sell things have this organism on/in several parts of their bodies. Nester *et al.*, (2001); Garg *et al.* (2009) and Farmer (2015) reported that chopping vegetables in different ways has been proven to cause microbial populations on/in various sections of their bodies to rise by six to seven times. Since certain coliforms, particularly *E. coli*, are frequently found in high concentrations in feces from both humans and animals, the presence of these coliforms may potentially be related to fecal contamination. Therefore, microbial pathogens cannot be completely eliminated by simply washing fruit surfaces. The primary sources of

microbiological contamination linked to the contamination of fresh fruit appear to be inadequate washing of the component of the fruits and utensils, use of dirty water, and bad personal hygiene and unsanitary production methods. The findings of this study demonstrate that consumers of cut fruit sold on the street face serious health concerns since such fruit is infested with dangerous microorganisms that might cause fatal infections. Since fruit is typically consumed unprocessed, vendors should follow correct processing procedures, stringent aseptic procedures and appropriate personal hygiene to limit microbial load and eradicate microbial contamination of the finished product. Gentamicin and Ciprofloxacin was found to be the most sensitive drug after the antibiotic's sensitivity test. The isolates low resistance to the other antibiotics. These findings tally with that of Chukwu *et al.*, (2010) who revealed in their study on microbiological quality of pre-cut fruits that Gentamicin was found to be the most sensitive antibiotic.

Oxygen and carbon dioxide concentrations in modified atmosphere is created by polyethylene bags which depend on its respiration rate, temperature of the environment, surface area, perforations and thickness of the polyethylene, permeability of gases to the film used in packaging and composition of air outside the polyethylene bag. Mahajan *et al.*, (2014). On the other hand, the water vapour permeability of the polyethylene and the respiration and transpiration activities both have an impact on the humidity of the microenvironment of fruits packed in polyethylene (Mahajan *et al.*, 2014). Packaging in polyethylene hinders contamination of fruits during retailing after washing, packaging of these fruits in polyethylene bags immediately after washing will extensively reduce microbial contamination on these ready- to- eat fruits (Akomolafe and Awe, 2017).

V. CONCLUSION

According to this study, all of the sliced fruits collected from various vendors in Port Harcourt had a high microbial load. Even though these microbes can be found in the fruits' epiphytic flora, their survival and growth are indicators of poor hygiene. The findings of this study demonstrate that fruits sold on the street constitute a severe health risk to customers since they are contaminated with numerous hazardous germs that can result in life-threatening illnesses. Since fruit is typically consumed unprocessed, to reduce microbial load and prevent microbial contamination of the finished product, vendors are urged to follow proper processing methods, strict aseptic techniques, and good personal hygiene. Additionally, clean water should be used to wash and clean the fruits and any utensils used before packaging.

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