# Characteristics of Fungi isolated from Vended fruits in Port Harcourt Metropolis

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***Abstract***
*The unhygienic handling and processing of fruits before they are sold to the unsuspecting public leaves much to be desired. This study was undertaken to isolate and identify fungi associated with vended fruits sold in Port Harcourt metropolis. A total of twenty-four (24) fruits were purchased from various vendors at different locations around the Port Harcourt metropolis for analysis for the presence of fungi. The fruits include apple (Malus domestica), banana (Musa sapientum), orange (Citrus sinensis), paw-paw (Carica papaya), pineapple (Ananas comosus), and watermelon (Citrullus vulgaris). The microbial analysis of the vended fruit was studied using standard analytical methods. Results show that fungi isolated from the fruits were Aspergillus niger, Fusarium avenaceum, Aspergillus fumigatus, Mucor piriformis, Fusarium solani, Aspergillus flavus, Penicillium digitatum and Rhizopus stolonifer. Percentage occurrence of the isolates show that the predominant fungi is Aspergillus niger with a frequency of 30% while Fusarium avenaceum (26%), Aspergillus fumigatus (13%), Mucor piriformis (10%), Fusarium solani (8%), Aspergillus flavus (5%), Penicillium digitatum (4%) and Rhizopus stolonifer (4%) in this order. This shows that these fungi species are of economic and public health significance in the deterioration of fruits and are capable of producing toxic compounds in food. The toxic substances can result to food poisoning or intoxication if consumed*

***Keywords:*** *Fungi, Fruits, Vendors, Diseases, Food poisoning*

## Introduction

Street vended fruits are ready-to-eat fruits that can be bought directly from street vendors or hawkers in streets and other similar public places and eaten immediately without necessarily having to cut, peel or rinse them as they have already been prepared by the vendors. In recent times, there has been a significant increase in the consumption of vended fruits because they are easily accessible, convenient and cheaper than the whole fruits. Fruits are a good source of nutrients, micronutrients, vitamins and fibre essential for health and wellbeing of human (Oranusi and Olorunfemi, 2011) but most worrisome is the manner in which it is prepared, however it is becoming a viable and important informal-sector industry (Nielsen, 2006; Sharmila, 2011). Many authors through their transactions reported that street food vending businesses are potentials for income generation and yield a substantial monthly income to families (Bromley 2000, Dipeolu *et al.*, 2007; Amoo *et al.*, 2012). In addition, it provides entrepreneurial opportunities to people that may not be able to afford to buy or rent fixed premises and therefore forms a potential source of income to the government through the levy of licensing fees, sales and value-added taxes.

However, vended fruits commonly consumed include apple, banana, orange, paw-paw, pineapple and watermelon, are perceived to be a major public health risk due to lack of basic

infrastructure and services and therefore portends danger of outbreak of food poisoning (Ghosh *et al.*,2007; Chukuezi, 2010; Nurudeen *et al*., 2014). Due to contamination during the unhygienic processes fruits undergo before sale to the public, they have aided the spread of fruit-borne diseases (Estrada-Garcia *et al*., 2004, Eni *et al*., 2010; Isa *et al.*, 2014). This is because they often expose their goods to the sun, air pollution, and contamination by passers-by.

In Nigeria, several studies carried out with regard to microbiological quality of ready-to-eat fruits discovered that vended fruits can be affected by factors such as improper handling and processing, use of contaminated water during washing, cross contamination from other fruits and vegetables or the use of dirty processing utensils like knives and trays (Nwachukwu *et al*., 2008; Chukwu *et al*., 2010; Oranusi and Olorunfemi, 2011; Daniel *et al.*, 2014).
Various pathogens are associated with the contamination of fruits, with different outbreaks of gastroenteritis and are associated with the consumption of contaminated fruits at various times (Jeddi *et al*., 2014). Pathogens implicated in contamination of fruits include *Escherichia coli, Salmonella* spp., *Listeria monocytogenes, Aeromonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Vibrio* spp. and *Pseudomonas* spp (Mensah *et al*., 2002; Falola *et al.*, 2011; Mbah *et al.*, 2012; Ossai, 2012; Oranusi and Braide, 2012; Nwachukwu and Chukwu, 2013; Senjuti *et al*., 2014). Most vended foods are usually sliced, packaged in polyethylene bags and sold by street vendors. The rate of consumption of these foods is high and the associated risk of disease to which consumers may be exposed, is a matter of great concern. It is also difficult for one to ascertain the level of hygiene of the processors and to the sanitary conditions at points of preparation. Moreover, the case is worsened by the fact that street vended foods are done without adequate storage conditions, thereby exposing the foods to flies and other disease-causing agents. Contamination or cross-contamination of street foods, especially sliced fruits and vegetables, can occur by unsanitary processing and poor preservation methods. The use of dirty utensils, as well as the open display of street food produce encourages sporadic visits by flies, cockroaches, rodents and dusts (Nwachukwu *et al*., 2008) hence they are predisposed for invasion by pathogenic mesophiles (Muinde and Kuria, 2005). This study therefore is aimed at determining the fungal isolates in vended foods sold in markets and streets around the Port Harcourt metropolis, Nigeria

## Materials and Methods

### Sample collection

A total of twenty-four (24) vended fruits samples including apple, banana, orange, pawpaw, pineapple and watermelon were purchased from the Mile 3 market,), and Iloabuchi, Diobu and the Rivers State University environs, Port Harcourt. The samples were collected with sterile polythene bags in an ice-parked cooler and immediately taken to the laboratory for analysis.

### Preparation of Sample for Inoculation

The laboratory bench was sterilized by cleaning with ethanol (99.9%) concentration and fruit samples were sliced aseptically using a sterile knife. About 10 g of the sliced fruit samples was weighed and homogenized in 100ml of sterile 0.1% (w/v) peptone water. Ten-fold serial dilutions of the homogenates were made; 0.1 ml of $10^{-4}$ dilutions of the homogenate was plated in duplicate on Sabouraud Dextrose Agar. The plates were incubated at 37$^{o}$C for 2-4 days after which the plates were observed for growth. The colonies of representative organisms were recorded.

**Identification of Fungal Isolates**

The technique of Oyeleke and Manga (2008) was adopted for the identification of the fungi isolates using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with x10 and x40 objective lenses. The morphological characteristics and appearance of the fungal isolates under the microscope were identified in accordance with standard scheme for identification of fungi (Samson and Varga, 2007; Adebayo-Tayo *et al*., 2012) like sexual and asexual reproduction structures like sporangia, conidial head, the vegetative mycelia and septate or non-septate hyphae while the the macroscopic assessment of fungal growth was done by observing colonial morphology: colour of colony, size, texture, shape and surface appearance.

**Results and Discussion**

Predominant fungal isolates from the vended fruits were characterized and identified as *Aspergillus niger, Fusarium solani, Aspergillus fumigatus, Rhizopus stolonifer, Aspergillus flavus, Fusarium avenaceum, Mucor piriformis* and *Penicillium digitatum* (Figs a-h and Table 1). The presence of these fungal species indicate that they may find their way into the fruits through processes such as harvesting, storing, packing and transportation of fruits which may encounter physical injury that may cause increases in post-harvest losses and the possibility of fungal contamination (Isa *et al.*, 2014). Vending conditions that favour contamination can be worsened by poor hygiene of the vendors using microbial unsafe containers. 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.

**Table 1: Morphological Characteristics and identification of fungal Isolates**

| Isolates code | Morphological Characteristics | Microscopic Characteristics | Probable organism |
|---|---|---|---|
| **1.** | Loose cotton wool-like aerial mycelium | Non septate mycelia, that bear long sporangiophores scattered all over the mycelium | *Mucor piriformis* |
| **2.** | Compact, clusters of dark colony | Hyaline conidiophore phylides borne on vesicles, clusters of dark walled conidia with septate hyphae. | *Aspergillus niger* |
| **3.** | Green dense velvet mycelium | Hyaline conidiophores, phialides borne on versicles. Green chain of conidia with septate hyphae. Smooth walled conidiophores | *Aspergillus fumigatus* |
| **4.** | Greenish-yellow hairy elevated surface | Septate hyphae with long conidiophores | *Aspergillus flavus* |
| **5.** | Orange coloured and oval shaped | Septate hyphae with long, slender to falcate macroconidia | *Fusarium avenaceum* |
| **6.** | Dark coloured and oval shaped | Septate hyphae with lightly curved, wide and thick walled conidia | *Fusarium solani* |
| **7.** | White coily elevated surface | Conidiophores with inflated branches at the top, conidia in chains | *Penicillium digitatum* |
| **8.** | Whitish grey cottony mycelium | Non-septate hyphae, large globose many spored sporangia on single sporangiophore | *Rhizopus stolonifer* |

**Fig 1:** Morphological features of fungal isolates on Sabouraud Dextrose Agar media and their appearance under Microscope

a) *Aspergillus niger*
Macroscopic Examination

*Aspergillus niger*
Microscopic Examination

b) *Fusarium solani*
Macroscopic Examination

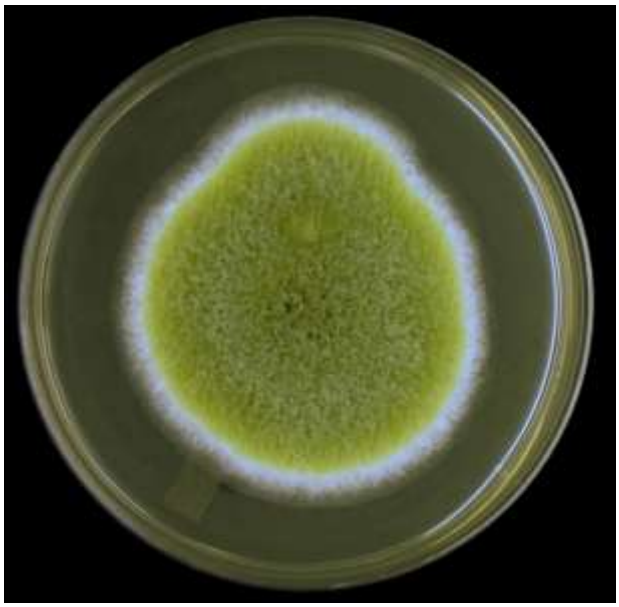
*Fusarium solani*
Microscopic examination

c) *Aspergillus fumigatus*
Macroscopic Examination

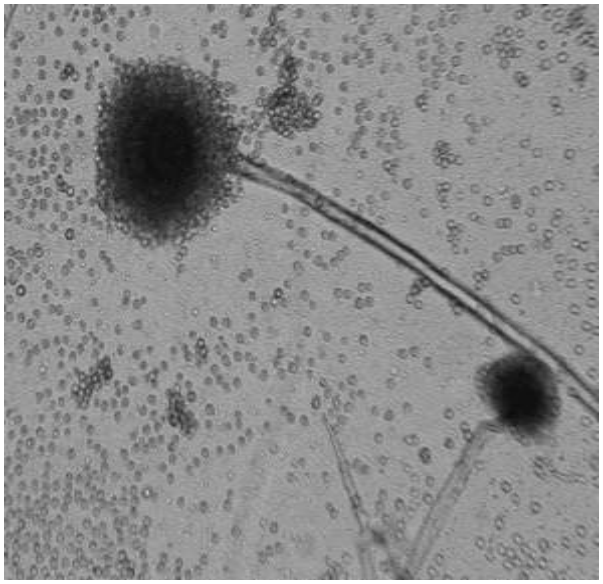
*Aspergillus fumigatus*
Microscopic Examination

d) *Rhizopus stolonifer*
Macroscopic Examination

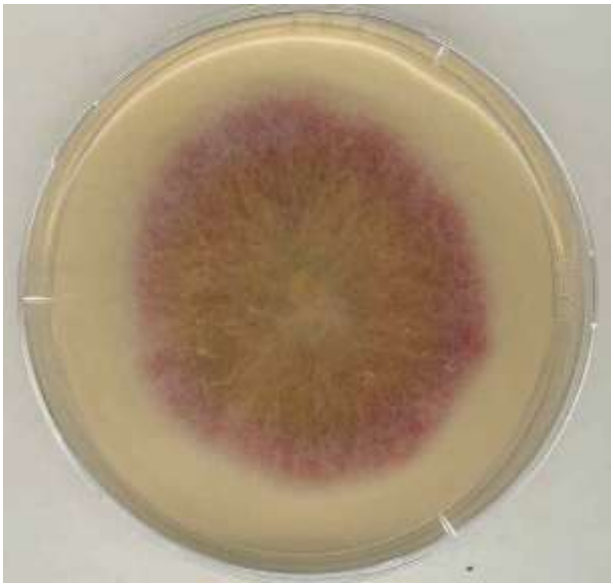
*Rhizopus stolonifer*
Microscopic Examination



e) *Aspergillus flavus*
Macroscopic Examination

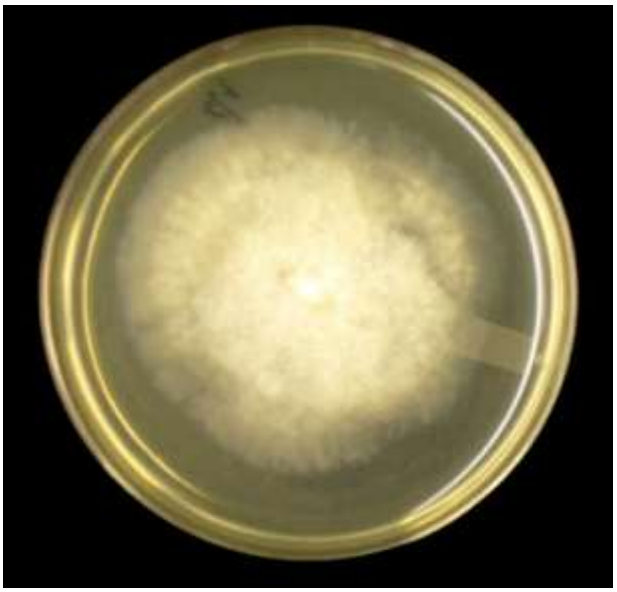


*Aspergillus flavus*
Microscopic Examination

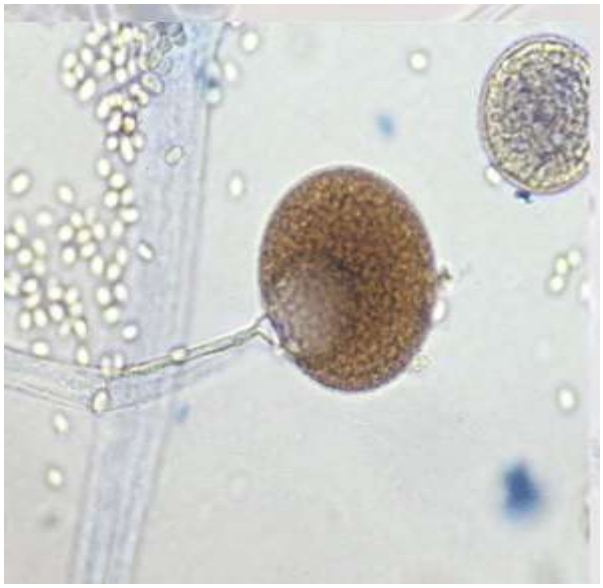


f) *Fusarium avenaceum*
Macroscopic Examination

*Fusarium avenaceum*
Microscopic Examination

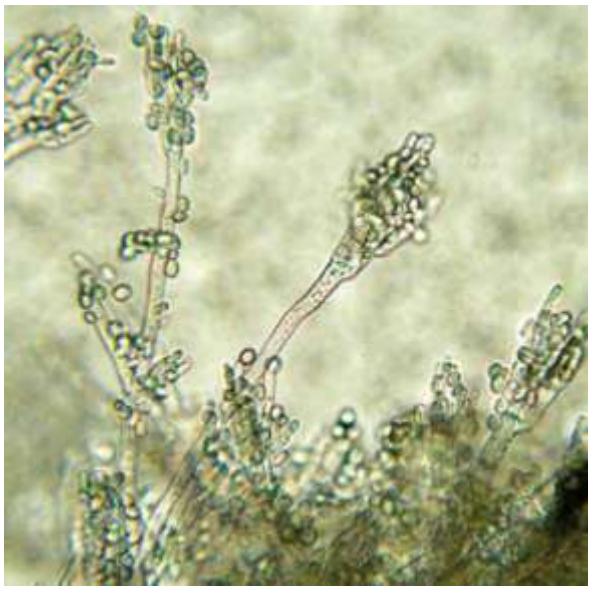


g) *Mucor piriformis*
Macroscopic Examination



*Mucor piriformis*
Microscopic Examination

h) *Penicillium digitatum*
Macroscopic Examination



*Penicillium digitatum*
Microscopic Examination

Table 2 shows the mean counts for the total heterotrophic fungi observed in this study. The apple fruit was found to have the highest fungal count of $1.8 \times 10^2$ while orange fruit has the lowest fungal count of zero. Contaminants may invade the interior surfaces of fruits during pealing, slicing, trimming, packaging or from handling by the vendor. Lack of storage facilities could also have heightened the chances of contamination (Baro *et al.*, 2007; Oranusi and Braide, 2012). The vended products are maintained throughout the day under the intense heat of the sun, this encourages proliferation of contaminants. This measure calls for strict adherence to standard food practices and effective HACCP application in food products.

**Table 2: Total counts of Total Heterotrophic Fungi in the Vended fruits**

| S/N | Fruits | RSU | Illoabuchi, Diobu |
|---|---|---|---|
| 1 | Apple | $1.8 \times 10^2$ | $1.5 \times 10^2$ |
| 2 | Pineapple | $1.7x\ 10^2$ | $1.3 \times 10^2$ |
| 3 | Pawpaw | $2.0 \times 10^2$ | $1.8 \times 10^2$ |
| 4 | Orange | NG | $1.0 \times 10^2$ |
| 5 | Watermelon | $1.4 \times 10^2$ | $1.0 \times 10^2$ |
| 6 | Banana | $1.5 \times 10^2$ | $1.1 \times 10^2$ |

**NG – No growth, RSU- Rivers State University**

The danger associated with migratory food vendors of unknown identity, untrained in food safety and of unknown educational background is enormous and must be discouraged. The way they are exposed to the highways can best be imagined and are at risk especially when one observe them meandering between vehicles on motion and in most cases run after vehicles for several meters to get their wares sold.

Amongst the fungi isolates, *Aspergillus niger* which causes black mold disease on some fruits and vegetables produces potent mycotoxins called ochratoxins that can be harmful to human beings and animals. However, Aspergillus *niger* had the highest occurrence of 30% followed by *Fusarium avenaceum* (26%), *A. fumigatus* (13%), *Mucor piriformis* (10%), *Fusarium solani* (8%), A. *flavus* (5%), *Penicillium digitatum* (4%), and *Rhizopus stolonifer* (4%) (Table 3; Fig2). This result is in tandem with reports by Tafinta *et al* (2013). Most of the fungal isolates in this study play a pivotal role in the deterioration of fruits and some of them are capable of producing toxic compounds in humans. The mycotoxins produced by these fungi can cause serious health hazards (Okojie and Isah, 2014). However, *Penicillium* species are common fungi that are often considered non-pathogenic to humans (Oshikata *et al*., 2013).

According to Foody and Tong, (2008), *Rhizopus stolonifer* is a significant agent of fruit disease. It is a threadlike mold and a heterotrophic species; it depends on sugar or starch for its source of carbon as food. It also uses food matter, mostly soft fruits, like grapes or strawberries, as a food source for growth, nutrition and reproduction. *Fusarium avenaceum* is a fungal plant pathogen that causes Panama disease of banana (*Musa* spp.), also known as *fusarium* wilt of banana. Some species may cause a range of opportunistic infections in humans especially in the nails (onychomycosis) and the cornea (keratomycosis or mycotic keratitis). Fusarial infections may occur in people whose immune systems are weakened but aggressive fusarial infections can disseminate infections and penetrate the entire body and bloodstream (Howard, 2003).
Fungal pathogens cause loss of quality and hygiene of fruits, resulting in major economic problems. More so, contaminated fresh fruits pose potential food safety hazard and poor

processing regime could lead to potential food safety problems. Poor handling can damage fresh fruits, rendering their products susceptible to the growth or survival of spoilage and pathogenic microorganisms (Gultie *et al.*, 2013). Fruit contamination can be prevented using physical and chemical methods. Lactic acid bacteria can play a vital role as natural preservatives, because it produces antifungal compounds such as carboxylic acids, fatty acids, ethanol, carbon dioxide, hydrogen peroxide, and bacteriocins (Pawlowska *et al.*, 2012).

**Table 3: Frequency of Occurrence of fungal isolates in Vended fruits**

| S/N | *Fungi Isolates* | Type of Fruit | Frequency of Occurrence (%) |
|---|---|---|---|
| 1 | *Aspergillus niger*, | Apple, pineapple, orange, watermelon and pawpaw | 30 |
| 2 | *Fusarium avenaceum* | Banana, orange, pawpaw and watermelon | 26 |
| 3 | *Aspergillus fumigatus* | Apple, pineapple, pawpaw and watermelon | 13 |
| 4 | *Mucor piriformis* | Apple | 10 |
| 5 | *Fusarium solani* | Banana, watermelon and pawpaw | 8 |
| 6 | *Aspergillus flavus* | Pawpaw | 5 |
| 7 | *Penicillium digitatum* | Apple | 4 |
| 8 | *Rhizopus stolonifer* | Orange | 4 |

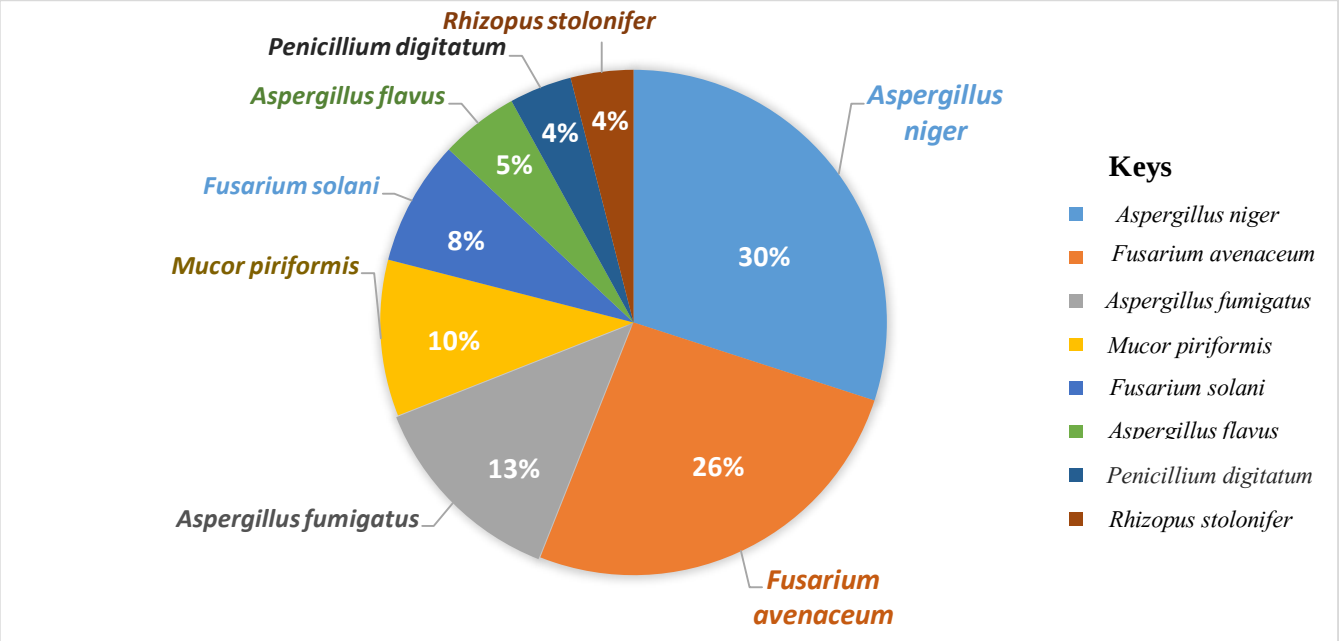



**Figure 2**: Frequency of occurrence of fungal isolates

## Conclusion

The pathogenic fungi species associated with vended fruits are of economic and public health significance. Therefore, vendors activities must be regulated to ensure proper and safe fruit processing practices which includes use of clean water for processing prior to sale, proper handling of these fruits, and use of sterilized/clean utensil such as knife, tray etc. during sales. Also, vendor must ensure that they acquire good storage condition for these fruits in order to reduce pathogenic microbial growth on the fruit. And they should purchase these fruits from wholesalers who have a good storage facility and avoid migrant traders who handles fruit in an unsafe manner. The health of consumers should be their priority.

They should as a matter of necessity wash the fruits with clean water prior to consuming them, in order to reduce if not eliminate possible contaminants on the body of the fruits.

However, proper sanitary condition must be practiced by food vendors. In addition, the government must have a better surveillance measure on the activities of street vendors in order to minimize the risk of disease outbreak associated with consumption of contaminated food produce

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