International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 6 Issue 8, August 2019

ISSN: 2394-4404

Evaluation Of Microbial Quality Of Ready–To-Eat Fruits Sold In Different Markets Of Enugu Metropolis, Enugu State, Nigeria

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Abstract: ***Fresh fruits provide nutrients and vitamins which are important to health and at the same time harbor pathogenic microorganisms of public health significance. Microbial quality of ready-to-eat fruits sold in different markets of Enugu metropolis were evaluated using microbiological methods. A total of forty (40) ready-to-eat fruits were screened for microbial counts. The mean bacterial load of the samples ranged from 6.0 x10^5cfu/g to 8.2 x10^5 cfu/g with pineapple and watermelon showing the highest counts while the mean fungal load ranged from 1.3x 10^5 cfu/g to 1.7x10^5 cfu/g with pawpaw showing the highest count. Five bacterial and two fungal species were isolated: Staphylococcus aureus, Klebsiella sp, Escherichia coli, Shigella sp, Salmonella sp, Candida sp and Aspergillus sp. Staphylococcus aureus 28 (70%) had the highest occurrence followed by Escherichia coli 25 (62.5%), Salmonella sp 20(50%), Klebsiella sp 16(40%), Shigella sp 15(37.5%), Candida sp 15(37.5%) and Aspergillus sp 7(17.5%) which had the least occurrence. The presence of these organisms in the screened fruits are an indication of poor sanitary practices, hence fruit vendors should be educated on the dangers of these organisms by the government and community health workers.***

Keywords: Microorganisms, Ready to eat fruits, Vendors, Microbial load, Fruit contamination

I. INTRODUCTION

Fruits are an extraordinary dietary source of nutrients, vitamins and fiber for humans, hence are important for health and well-being of individuals (Orji *et al*., 2016). Well balanced diets rich in fruits have been reported to help to prevent vitamin C and vitamin A deficiencies thereby reducing the risk of several diseases (Kalia and Gupta, 2006). Fruits are also rich in riboflavin (B_2), zinc, calcium, potassium and phosphorus (Iyoha and Agoreyo, 2015). These ready-to-eat fruits are fruits that have been cut open and carried around by street vendors or hawkers at local markets and such fruits are eaten immediately without necessarily having to cut, peel or rinse them before consumption because they have already been prepared or packaged by the vendors (Kaplan and Compbell, 1982; Lund, 1992; De Rover, 1998). They are usually packaged in small polyethene bags for sale (Orji *et al*., 2016). The vendors usually blow air into the polyethene bags with their mouth with the view of opening it before packaging thereby introducing some microbial flora into the bags. These fruits are exposed to microbial contamination through contact with soil, dust, dirty environment, water and by mal-handling during harvest or post-harvest processing, hence harboring a diverse range of microorganisms (Kalia and Gupta, 2016). These fruits are consumed because they are accessible, cheaper than the whole fruits as well as lack of time to prepare a good meal. Their increased consumption coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern (Orji *et al*., 2016; Odebisi-Omokanye *et al*., 2015). Sometimes, it is difficult for one to attest to the hygiene of the processors or the sanitary conditions at the point of slicing. In addition, these fruits are prepared without adequate storage conditions, thereby exposing them to flies, dust and other pathogens (Barro, 2007). These fruits such as watermelon, pineapple, carrots, cucumber, tiger nuts, and pawpaw are sold by inexperienced vendors or local hawkers who lack proper education and know little about knowledge on food hygiene (Muinde and Kuria,

2005). These therefore increase the risk of food-borne diseases caused by a wide range of pathogens such as (*Salmonella* sp, *Staphylococcus aureus*, *Enterobacteriaceae*), fungi, viruses and parasites (Mensah *et al*., 1999). These microorganisms may invade the interior surfaces of the fruits during washing, peeling, slicing, trimming, packaging, handling and marketing (Barro *et al*., 2007; Khali and Mazhar. 1994). The use of dirty utensils as well as the open display of these fruits in a dirty environment encourage visits by flies, cockroaches, other insects and dust (Bryan *et al*., 1992).

This study therefore aimed at evaluating the microbiological quality of ready-to-eat fruits sold at different markets in Enugu metropolis, Enugu State, Nigeria, with the view of highlighting the health implications of consuming such fruits.

II. MATERIALS AND METHODS

STUDY AREA

This study was conducted in Applied Microbiology Laboratory unit, Enugu State University of Science and Technology. The fruit samples were collected from different fruit vendors in Abakpa market, Eke-Agbani market and Ogbette main market , Enugu State .which are named as A, B and C respectively.

COLLECTION OF SAMPLES

A total of forty (40) ready-to-eat fruit samples consisting of pawpaw (*Carica papaya*), cucumber (*Cucums sativus* L.),water melon(*Citrullus lanatus* Thumb.) and pineapple (*Ananas comosus* L.) were collected and put into different white polyethene bags to differentiate them based on the vendors/ markets they were bought from. They were taken to the laboratory for microbial enumeration.

SAMPLE PREPARATION

About 1g each of the fruit samples were weighed and mashed into a test tube containing 10ml of sterile peptone water as described by Oranusi and Olorunfemi (2011). This was mixed evenly in order to homogenize the mixture and this was labelled as the stock. A ten-fold dilution of the homogenates were made with sterile normal saline as diluents (Cheesbrough, 2006). A total of 9ml of the sterile normal saline was measured into eight test tubes. 1ml of the stock was collected using a pipette and serially diluted into the first test tube till the fifth test tube. 10^{-5} and 10^{-6} were used as the dilution factors and 0.1ml was taken from each factor and dispensed into a sterile petri-dish before the prepared media were poured. This was swirled properly and allowed to gel. These were done in duplicates. After gelling, the petri-dishes containing mannitol salt agar, nutrient agar, MacConkey agar and Salmonella Shigella agar were incubated at 37^{o}C for 24hr to obtain the bacterial counts while the petri-dishes that contained Sabouraud dextrose agar(SDA) were incubated at room temperature for 72 hrs to obtain the fungal counts. After incubation, the representative colonies on the plates were counted using colony counter which was then transferred into nutrient agar and SDA slants respectively for identification.

CALCULATION OF COLONY FORMING UNIT

The method described by Collins *et al*. (1989) for estimating bacterial counts was used to enumerate the total viable counts of the isolates. The number of colonies of the plates were multiplied by the reciprocal of the dilution factor and calculation was done for 0.1ml of the original sample and plating was done in duplicates for each dilution. An average count was taken to obtain the total counts.

CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATES

All the organisms isolated were sub-cultured in nutrient agar plates to obtain the pure cultures. The organisms were characterized to confirm them. Gram staining and other biochemical tests were carried out based on the method of Cheesbrough (2006). The biochemical tests performed included catalase, coagulase, oxidase, citrate, indole, methyl red test, glucose, lactose, sucrose, fructose and mannitol.

IDENTIFICATION OF FUNGAL ISOLATES

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation. The technique was also adopted for the identification of the isolated fungi using lactophenol cotton blue 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 ×10 and ×40 objective lens for its morphological characteristics (Cheesbrough, 2006).

STATISTICAL ANALYSIS

Statistical Package for the Social Science (SPSS) was used for the data analysis. Analysis of variance (ANOVA) was used to compute and arrived at statistical decision.

III. RESULTS

MICROBIAL LOAD OF READY- TO- EAT FRUIT SAMPLES

The different fruit samples were examined and their microbial loads were determined. The result is shown in Table 1.

Samples	Markets	Bacterial count (cfu/g)	Average bacterial count (cfu/g)	Fungal count (cfu/g)	Average fungal count (cfu/g)
Cucumber	A	$5.7x10^5$	$6.0x10^5$	$1.2x10^5$	$1.3x10^5$
	B	$6.8x10^5$		$1.1x10^5$	
	C	$5.4x10^5$		$1.5x10^5$	
Pineapple	A	$6.7x10^5$	$8.2x10^5$	$2.0x10^5$	$1.4x10^5$
	B	$8.3x10^5$		$1.0x10^5$	
	C	$9.7x10^5$		$1.2x10^5$	
Watermelon	A	$6.5x10^5$	$8.2x10^5$	$1.8x10^5$	$1.5x10^5$
	B	$9.3x10^5$		$1.5x10^5$	
	C	$8.9x10^5$		$1.3x10^5$	
Paw Paw	A	$6.3x10^5$	$6.9x10^5$	$1.9x10^5$	$1.7x10^5$
	B	$7.8x10^5$		$2.1x10^5$	
	C	$6.7x10^5$		$1.1x10^5$	

Table 1: The mean microbial load of the samples (cfu/g)

MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATES

Identification of the isolates were further confirmed by colony characteristics using Salmonella Shigella agar, MacConkey agar and Mannitol salt agar. The result is presented in Table 2.

Media used	Choromogenesis	Elevation	Shape	Suspected Organisms
Mannitol salt agar	Yellow	Convex	Round	*S. aureus*
MacConkey agar	Pink	Raised	Mucoid - Round	*Klebsiella* sp
MacConkey agar	Dark pink	Convex	Round	*E. coli*
Salmonella Shigella agar	Colorless	Convex	Round	*Shigella* sp
Salmonella Shigella agar	Black	Convex	Round	*Salmonella* sp

Table 2: Morphological characteristics of the isolates

BIOCHEMICAL IDENTIFICATION OF THE BACTERIAL ISOLATES

The isolates were further identified and characterized using Gram stain and other biochemical tests. The result is shown in Table 3.

S/N	Isolates	Gram reaction	Catalase test	Methyl red test	Citrate test	Coagulase test	Oxidase test	Indole test	Sugar fermentation: Lactose	Sucrose	Glucose	Mannitol	Fructose
1	*Staphylococcus aureus*	+ve cocci	+ve	+ve	+ve	+ve	-ve	-ve	A/G	A/G	A/G	A/G	A/G
2	*Escherichia coli*	-ve rod	+ve	+ve	-ve	-ve	-ve	+ve	A	A	A/G	A/G	-ve
3	*Klebsiella* sp	-ve rod	+ve	-ve	+ve	-ve	-ve	-ve	A	A/G	A/G	A	A/G
4	*Salmonella* sp	-ve rod	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	A/G	A/G	A
5	*Shigella* sp	-ve rod	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	A/G	A/G	A

Keys: A= Acid production G= Gas ProductionA/G= Acid and Gas production

Table 3: Results of biochemical test.

MACROSCOPIC AND MICROSCOPIC IDENTIFICATION OF FUNGI ISOLATED FROM THE SAMPLES

The fungi isolates which was isolated from the samples were characterized and identified macroscopically and microscopically. The isolated fungi were *Aspergillus* sp *and Candida* sp. The result is shown in Table 4.

MACROSCOPIC IDENTIFICATION	MICROSCOPIC IDENTIFICATION	ORGANISMS SUSPECTED
Creamy and glistering colour, with smooth surface	The cells were round to oval in shape, some few budding cell were observed. The cells stain retained crystal violet colour	*Candida* sp
Produce yellow to white colonies and hyphae turning black with the formation of conidia after five days	Septate hyphae	*Aspergillus* sp

Table 4: Macroscopic and Microscopic Identification of Fungi Isolated From The samples

PREVALENCE OF BACTERIA ON THE SAMPLES EXAMINED

Out of 40 ready to eat fruits (pineapple, watermelon, pawpaw and cucumber) samples that were analyzed, *Staphylococcus aureus* had the overall highest number of occurrence 28(70%) followed by *Escherichia coli* 25(62.5%), *Salmonella* sp 20(50%), *Klebsiella* sp 16(40%) and *Shigella* sp 15(37.5%). The result is presented in Table 5.

Sample	Total no of sample	*S. aureus*	*Klebsiella* sp	*Salmonella* sp	*Shigella* sp	*E. coli*
PINEAPPLE	10	6 ± 0.58a (60%)	3 ± 0.57a (30%)	4 ± 0.57a (40%)	3 ± 1.15a (30%)	7 ± 0.57a (70%)
WATERMELON	10	6 ± 1.15a (60%)	4 ± 1.15a (40%)	6 ± 0.58a (60%)	4 ± 0.58a (40%)	5 ± 0.58b (50%)
PAWPAW	10	9 ± 0.58b (90%)	7 ± 1.15b (70%)	5 ± 1.15a (50%)	6 ± 1.15b (60%)	6 ± 0.58a (60%)
CUCUMBER	10	7 ± 1.15a (70%)	2 ± 0.58a (20%)	5 ± 0.58a (50%)	2 ± 0.57a (20%)	7 ± 0.58a (70%)
TOTAL	40	28 (70%)	16 (40%)	20 (50%)	15 (37.5%)	25 (62.5%)

Table 5: Prevalence of bacterial isolates on the samples

Mean values with different alphabetic superscripts differ significantly within sample treatments (in a column) duration based on LSD analysis at $p < 0.05$, ($n = 3$).

PREVALENCE OF FUNGI ON THE SAMPLES EXAMINED

The samples were analyzed, *Candida* sp and *Aspergillus* sp were isolated from different fruit samples. Out of 40 ready to eat fruits (pineapple, watermelon, pawpaw and cucumber) samples that were analyzed, *Candida* sp 15(37.5%) had the highest number of occurrence than *Aspergillus* sp 7(17.5%). The result is presented in Table 6.

Sample	Total no of sample	*Candida sp*	*Aspergillus sp*
Pineapple	10	4 ± 1.15a (40%)	-
Watermelon	10	2 ± 0.57a (20%)	4 ± 0.57a (40%)
Pawpaw	10	3 ± 0.57a (30%)	-
Cucumber	10	6 ± 0.57b (60%)	3 ± 1.15a (30%)
Total	40	15(37.5%)	7(17.5%)

Table 6: Prevalence of fungal isolates on the samples

Means values with different alphabetic superscripts differ significantly within sample treatments (in a column) duration based on LSD analysis at $p < 0.05$, ($n = 3$).

IV. DISCUSSION

The microoganisms present in fruits in this study are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage and processing of the produce (Beuchat, 1996; Ray and Bhunia, 2007). In this study, the mean bacterial load of the samples showed that pineapple and watermelon gave a value of 8.2 x 10^5 cfu/g respectively while cucumber had a value of 6.0 x10^5 cfu/g and pawpaw gave a value of 6.9x10^5 cfu/g while the mean fungal load of watermelon and pawpaw are 1.5x10^5 cfu/g and 1.7x10^5 cfu/g respectively (Table 1). These values from different markets are high and could be as a result of mishandling and the practice of using the same bucket of water to wash all the fruits (Khali and Mazhar, 1994) as well as cross contamination using the same utensils to cut and display the fruits. The values obtained in this study is higher than the values gotten from the study done by Odebisi-Omokanye *et al.*(2015) whose mean total aerobic plate count ranged from 1.2 x 10^4 to 2.0 x10^4 cfu/g and fungal count ranged from 0.5 x 10^2 to 1.6 x10^2 cfu/g. This study is in agreement with the work done by Orji *et al*. (2016) who obtained the total aerobic count of 5.6 x10^5 in pineapple, 3.5 x10^5 in cucumber and 1.0 x10^6 in watermelon while the fungal count ranged from 1.1 x10^5 to 1.42 x10^6 cfu/ml. This study revealed the present of *Staphylococcus aureus*, *Klebsiella* sp, *Escherichia coli*, *Shigella* sp, *Salmonella* sp, *Candida* sp and *Aspergillus* sp (Tables 2 and 4). This study is in agreement with the works done by Odebisi- Omokanye *et al.*(2016); Orji *et al*. (2016); Jolaoso *et al*. (2010); Oranusi and Olurunfemi(2011) and Tambeker *et al*. (2009).These organisms isolated in this study may have been introduced into these fruits through faecally polluted water used in washing utensils like knives, trays and polyethene bags used for the packaging of the fruits after silicing or cutting and also exposure to low temperatures which encourage the microbial growth of these pathogens (Daniyan and Ajibo, 2011). Their presence could be through unclean hands of the vendors, contact with sewage and contaminated water (De Rover, 1998). This implies that the fruits samples could serve as a vehicle in the transmission of these pathogens to the consumers of these contaminated fruits (Orji *et al*., 2016).The dusty environment of the motor parks, busy roads coupled with unclean water used to sprinkle on the fruits could be contributing factors that aid the survival and multiplication of these microorganisms (Oranusi and Braide, 2012). The presence of these organisms in fruits could also be due to nutritional composition and availability of water in the fruit which are essential for the growth and survival of the microorganisms (Nwachukwu and Osuocha, 2014). This study revealed the percentage occurrence of the isolated organisms as thus: *Staphylococcus aureus* 28(70%), *Klebsiella* sp 16(40%), *Escherichia coli* 25(62.5%), *Shigella* sp 15(37.5%), *Salmonella* sp 20(50%), *Candida* sp 15(37.5%) and *Aspergillus* sp 7(17.5%)(Tables 5 and 6). This study is in agreement with the work done by Orji *et al*. (2016) even though *Escherichia coli* had highest percentage occurrence of 83.3%. The highest occurrence of *Staphylococcus aureus* in this study is in line with the works done by Eni *et al*. (2010); Kumar and Ganguli (2006). *Staphylococcus aureus* isolated from most of the fruits may have entered the fruits during packaging or handling since the organism is a normal flora of the human skin and nasal cavity (Hunter, 1993), hands and skins of healthy individuals (Nester *et al*., 2010). The presence of *Staphylococcus aureus* in fruits is of public health significance because it is usually responsible for Staphylococcal food poisoning (Frazer and Westhoof, 1995) and food intoxication (Nester *et al*., 2010). The presence of *Escherichia coli* in this study is similar to the works done by Daniyan and Ajibo (2011); Daniel *et al*. (2014); Iyoha and Agoreyo, (2015) and Odebisi-Omokanye *et al*. (2015).This could be through unclean hands of the vendors, contact with sewage and faecal contaminated water (De Rover, 1998). Faecal contamination can be through the customers who pick and drop to make choice thereby introducing microbes into the fruits. *Escherichia coli* is regarded as a primary indicator for faecal contamination, hence their presence showed that the fruits are not safe for human consumption. Some of these fruit vendors collect their water from dirty streams and could use very little quantity of water to wash or rinse all the fruits (Orji *et al*., 2016). The presence of *Aspergillus* sp and *Candida* sp in this study is similar to the work done by Odebisi-Omokanye *et al*. (2015). Their presence in fruits are of medical importance. These fungi are ubiquitous in nature, hence can thrive on fruits for nutrients. They can tolerate high concentration of sugar and salt (Odebisi-Omokanye *et al*. (2015).The production of spores by these fungi made it possible to thrive and multiply.

From this study, the fruits were found to contain bacteria and fungi which could lead to multiplication and food spoilage, hence rendering the fruits unsafe for human consumption. It is important to note that these fruits did not show any sign of spoilage when sampled, thus the outward appearance cannot be used to determine the good quality of

any fruits. Hence, any fruits bought should be washed before consumption.

V. CONCLUSION

The result from this study showed that poor hygienic practices by vendors, consumers and environmental factors could lead to microbial contamination of these vended fruits. Hence, fruit vendors should be educated on the effect of using untreated water for washing fruits and avoid blowing air into the polyethene bags used for packaging. The vendors should be educated to cover the fruits to avoid flies perching on them. Hands, utensils and trays used for cutting and slicing should be cleaned properly. Government and community health officers should help out in monitoring these vendors in order to minimize the risk of disease outbreak associated with consumption of contaminated fruits.

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