Evaluation of Fungal Contamination in Whole and Sliced Ready-to-Eat Fruits Sold in Port Harcourt, Nigeria

Abstract

Foodborne illnesses remain a pressing public health concern globally, especially in regions with inadequate regulatory oversight. This study aimed to assess the extent of fungal contamination in whole and sliced ready-to-eat (RTE) fruits sold in various markets across Port Harcourt, Nigeria. The methodology involved a simple random sampling of 30 fruit samples, both whole and sliced apples, cucumbers, oranges, pawpaws, and watermelons. These samples were collected from six major markets: Fruit Garden Market, Mile 1 Market, Mile 3 Market, Oil Mill Market, Rumuokoro Market, and Town Market. Microbiological analyses were conducted using serial dilution, culturing, and isolation methods, with Total Fungal Count (TFC) determined on Sabouraud Dextrose Agar and statistical evaluation via ANOVA and Tukey's post hoc tests. Results showed significant variability in contamination levels across different markets. The highest TFC in whole fruits was recorded in the Oil Mill Market (4.58×10^2 CFU/ml), while Rumuokoro and Mile 1 had no detected fungal contamination. Among sliced fruits, the highest TFC was in the Oil Mill Market (6.60×10^2 CFU/ml), with Rumuokoro showing the lowest (1.53×10^2 CFU/ml). Pawpaw and watermelon had the highest TFC among all tested fruit types, indicating greater susceptibility to fungal growth due to their nutrient composition and water content. The study concluded that RTE fruits sold in Port Harcourt are susceptible to significant fungal contamination, posing health risks to consumers. It highlighted the need for better hygiene practices among vendors and stricter regulatory measures to ensure food safety. Recommendations include proper pre- and post-harvest handling practices, the use of potable water for washing fruits, and food safety training for vendors to mitigate contamination risks.

Keywords: Fungal contamination, ready-to-eat fruits, Port Harcourt, food safety, microbial analysis, fruit markets**.**

INTRODUCTION

Food is vital for satisfying hunger and meeting the biological requirements of living organisms. It also plays an integral part in Maslow's hierarchy of needs (Takgbajouah and Buscemi, 2022). Moon highlighted that food consumption represents the fulfilment of these biological necessities (Feliciano et al., 2022). Ensuring food safety means making certain that food does not pose any harm to consumers when it is correctly prepared and consumed (Walaszczyk and Galińska, 2020). This includes preserving the physical, chemical, and biological integrity of food to maintain its nutritional value (Walaszczyk and Galińska, 2020). Monitoring food safety requires constant attention from the point of purchase through to consumption (Abdulah, 2016), and one of the major objectives of Healthy People 2020 is to reduce foodborne infections caused by key pathogens (Stenger et al., 2014). Food handlers are essential to maintaining food safety, as poor handling practices can lead to foodborne illnesses (Adane et al., 2018).

According to the WHO, 600 million people worldwide become ill each year due to contaminated food, resulting in 420,000 deaths. In the U.S. alone, foodborne diseases affect 48 million individuals annually, leading to 128,000 hospitalizations and 3,000 deaths (WHO, 2015). The highest rates of foodborne illness and fatalities occur in Africa and Southeast Asia (Adane et al., 2018). This ongoing issue is largely driven by unhygienic food handling and inadequate preservation techniques, which result in contamination (WHO, 2016). Moreover, food handlers can contribute to the spread of foodborne diseases through cross-contamination (Akabanda et al., 2017).

Implementing proper food safety practices, particularly in terms of hygiene, is critical for reducing the frequency, severity, and fatalities associated with foodborne illnesses (Vlasin-Marty et al., 2016). Outbreaks of foodborne pandemics are often due to a lack of knowledge or neglect of hygiene and safety regulations (Osaili et al., 2018). Marginalized groups, especially those working in food retail or service industries, face a greater risk of exposure to foodborne pathogens (Quinlan and Jennifer, 2013), with diarrheal diseases being the most prevalent consequence of food contamination (Khairuzzaman et al., 2014).

Ready-to-eat fruits (RTEFs) like oranges, apples, and bananas have gained popularity for their nutritional benefits and convenience. However, they are susceptible to microbial contamination during harvesting, postharvest processes, and handling (Lima et al., 2019). Reports indicate a rise in foodborne illness linked to fresh fruits (Pradhan et al., 2019), with bacteria such as Listeria monocytogenes identified as potential sources of serious infections, particularly in vulnerable groups (Bierne et al., 2018).

Due to the nature of fruits, they are especially prone to microbial contamination, and improper handling only increases the risk of spoilage (Mailafia et al., 2013). Bacterial pathogens like Escherichia coli and Salmonella are frequently linked to contaminated fruits (Wiley et al., 2013). Furthermore, contamination may be exacerbated by the use of untreated wastewater and organic manure in fruit cultivation in some areas (WHO, 2015). The growing consumption of contaminated RTEFs in Nigeria has led to outbreaks of foodborne diseases, underscoring the need for enhanced assessment and stricter food safety protocols (Pradhan et al., 2019). Consequently, this study focused on assessing the microbial contamination and identifying bacterial strains isolated from whole and sliced ready-to-eat fruits in Port Harcourt, Nigeria.

METHODOLOGY

Study Area

The study area of this research was in Port Harcourt, the capital and largest city in Rivers State, Nigeria.

Study Design

The study employed a simple random sampling technique. A total of 30 fruit samples, including both whole (3) and sliced (3) samples of five different types of fruits including apple, cucumber, orange, pawpaw, and watermelon were collected. These samples were purchased from six different markets: Fruit Garden Market (FGM), Mile 1 Market (M1M), Mile 3 Market (M3M), Oil Mill Market (OMM), Rumuokoro Market (RM), and Town Market (TM).

Sample Collection

All samples of whole and sliced fresh fruits were collected from six different markets in Port Harcourt using sterile polythene bags and transported in an insulated ice box to maintain temperatures between 4°C and 6°C. The samples were immediately taken to the Microbiology Laboratory Unit of Rivers State University Teaching Hospital, Port Harcourt, and analyzed within one hour of arrival. All necessary materials for analysis, including media and glassware, were sterilized and prepared before sample collection. The samples were collected from both wholesale and retail vendors between February 2022 and July 2022.

Preparation of Sample

The method by Kaur and Rai (2015) was followed. Twenty grams (20 g) of each fruit sample were weighed and transferred aseptically into sterile beakers containing 200 ml of sterile distilled water. Sliced fruits were homogenized using an electric blender, while whole fruits were rinsed, and the wash water was used as the stock for culturing. Ten-fold serial dilutions of both whole and sliced fruit stock samples were prepared using sterilized peptone water. Four test tubes, each containing 9 ml of sterilized peptone water, were used for the dilutions. The peptone water was sterilized by autoclaving at 121°C for 15 minutes. After cooling, 1 ml of stock was added to the first test tube to create a 10-fold dilution, and the process was repeated for the remaining tubes. Contamination was prevented by swabbing the workbench with 70% alcohol, working near a Bunsen flame, and using sterile materials.

Enumeration and Isolation of Microorganisms

After serial dilution, 1 ml from each of the four dilutions was aseptically dispensed into labelled Petri dishes using the spread plate method with a bent glass rod, in duplicates. Sabouraud Dextrose Agar (SDA) plates for Total Fungal Count (TFC) were incubated for seven (7) days at 25^{O}C. Colony morphology, including colour, shape, and size, was examined microscopically. All experiments were conducted in duplicate to ensure reproducibility.

Purification of Isolates

When in use, each of the test isolates were first purified by sub-culturing on freshly prepared Saboraud Dextrose Agar using the streak method and then incubated for 2 days (48 hours) for fungal isolates, which were identified on the basis of standard cultural, microscopic and morphological characteristics.

Statistical Analysis

The data was analyzed statistically using SPSS software (version 23.0). Descriptive statistics, including mean and standard error, were calculated for Total Fungal Count (TFC). A one-way Analysis of Variance (ANOVA) was performed to determine differences at a 0.05 significance level, followed by Tukey's post hoc test to assess significance between groups.

RESULTS

Table 1a showed that the Total Fungal Count (TFC) ranged between zero CFU/ml in Rumuokoro and Mile 1 to 4.58 x10^2 CFU/ml in Oil mill market. The Total fungal Count (TFC) showed that the fungal present in selected whole fresh fruits was highest in Oil mill followed by Mile 3, Fruit Garden while Rumuokoro and Mile 1 had no fungal contamination.

The results from Table 1b indicates a significant mean difference in the Total Fungal Count (TFC) among selected whole fresh fruit sample ($F_{5, 84} = 29.297; P < .05$). Therefore, there is a significant mean difference in the Total Fungal Count (TFC) amongst selected whole fresh fruits based on the market type.

Table 1a: Mean Fungal Count (TFC) and CFU/ml of selected whole fresh fruits based on market type at 10^1 CFU/ml.

Selected Markets	N	Mean	SD	CFU/ml
Rumuokoro	15	.00	.000	0
Mile 1	15	.00	.000	0
Fruit Garden	15	28.93	6.777	2.89×10^2
Oil Mill	15	45.80	3.373	4.58×10^2
Mile 3	15	34.87	1.774	3.49×10^2
Town	15	15.30	3.556	1.53×10^2
Total	**90**			

N **=** *Number of samples examined*

Table 1b: ANOVA Test showing the mean difference of Total Fungal Count (TFC) of whole fruits among market types.

Sources of Variation	Sum of Squares	Df	Mean Square	F	Sig. (p value)	Decision
Between Groups	53536.517	5	10707.303	29.297	.000	**Significant**,
Within Groups	63592.433	84	365.474			$P < 0.05$.
Total	117128.950	89				

Key: Df = Degree of freedom, F = F value, P = P value, Sig. = Significance, Between groups = Different fruit sample from the markets, Within groups = The same fruit samples from the markets

Table 2a showed that the Total Fungi Count (TFC) ranged between 1.53 x 10^2 CFU/ml in Rumuokoro to 6.60 x10^2 CFU/ml in Oil mill market. The Total fungal Count shows that the incidence of fungal present in selected sliced fresh fruits was highest in Oil mill market followed by Mile 1 market, Mile 3 market, Fruit Garden market and Town market while Rumuokoro had the least fungal contamination.

The results from Table 2b indicates a significant mean difference in the Total Fungal Count (TFC) among selected sliced fruit sample ($F_{5, 84} = 19.04; P < .05$). Therefore, there was a significant mean difference in the Total Fungal Count (TFC) amongst selected sliced fresh fruits based on the market type.

Table 2a: Mean Total fungal Count and CFU/ml of selected sliced fresh fruits based on Market type at 10^1 CFU/ml.

Selected Markets	N	Mean	SD	CFU/ml
Rumuokoro	15	15.33	5.842	1.53 x 10^2
Mile 1	15	57.93	5.875	5.79 x 10^2
Fruit Garden	15	29.20	4.865	2.92 x 10^2
Oil Mill	15	66.07	2.860	6.60 x 10^2
Mile 3	15	36.47	3.872	3.65 x 10^2
Town	15	22.33	3.479	2.23 x 10^2
Total	**90**			

***N** = Number of samples examined*

Table 2b: ANOVA Test showing the mean difference of Total Fungal Count (TFC) of sliced fruits among market types.

Sources of Variation	Sum of Squares	Df	Mean Square	F	Sig. (p value)	Decision
Between Groups	60720.44	5	12144.089	19.038	.000	**Significant**,
Within Groups	110993.33	84	637.893			P < 0.05.
Total	171713.77	89				

Key: Df = Degree of freedom, F = F value, P = P value, Sig. = Significance, Between groups = Different fruit sample from the markets, Within groups = The same fruit samples from the markets

Table 3a showed the Total Fungal Count (TFC) which ranged between 1.11 x 10^2 CFU/ml in apple fruit and 2.64 x 10^2 CFU/ml in pawpaw fruit. The Total Fungal Count (TFC) shows that the incidence of fungal present in whole fresh fruit is highest in pawpaw followed by watermelon, orange and cucumber while, apple had the lowest level of fungal contamination.

The results from Table 4.b using the ANOVA indicates that the mean difference in the Total Fungal Count (TFC) among selected whole fruit sample is not significant ($F_{4, 175} = 1.86; P > .05$). This implied that the mean level of fungal contamination among the fruits are closely distributed and do not have marked differences.

Table 3a: Mean Fungal Count (FC) and CFU/ml of selected whole fresh fruits.

Selected Fruits	N	Mean	SD	CFU/ml
Watermelon	36	22.78	4.068	2.27×10^2
Orange	36	22.58	4.203	2.25×10^2
Apple	36	11.11	2.716	1.11×10^2
Cucumber	36	21.17	4.977	2.11×10^2
Pawpaw	36	26.44	4.775	2.64×10^2
Total	**180**			

Number of samples examined

Table 3b: ANOVA Test showing the mean difference of Total Fungal Count (TFC) among selected whole fruit sample.

Sources of Variation	Sum of Squares	Df	Mean Square	F	Sig. (P value)	Decision
Between Groups	4786.53	4	1196.633	1.864	.119	**Not Significant**,
Within Groups	112342.41	175	641.957			P > 0.05.
Total	117128.95	179				

Key: Df = Degree of freedom, F = F value, P = P value, Sig. = Significance, Between groups = Different fruit sample from the markets, Within groups = The same fruit samples from the markets

Table 4a showed the Total Fungal Count (TFC) which ranged between 1.86 x 10^2CFU/ml in Cucumber fruit and 5.72 x 10^2 CFU/ml in Pawpaw. The Total Fungal Count shows that the incidence of fungal present in sliced fresh fruit is highest in Pawpaw followed by Watermelon and Orange, while Cucumber and Apple had the lowest level of fungal contamination.

The results from Table 4b using the ANOVA indicates that the mean difference in the Total Fungal Count (TFC) among selected sliced fruit sample is significant ($F_{4, 175} = 14.58; P < .05$). As a result of this, Tukey test was also used for ranking the mean and for measuring the pairwise difference among selected fruit sample.

The Tukey post hoc test in Table 4c revealed that the level of fungal contamination was significantly higher in pawpaw (5.72±3.32) and watermelon (5.43 ± 5.17) when compared to cucumbers (1.86±4.65), orange (3.41±4.38) and apple (2.50 ±4.85 [a]). However, there was no statistical difference in the level of fungal contamination between watermelon and pawpaw, as well as amongst cucumbers, orange and apples.

Table 4a: Mean Fungal Count and CFU/ml of selected sliced fresh fruits.

Selected Fruits	N	Mean	SD	CFU/ml
Watermelon	36	54.33	5.174	5.43 x 10^2
Orange	36	34.17	4.381	3.41x 10^2
Apple	36	25.06	4.855	2.50x 10^2
Cucumber	36	18.67	4.652	1.86x 10^2
Pawpaw	36	57.22	3.323	5.72x 10^2
Total	**180**			

N = Number of samples examined

Table 4b: ANOVA Test showing the mean difference of Total Fungal Count (TFC) among selected sliced fruit sample.

Sources of Variation	Sum of Squares	Df	Mean Square	F	Sig. (P value)	Decision
Between Groups	42920.66	4	10730.16	14.58	.000	**Significant**,
Within Groups	128793.11	175	735.96			P < 0.05.
Total	171713.78	179				

Key: Df = Degree of freedom, F = F value, P = P value, Sig. = Significance, Between groups = Different fruit sample from the markets, Within groups = The same fruit samples from the markets

Table 4c: Tukey Test for ranking of Total Fungal Count (TFC) (Mean ± SE) of Selected sliced fruits at 10^2 CFU/ml.

Fruit Sample	(Mean ± SD)
Cucumber	1.86 ±4.65[a]
Apple	2.50 ±4.85 [a]
Orange	3.41 ±4.38[a]
Pawpaw	5.72 ±3.32 [b]
Watermelon	5.43 ±5.17 [b]

Each value is the mean of 2 replicates from three samples of each fruit type. Means of fruit sample in each column followed by the same letter (at least one identical letter) are not significantly different (P>0.05) by Tukey's test while, fruit type having mean with different letters (no identical letter) are statistically Significant (P<0.05).

DISCUSSION

This study aimed to evaluate fungal contamination in whole and sliced ready-to-eat fruits sold in Port Harcourt, Nigeria. The result obtained from the Total Fungi Count (TFC) ranged between 1.53 x 10^2 CFU/ml in Town and 4.58 x10^2 CFU/ml in Oil mill market. The Total fungal Count (TFC) showed that the incidence of fungal presence in selected whole fresh fruits was highest in Oil mill market followed by Mile 3 market, Fruit Garden market and Town market while Rumuokoro market and Mile 1 market had no fungal contamination.

The highest fungal contamination in Oil Mill market could be as a result of the fact that the location of the market was not close to any water body and the place is relatively dry and free from moisture. The spores of fungi are transmitted through air from one place to another which germinates on the fruits and foods materials when they fall on them. The relatively dry nature of the market enhances the free transmission of the spore from place to place.

The Town market which was closer to a water body (rivers) had relatively low level of fungal contamination. Mile 1 market was closer to Abonnema Wharf River and Rumuokoro market was close to Ntawogba creek had no fungal occurrence as a result of the evaporation of water from the water bodies. The evaporation of water from the water bodies makes the air to be dense and does not allow the free movement of the airborne fungal spore to be transmitted from place to place.

However, the high microbial count on whole fresh fruits in these markets may also be attributed to the unhygienic practices right from farm to the market and exposure to potential microbial contaminations at every step including cultivation, harvesting, transporting, packaging, storage and selling to the final consumer (Ntuli *et al*., 2017).

The result of the Total Fungal Count (TFC) for whole fresh fruits ranged between 1.11 x 10^2 in apple fruit and 2.64 x 10^2 CFU/ml in pawpaw fruits. The Total Fungal Counts (TFC) showed that the incidence of fungal present in whole fresh fruit was highest in pawpaw followed by watermelon, orange and cucumber while apple had the lowest level of fungal contamination. In this study, the level of fungal contamination was highest in pawpaw amongst all the fruits samples analysed.

The study is in line with the study of Adebayo-Tayo and Okonko, (2013) in Nigeria which reported a lower mean cfu/ml (10^2) of fungal count in all samples analysed. However, the study was not in

line with the study of Adebayo-Tayo and Okonko, (2013) in Nigeria which reported a lower mean fungal count of $1.7x\ 10^2 – 3.9\ 10^3$ CFU/ml in cucumber and watermelon juice.

The reason for the high mean Total Fungal Count (TFC) in pawpaw could be attributed to the fact that pawpaw provides a better nutrition and growth environment for fungal growth than the other fruits samples analysed. Statistically, there is no significant mean difference in the Total Fungal Count (TFC) amongst the selected whole fresh fruits samples analysed. This indicates that the mean difference in the Total Fungal Count (TFC) among selected whole fruit sample was not significant. This implies that the mean level of fungal contamination among the fruits are closely distributed and do not have marked differences.

The Tukey post hoc test revealed that the level of fungal contamination was significantly higher in pawpaw and watermelon when compared to cucumber, orange and apple, even though, there was no statistical difference in the level of fungal contamination between watermelon and pawpaw, as well as amongst cucumber, orange and apple. This was because the level of fungal contamination between watermelon and pawpaw was not too far apart.

CONCLUSION

The present research has shown that ready-to- eat fruits sold by street vendors in Port Harcourt markets are not safe for human consumption and consumers are at health risk in terms of microbial quality. Also, continuous consumption of food at a tolerable level is also a health risk coupled with the type of pathogenic microorganisms isolated. Contamination from farms or production areas, improper food handling while processing, non-hygienic practices while packaging and environmental conditions are major factors responsible for high fungal load on the whole and sliced fruits.

Thus, proper use of potable water for fruit washing and processing equipment, regular hand washing with soap and clean water, and the use of protective disposable polyethylene gloves and good hygiene practices are recommended to fruit vendors. Also, it is imperative to maintain adequate pre- and post- harvest practices of the fruits vended.

Therefore, the results obtained in this report concluded that the association of whole and sliced fresh produce with these fungi may possibly cause contamination that will lead to outbreaks of

human diseases. Thus, proper whole and sliced fresh fruits processing methods that could inhibit the growth or kill these fungi are suggested to ensure the safety of these fruits. From the results obtained above, it clearly shows that these fruits are contaminated and may cause health hazard when consumed.

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