Microbiological Assessment of different species of apples (Granny smith, Red delicious and Goldrennette) sold in Owerri Metropolis, Imo State.

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Abstract: The microbiological assessment of different species of apples (Granny smith, Red delicious and Gold rennette apples) sold in Owerri Metropolis, Imo Slate was carried out using standard microbiological procedures. Three species of apples were bought from four different vendors at different locations within Owerri. Total viable bacterial counts recorded ranged from 1.0 x 10^3cfu/g to 1.32 10^5cfu/g. Total coliform counts recorded ranged from 1.0 x 10^3cfu/g to 1.6 x 10^4cfu/g. Total fungal counts recorded ranged from 2.0 x 10^3cfu/g to 1.4 x 10^4cfu/g. Bacterial isolates from the apples were; *Staphylococcus aureus* 3(9.68%), *Pseudomonas aeruginosa* 4(12.90%), *Corynebacteriumspecies* 8(25.81%). *Bacillus* species 5(16.13%), *Proteus* species 4(12.90%), *Micrococcus* species 5(16.13%) and *Klebsiella* species 2(6.45%). Fungal species isolated from the apples were; *Penicilliumspecies* 6(35.29%), *Saccharomyces* species 4(23.53%), *Kluyveromycesspecies* 3(17.65%) and *Mucor* species 4(23.53%).The prcsenceof pathogenic organisms on the fruits is of public health concern. The fruits should be properly washed before consumption.

Keywords: Assessment, isolates, apples, locations, vendors.

Introduction

Fruits are part of a flowering plant that are derived from specific tissues of the flowers (one or more ovaries) and in some cases accessory tissues (Lewis, 2002). They are widely distributed in nature and have high health as well as economic values. But one of the limiting factors that influence the economic values of fruits is the relatively short shelf-life period caused by pathogens.

Fruits are widely exposed to microbial contaminants through contact with soil, dust and water, and by handling at harvest or during post-harvest processes (such as; peeling, slicing etc). They, therefore, harbor a diverse range of microorganisms including plant and human pathogens. Differences in microbial profiles of various fruits result largely from unrelated factors such as; resident microflora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor *et al.*, 2009).

It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006). The apple is a deciduous tree comprising fruits with exclusive popularity and anti-oxidant traits with the capacity to reduce the risk of various diseases (Marchand *et al.*, 2000 and Boyer *et al.*, 2010). Apples are rich source of nutrients and phytochemicals that have been reported to reduce risk of cardiovascular disease, asthma, diabetes, cataracts, Alzheimer's disease or cognitive decline and pulmonary functions (Gercia *et ai,* 2005; Chan and Shea, 2009; Hyson, 2011; Chan *et al.*, 2016).

Despite the health benefits of fruits (such as apples) to human's health, there are several reports on the contamination of fresh fruits (Buck *et al.*, 2003; Eni *et al.*, 2010; Acharjee *et al.*, 2013; Dulta *et al.*, 2013). The surfaces of apple fruits can harbor microorganisms depending on the mechanical handling of the fruits. Microorganisms can adhere to surface, invade or penetrate the surfaces of apple and multiply within the tissue. Contamination could be from human handling, transport vehicles, insect, dust, and rinse water, harvesting equipment, soil, faeces, irrigation water, water used to apply fungicides and insecticides, manure, wild and domestic animals (Burnett and Beuchat, 2001; and Buck *et al.*, 2013).

Microorganisms may gain access more frequently to the surface of the fresh fruits due to poor handling in stores and retail markets (Chen *et al.*, 2004; Mudgil *et al.*, 2004; Aycicek *et al.*, 2006; Eniet *al.*, 2010; Mukhtar *et al.*, 2010; Otaiment *et al.*, 2012). Fungal pathogens are the main cause of post-harvest losses of apples that can be high as 50% (Spadaro *et al.*, 2004). Initial contamination sources include flume and sorting water, unclean storage crates or storage facilities (Tournas, 2005; Badosa *et al.*, 2008).

Considering the way in which apple fruits are indiscriminately displayed for sale in Nigeria at motor parks, busy dusty roads, hawked under hot weather conditions, security check points, etc, and often purchased and eaten without sufficient washing by the buyers, there is the tendency of them being contaminated with pathogenic microorganisms which could cause health challenges to the consumers. This study, therefore, dwelt on the assessment of different species of apple sold in Owerri metropolis.

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Materials and Methods

Source/collection of samples

Different species of fresh apples of; Granny smith (*Malusdomestica* "Granny Smith"), Red delicious (*Malusdomestica*) and Gold rennette (*Maluspumila*) were bought from four different sites identified to be the major sales location for apple fruits in Owerri and also locations considered as places where the fruits are bought as ready-to-eat for immediate consumption without washing or treatment of any sort. The sampling sites included two tertiary institutions of learning, one major motor park and one major/busy road.

Preparation of media/Isolation of microorganisms from the apple surface

Nutrient agar and plate count agar (Oxoid, England), Eosin Methylene Blue (EMB) agar (Oxoid) and MacConkey agar (Fluka), Sabouraud Dextrose agar (Fluka) were prepared according to the manufacturer's instruction and autoclaved at a temperature of 121°C, 15 psi for 15 minutes. The media were poured into different sterile petridishes and kept for analysis.

Each apple sample was rinsed out in 10 mL sterile peptone water. The rinse/wash water was diluted to get dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The spread plate technique was adopted in plating aliquots of 0.1 mL of the dilutions in duplicates onto different media plates; Nutrient agar and plate count agar (Oxoid, England) for total aerobic plate count, Eosin Methylene Blue (EMB) agar (Oxoid) and MacConkey agar (Fluka) for coliform count, Sabouraud Dextrose agar (Fluka) for fungal count and the plates were incubated for 24 h at 37°C. Sabouraud Dextrose agar was however; left at 28°C for 72 h. Colonies were counted after the incubation time using digital colony counter (Stuart Scientific, United Kingdom). Distinct discrete colonies on the different media were isolated and purified on nutrient agar by repeated sub-culturing. Further characterization of pure cultures stored on agar slants at 4°C was by the methods described by Speck (1976).

Identification of Bacterial isolates

Typical colonies stored on nutrient agar slants at 4°C, were Gram-stained and confirmed (Cheesbrough, 2005). Cultural characteristics and biochemical tests: motility, Oxidase, Catalase, Coagulase, sugar production test, citrate utilization test, were carried out.

Identification of Fungal isolates

Identification was based on their macroscopic and microscopic characteristics as seen in culture morphological characteristics, needle mount and slide culture. Reference was made to standard identification keys and atlas (Fawole and Oso, 1986; Tsuneo, 2010).

Results

Table 1: Microbial loads of the apples

Samples	TVBC (cfu/g)	TCC (cfu/g)	TFC (cfu/g)
GSA1	1.8×10^4	3.0×10^3	5.0×10^3
GSA2	2.8×10^4	4.0×10^3	2.0×10^3
GSA3	4.2×10^4	1.2×10^4	4.0×10^3
GSA4	3.2×10^4	1.0×10^3	3.0×10^3
RDA1	4.8×10^4	2.0×10^3	3.0×10^3
RDA2	1.32×10^5	1.0×10^3	1.0×10^4
RDA3	NG	NG	2.8×10^4
RDA4	NG	NG	2.0×10^3
GR1	2.8×10^4	NG	1.4×10^4
GR2	4.0×10^3	NG	6.0×10^3
GR3	2.0×10^3	1.6×10^4	5.0×10^3
GR4	5.4×10^4	NG	6.0×10^3

Keys: cfu/g = colony forming unit per gram
NG = No growth
TVBC = Total viable bacterial counts
TCC = Total coliform counts
TFC = Total fungal counts
GSA = Granny smith apple
RDA = Red delicious apple
GR = Gold renette apple

The results of total aerobic plate count, coliform count and fungal counts are shown in Table 1. Total viable bacterial counts recorded ranged from 1.0 x 10^3cfu/g to 1.32 10^5cfu/g. Total coliform counts recorded ranged from 1.0 x 10^3cfu/g to 1.6 x 10^4cfu/g. Total fungal counts recorded ranged from 2.0 x 10^3cfu/g to 1.4 x 10^4cfu/g. Two samples of red delicious apples used in this study had no bacterial and coliform growths, also two Gold renette apple samples had no coliform counts.

Table 2: Identification and characterization of bacterial isolates

MorphologicalCharacteristics	Gram reaction	Oxidase test	Indole test	Spore test	Catalase test	Citrate test	Coaguase test	Motility test	SFT S	SFT B	SFT G	SFT H_2S	Possible bacteria
Milkish. raised, non- mucoid colonies	Gram positive cocci in clusters	-	-	-	+	-	+	-	No Reaction			-	*Staphylococcus* species
Pale, flat, non-mucoid elongated colonies	Gram negative rods	-	+	-	-	+	-	+	R	Y	+	+	*Proteus* species
Bluish-green, flat, non-mucoid colonies	Gram negative rods	+	-	-	+	-	-	+	R	R	-	-	*Pseudomonas* species
Milkish, enlarged, non-mucoid regular shaped colonies	Gram positive cocci	-	-	-	-	-	-	-	No Reaction		-	-	*Micrococcus*spp
Milkish, flat, rhizoid-like colonies	Gram positive rods	-	-	+	-	-	-	-	Y	Y	+	-	*Bacillus* species
Milkish, raised, needle-pointed non-mucoid colonies	Gram positive rod	-	-	-	+	-	-	-	No Reaction			-	*Corynebacterium*species
Pinkish, raised, non-mucoid enlarged colonies	Gram negative rods	-	-	-	-	+	-	+	R	Y	+	+	*Klebsiella* species

KEY: -= Negative += Positive S= color of slope B= color of butt G= Gas production H2S= Hydrogen sulphide production (blackening) R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

The bacterial species isolated include; *Staphylococcus* species, *Bacillus species*, *Corynebacterium*species, *Pseudomonas* species, *Proteus* speciesand *Klebsiella* species as shown in the Table 2 above.

Table 3: Frequency (%) of bacterial occurrence on apple samples.

Bacteria Species	Frequency	Percentage occurrence (%)
Pseudomonas aeruginosa	4	12.90
Staphylococcus aureus	3	9.68
Bacillus species	5	16.13
Klebsiella species	2	6.45
Corynebacterium species	8	25.81
Proteus species	4	12.90
Micrococcus species	5	16.13
Total	31	100.0

Key: % = percentage

The frequency (%) of bacterial occurrence on the apples were *Staphylococcus aureus* 3(9.68%), *Pseudomonas aeruginosa* 4(12.90%), *Corynebacterium* species 8(25.81%), *Bacillus* species 5(16.13%), *Proteus* species 4(12.90%), *Micrococcus* species 5(16.13%) and *Klebsiella* species 2(6.45%). *Corynebacterium* species had the highest occurrence 8(25.81%) while *Klebsiella* species had the least occurrence 2(6.45%) as Table 3 above shows.

Table 4: Identification and characterization of fungal isolates

Cultural morphology	Microscopy	Possible fungi
Whitish, fluffy, enlarged colonies with grey center	Non-branched hyphae	*Mucor* species
Whitish cottony broom-like colonies with greenish centre	Septate hyphae with spores	*Penicillium* species
Creamy, convex, circular, enlarged colonies	Budded yeast cells	*Kluyveromyces* species
White, creamy, oblong colonies	Budded yeast cells in diploid	*Saccharomyces* species

Fungal species isolated were *Penicillium* species, *Saccharomyces* species, *Klyveromyces* species and *Mucor* species.

Table 5: Frequency (%) of fungal occurrence on apple samples

Fungi Species	Frequency	Percentage occurrence (%)
Mucor species	4	23.53
Penicillium species	6	35.29
Saccharomyces species	4	23.53
Kluyveromyces species	3	17.65
Total	17	100.0

As is indicated in the Table 5 above, the frequency (%) of fungal occurrence on the apples were *Penicillium* species 6(35.29%), *Saccharomyces* species 4(23.53%), *Kluyveromyces* species 3(17.65%) and *Mucor* species 4(23.53%). *Penicillum* species had the highest occurrence 6(35.29%) while *Kluyveromyces* species had the least occurrence 3(17.65%).

Discussion

Fruits harboura diverse range of microorganisms of plant and human origin (Ray and Bhunia, 2007; Ofor*et al*., 2009).The bacterial isolates were; *Staphylococcus aureus* , *Pseudomonas aeruginosa, Corynebacterium* species, *Bacillus* species, *Proteus* species, *Micrococcus* species 5 and *Klebsiella* species. *Corynebacterium* species had the highest occurrence while *Klebsiella* species had the least occurrence. Oranusi and Wesley (2012) reported the presence of similar organisms in apples.

From the result obtained, the total aerobic plate counts recorded ranged from 10^3 - 10^5cfu/g. According to the guidelines under Hazards Analysis and Critical Control Point-Total Quality Management (HACCP-TQM), microbial quality for raw foods containing aerobic plate count of $<10^4$cfu/g is regarded as "Good",10^4 - 10^6 cfu/g as "average", 10^6 - 10^7 cfu/g as "poor" and $>10^7$cfu/g as "spoilt" (EC-SCF, 2002, Aycicek*et al.*, 2006). The total aerobic plate count recorded in this study is within the range of "good and average".

The total aerobic plate counts recorded in this study are lower than those reported by Oranusi and Wesley (2012); Dee Giusti *et al*., (2010) and Jocelyn *et al*. (2012). Coliforms are indicator organisms for water and foods. The coliform count of 10^4 - 10^6cfu/g is cause for concern since the apples are usually eaten without further processing, or washing by some people.

Staphylococcus aureus and *Bacillus* species are common food contaminants from man and the environment. Their presence in the apple could be from touching by the customers, soil or container (Mudgil *et al*., 2004; Oranusi *et al*., 2004). However, their presence needs to be controlled because they have been reported to cause major food-borne illness (Ochei and Kolhatkar, 2000; Oranusi *et al*., 2007).

The presence of *Pseudomonas aeruginosa, Proteus species and Klebsiella species* could be traced to the water used by the vendors in washing the apples. Eni*et al.* (2001); Oranusi*et al*. (2007) reported isolation of *Pseudomonas aeruginosa, Shigella* species *and Proteus* species and *Staphylococcus aureus* from fruits. The presence of these bacteria in the apple is of public health concern as they are involved in causing infection in man. The use and reuse of water by the vendors and lack of potable water within the market where these apples are sold could account for their presence.

The fungi isolated from this study have been reported to be environmental contaminants (Tournas, 2005; Badosa *et al*., 2008). Their presence in the apples indicated that the environments where these apples are sold are not hygienic. The spores of these fungi have been reported to be present in the air and could drop on the apples during display for sale in the market. Some of the fungi have been reported to produce my cotoxins and implicated in cases of mycoses (Tournas, 2005; Kathrine *et al*., 2006).

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