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MICROBIOLOGICAL EXAMINATION OF FRUITS SOLD AT ISI-GATE UMUAHIA, ABIA STATE.

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ABSTRACT

The presence of microorganisms on five fruits (Apple, Banana, water melon, pineapple and paw-paw were examined. Five genera of pathogenic bacteria: (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp, *Pseudomonas aeruginosa*, *Streptococcus spp*.) And three fungal genera (*Aspergillus spp*, *Saccharomyces cerevisae* and *Penicillium* spp). were successfully isolated from this study. Water melon carry the highest bacterial load $2.91x10^3$cfu/ml, while the lowest count was observed with Apple $1.10x10^3$cfu/ml, The Total Fungal Count ranged from $1.10x10^3$cfu/ml to $2.63x10^3$cfu/ml. Banana had the higher fungal count: 2.63 $x10^3$ cfu/ml while Apple had the lowest count: 1.10 $x10^3$ Cfu/ml. The Percentage occurrences of bacteria isolated from the samples revealed both *Staphylococcus aureus* (100%) and *Bacillus* spp (100%) to have the highest percentage occurrence while *Pseudomonas aeruginosa* (40%) had the least percentage occurrence. The percentage occurrence of fungal isolates reveals *Saccharomyces* spp. (80%) to have the highest percentage occurrence, while *Penicillium* spp. (40%) had the least percentage occurrence. The presence of these organisms in the fruit juice indicates that their consumption could be hazardous to health. This therefore calls for more hygienic practices in the production of these products.

Keyword: Fruits, Bacteria, Fungi, Microbial Examination

1. INTRODUCTION

Among horticultural crops, fruits are of great importance for an adequate and balance human diet. In certain part of the world, fruits are the major dietary staple (Eni *et al.*, 2010 ; Amoah *et al.*, 2009).

Fruits are cultivated in areas where the environmental factors are suitable for their growth. Umuahia Abia state is considered one of the areas that have good cultivating land for great yield of fruits and this is done usually during rainy season or use of irrigation during dry season. Irrigation water is achieved by different sources like lake, stream, river, ponds etc. which may be polluted with animal and human feaces. Due to high number of eggs, cyst and larvae of human intestinal parasites present in the waste water, the use of excreta polluted water is a health risk to both the farmers and the consumers that eat the produce raw and fresh, like apples, guava, pear and mango (Scolf, 1992).

Fresh fruits promote good health but harbor a wide range of microbial contaminations (Eni *et al.*, 2010). Increasing health awareness has led to an increased consumption of minimally processed fruits in recent years (Warriner *et al.*, 2009), as these do not require elaborate preparations (Amoah *et al.*, 2009). Microbial spoilage and contaminating pathogens pose a serious problem in food safety (Abadias, *et al.*, 2008; Warriner *et al.*, 2009). Fruits from sources like super markets may be protected from contamination and spoilage during subsequent handling, packaging, transport and storage (Park *et al.*, 2012).

Bacteria, viruses and parasites on fruits and vegetables have been linked with illness. Several cases of an outbreak of typhoid fever has been associated with eating contaminated vegetables grown in/or fertilized with contaminated soil or sewage (Beuchat, 1998). These outbreaks differ from a few persons to many thousands affected (SCF, 2002). Olsen *et al*., (2000) reported that diseases associated with the consumption of fruits and vegetables doubled between 1973 – 1987

and 1988 – 1992. In Canada, 18 outbreaks were documented from 1981 to 2000, with approximately 2000 people affected and 18. In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor for the outbreak of diseases (Amoah *et al*. 2009, Olayemi, 1997). The differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of non-resident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ofor *et al*., 2009).

Pineapple, water melon, apple, banana, and pawpaw are some of the fruits that are normally consumed raw in order to obtain their valuable nutrients in the best form. Fruits are good sources of nutrients for growth, repair and control of body processes as most of them contain sugar, vitamins, mineral elements ,small quantities of protein and oil (Duckworth,1996).

Due to their high nutritional contents, particularly sugar, and low pH, fruits and their products serve as breeding substrates for microorganisms whose activities constitute the most important causes of spoilage. Microorganisms from many sources such as agricultural environment, the vegetation as well as dead decaying materials, can contaminate fruits.

The ground where product is grown plays a vital role in safety of the product. If the area has a history of use for chemical waste or the processing of biosolids, this would present a potential source of contamination for crops. Adjacent land use also affects the safety of the crop grown. If fruit and vegetables are grown next to an animal-rearing operation, there is a potential for product to become contaminated by animals.

Zweietering (2002) stated that four categories of Microbiological quality have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous. Satisfactory results indicate good microbiological quality. No action required.

Marginal results are borderline in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the handling of the fruits. Unsatisfactory results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. Potentially hazardous, the levels in this range may cause food borne illness and immediate remedial action should be initiated. Actions consideration should be given to the withdrawal of suspected fruits still available for sale or distribution and recall of marketed fruits. In Nigeria, sellers as well as consumers are not well aware of the hygienic management in all steps of fruit production, processing, transportation, marketing and consumption (Rahman *et al.*, 2011). Report on water used for the washing of fresh tomatoes in major markets in south-eastern Nigeria has been shown to possess a high level of microbial contamination (Ofor *et al.*,(2009). The aim of this study is to evaluate the microbiological quality of fruits sold at Isi-gate Umuahia, Abia State

2. MATERIALS AND METHOD

2.1 STUDY LOCATION

This present study was conducted at Isi-gate, Umuahia metropolis, Abia state, Nigeria. Abia state is located in South-Eastern Nigeria. It is surrounded by four states namely Imo, Riverse, Akwa-Ibom and Ebonyi state. It has three senatorial districts and 27 local governments.

2.2 COLLECTION OF SAMPLES

Freshly harvested fruits samples; Paw-Paw (*Cacrica papaya*), Pineapple, Water melon, Apple (*Malus domestica*), and Banana (*Musa* sp) were bought from Isi-gate, Umuahia following the methods described by (Andrews and Hammock, 2003). They were collected using standard microbiological method of collection employing aseptic techniques according to Cheesebrough, (2005). They were packed in pre-sanitized plastic containers and transported to the Microbiology laboratory of Michael Okpara University of Agriculture, UmudikeAbia State Nigeria within 2-3 hours after collection.

2.3 MEDIA PREPARATION

The media used in the isolation of bacteria and fungi includes; Nutrient Agar (NA), MacConkey Agar (MA), Sabroaurd Dextrose Agar (SDA) and *Salmonella Shigella* Agar (SSA). All the media were prepared according to the manufacturer's specification as reported by Eliner *et al*. (1992), Cheesbrough (2003) and Oyeleke andManga (2008) and sterilized by autoclaving at 121°C for 15 minutes at 15 Psi, except for *Salmonella Shigella* Agar which was boiled up to 45°C. They were allowed to cool before pouring into sterile petri dishes. They were allowed to cool and solidified before inoculation of fruits samples. Nutrient agar slants were prepared for purification and storage of microorganisms. This was done by pouring the already prepared nutrient agar into a borju battle, it was allow to solidify by bending the bottle one side and it was stored into the refrigerator.

2.4 MICROBIOLOGICAL ANALYSIS

2.4.1 Sample preparation

The purchased fruits were washed and each sample was homogenized using an electric blender. 1g of each homogenate was aseptically dispensed into 9mls of sterile water in a test

tube. After mixing well, 1ml portion of the mixture was aseptically transferred to a separate test tube containing 9mls of distilled water. This was stirred very well using sterile glass rod. Then 10 fold serial dilutions (10^{-10}) were carried out. 1ml was transfer from the 1st test tube to the 2nd test tube, this was repeatedly done for all the test tubes till the 10th tube. From 10^{-3} dilution, 0.1 ml was plated on a different prepared media plates employing spread plate method. The aliquots were spread over the media using hockey stick for even spread and allowed to settle about 10 minutes before incubation. All the plates were incubated at 37°C for 24 hr, while SDA were kept under the room temperature for 5-7 days.

2.4.2 Total bacterial and fungal counts

The colonies that grew on the plates were counted using automated scan $_R$ 500 colony counter and the values are expressed as colony forming units (cfu)/ml.

2.4.3 Isolation of pure cultures

Pure cultures of isolates were obtained by repeated sub-culturing onto freshly prepared Nutrient media and the cultures were maintained on agar slants for further identification.

2.5 IDENTIFICATION OF BACTERIAL AND FUNGAL ISOLATES

Characterization and identification of bacterial isolates

Bacterial isolates were characterized based on microscopic appearance, colonial morphology and biochemical tests. The isolates were identified by comparing their characteristics with those of known taxes, as described by (Cheesbrough (2003), and Oyeleke and Manga (2008). Microscopic examination of each isolate was done to establish the shape and arrangement of the

cells as well as its reaction to general and specific dyes (stain) which in form show the presence and absence of specific features.

3. RESULTS AND DISCUSSION

The Total Bacteria Count (TBC) presented in Table 1 revealed the highest bacterial count with sample 3 Water melon, ($2.91x10^3$cfu/ml) followed by sample 4 (pineapple) ($2.88\ x10^3$ cfu/ml) while the lowest count was observed with sample 1 (Apple) ($1.10x10^3$cfu/ml).

The Total Fungal Count (TFC) were presented in Table 2. The Total Fungal Count ranged from $1.10x10^3$cfu/ml to $2.63x10^3$cfu/ml with the highest been recorded against Banana ($2.63\ x10^3$ cfu/ml), and the least recorded against Apple ($1.10\ x10^3$ cfu/ml).

Five genera of bacteria were isolated, characterized, and identified in this investigation. They are presented in Table 3. They include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus* sp and *Bacillus* sp.

Three fungal species were isolated from this work , they are presented in Table 4. They included *Aspergillus* sp, *Saccharomyces cerevisae* and *Penicillium* sp. They were identified based on their morphology and microscopy.

Percentage occurrences of bacteria isolated from the samples were presented in Table 5. Both *Staphylococcus aureus* (100%) and *Bacillus* sp (100%) has the highest percentage occurrence, while *Pseudomonas aeruginosa* (40%) had the least percentage occurrence.

The percentage occurrence of fungal isolates is presented in Table 6. Of the 5 samples used, *Saccharomyces* sp. (80%) has the highest percentage while *Penicillium* sp. (40%) had the least percentage occurrence.

Five bacteria and three fungal species were isolated and identified in this study namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus sp*, *Bacillus* sp, *Aspergillus* sp, *Saccharomyces cerevisae* and *Penicillium* sp. All the five bacteria and three fungi isolated in this study have previously been isolated from fruits and vegetables in other studies, both in Nigeria and elsewhere (Dunn *et al*., 1995; Oluwafemi and Simisaye, 2005). The microorganisms present in fruits are a direct reflection of the sanitary quality, harvesting, transportation, storage, and processing of the produce (Beuchat, 1996).

The Total Bacterial Count (TBC) revealed the highest bacterial count with water melon ($2.91x10^{-3}$cfu/ml) while the lowest count were observed with Apple ($1.10x10^{-3}$cfu/ml).The high microbial contamination observed in this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same water by the vendor or processor. More importantly, bacteria on the produce may multiply over time depending on the storage conditions and also be related to the hygiene level of the individual vendors (Montville and Matthews, 2008; Abadias *et al*., 2008) the high microbial load observed with water melon could be as the result of its crippling in nature and also it can be contaminated during the cutting process or when it been washed with contaminated water.

The Total Fungal Count (TFC) revealed the highest count with Banana ($2.63x10^{-3}$cfu/ml) while the least count were observed with Apple ($1.10x10^{-3}$cfu/ml). in this study, the low microbial

count observed with apple could be as the result of its high sugar content which is not favorable for bacterial growth and also its thick cover which makes it difficult for bacterial penetration . The high fungal count might be as a result of un-hygienic practices involved during harvesting of the fruit, dusty environment, presence of housefly perching on the fruit when in stale and using same bucket to wash all the fruits could lead higher microbial count (Wanyenya *et al*., 2004).

The presence of the fungal isolates *Aspergillus* sp, *Penicillium* sp and *Saccharomyces cerevisae* in this work can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing, cross-contamination from other fruits and or the use of dirty processing utensils like knives and trays (Bryan *et al*, 1992; Khali *et al,* 1994).

More so the presence of *Penicillium* and *Aspergillus* sp presence in this study could be due to the fact that these organisms are spore formers and are known common environmental contaminants; although, they have been implicated as food borne pathogens (Oluwafemi and Simisaye, 2005).

The presence of *E. coli*, *S. aureus*, *Bacillus* sp, *Streptococcus* sp, and *Pseudomonas aeroginosa* is generally an indication of faecal contamination of the water often used by vendors for washing their utensils, hands before selling the fruits.

The percentage occurrence of bacterial specie revealed *S. aureus* (100%) and *Bacillus* sp (100%) as the most prevalence of all the bacterial isolated the reason behind this could be that *S. aureus* are frequently found in the human respiratory tract and on the skin. They could have penetrated through the hands and displaying of the fruits samples. More so, the high occurrence of *Bacillus sp* in this study could be as a result of their ubiquitous in nature.

S. aureus may be introduced into the pre-packaged fruits during harvesting or washing of the fruits. The high rate of *S. aureus* as shown in this work is similar to the report of Kumar and

Ganguli (2006), where they reported high occurrence rate of *S. aureus* in food *S. aureus, E. coli* and *Bacillus* sp isolated in this study could be associated with the general poor sanitary environmental conditions under which the fruits were handled (Muinde and Kuria, 2005).

The percentage occurrence of fungal isolates in this survey observed highest percentage rate for *Saccharomyces* sp (80%) while in contrast, the least percentage was *Penicillium* sp (40%) and *Aspergillus* sp (60%). The high percentage occurrence of *saccharomyces* in this study could be as a result of their natural habitat on the surface of fruits.

Despite the high microbial counts obtained for some of the samples in this study, it is important to note that these samples did not show any visible signs of spoilage. Thus outward appearance may not be a good criterion for judging the microbial quality of fruit. All fruits should therefore be adequately washed before consumption either by the consumer or the processor and where possible.

Conclusion

In conclusion, the practice of having fruits sold by street vendors who may not be licensed and/ or untrained in food hygiene practices is not healthy because their method of preparation of such fruits are most times unsanitary and unhygienic, which exposes the consumer of the fruits to pathogens of public health importance.

As a result of the occurrence of potential food borne pathogens and the possible outbreaks of food poisoning, retailers and consumers are advised to wash fresh fruits properly before peeling, slicing or cutting. Also to handle and cut fruits with clean and sanitized utensils and surfaces and to store cut fruits at 4°C or below until sold or consumed. Generally, there is a need to

thoroughly educate the populace on the impending danger of poor hygiene with respect to vended unwashed fruit in order to reduce the occurrence of these organisms.

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Table 1: Total Bacterial Count

Sample	Total bacterial count (cfu/ml)x10^3
Apple	1.52 x10^3 cfu/ml
Banana	2.10 x10^3 cfu/ml
Water melon	2.91 x10^3 cfu/ml
Pineapple	2.88 x10^3 cfu/ml
Paw-paw	2.73 x10^3 cfu/ml

Table 2: Total Fungal Count

Sample	Total fungal counts (cfu/ml)x10^3
Apple	1.10 x10^3 cfu/ml
Banana	2.63 x10^3 cfu/ml
Water melon	2.34 x10^3 cfu/ml
Pineapple	1.50 x10^3 cfu/ml
Paw-paw	1.12 x10^3 cfu/ml

Table 3: Percentage occurrence of bacterial isolates

Sample	*Staph aureus*	*Bacillus* sp	*Pseudomonas spp*	*E- coli*	*Strep* sp
Apple	+	+	-	-	-
Banana	+	+	+	+	+
Water melon	+	+	-	+	-
Pineapple	+	+	+	+	+
Paw-paw	+	+	-	+	+
No positive	**5**	**5**	**2**	**4**	**3**
%occurrence	**100**	**100**	**40**	**80**	**60**

Keys: + = Present

- Absent

Table 4: Percentage occurrence of fungal isolates

Sample	*Saccharomyces* spp	*Penicillium* spp	*Aspergillus* spp
Apple	+	+	-
Banana	+	-	+
Water melon	-	-	+
Pineapple	+	-	-
Paw-paw	+	+	+
No positive	**4**	**2**	**3**
% occurrence	**80**	**40**	**60**

Key: + = Present

- = Absent