MICROBIAL QUALITY ASSESSMENT OF SLICED PINEAPPLE AND WATERMELON SOLD IN SOME SELECTED MARKETS IN KADUNA METROPOLIS, KADUNA STATE.

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ABSTRACT

Spoilage of fruits which results from the contamination by microorganisms into fruits through irrigation water, harvesting/processing equipment, improper storage, packaging and transporting, personal handling, soil, dust and even manure is a source of concern to human existence. To assess their microbial quality, sliced fruits sold at some selected markets within Kaduna metropolis were analyzed to determine the microorganism associated with the contamination. The bacterial mean counts in all the markets for Pineapple and Watermelon ranges between 1.5 x 10^5 – 9.5 x10^5, and 1.4 x 10^5 – 9.9 x 10^5 respectively. The fungal mean count of pineapple and watermelon in the markets ranges from 1.0 x 10^5 – 1.2 x 10^5 and 1.1 x 10^5 – 2.0 x 10^5 respectively. The bacterial isolates were *Staphylococcus spp, Escherichia coli, bacillus spp. While the f*ungal isolates includes, *Aspergillus spp, penicillum, Rhizopus, Fusarium* and yeast. The result reveals that the above microorganisms are responsible for microbial contamination of the fruits sold in the selected markets within Kaduna Metropolis. This therefore calls for serious attention in creating awareness in the control of human infectious diseases associated with consumption of contaminated raw fruits within the metropolis.

KEY WORDS ; Microbial quality, assessment, foodborne pathogens, pineapple, watermelon, markets.

INTRODUCTION

Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans are thus vital for health and well-being. Well balanced diets rich in fruits are especially valuable for their ability to prevent vitamin C and vitamin A deficiency and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006). Fruits are widely exposed to microbial contaminations through contact with soil, dust and water and by handling at harvest or during post-harvest processing. They therefore harbor a diverse range of micro-organisms including plant and human pathogens (Nguyen and Carlin, 1994; Dunn *et al*., 1995; Carmo *et al*., 2004). Differences in microbial profiles of various fruits result largely from unrelated factors such as resident microflora in the soil, application of non-resident

microflora in the soil via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ofor *et al*., 2009). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits is a major contributing factor to contaminations (Olayemi, 1997; Amoah *et al*., 2009).Fresh cut fruit consumption is increasing due to the rising public demand for convenience and awareness of fresh cut fruits for health benefits. The entire tissue of fruits is rich in bioactive compounds such as phenolic compounds, carotenoids and vitamins. Bacteriologically safe fruit are essential to maximize the health benefits promised by adequate consumption of those produce. Proper washing of fruits is essential for decontamination. Water supplemented with varying concentrations of organic acids such as acetic, citric and ascorbic acids has been shown to reduce microbial populations on fruits. Previous studies revealed that a vinegar dip resulted in a 3 to 6 log 10 decreases in the number of aerobic bacteria on parsley leaves, depending on vinegar concentration used and incubation time (Beuchat, 1998). Eating fresh fruits are essential for good health and balance diet, a large number of epidemiological studies have demonstrated that people who eat a diet rich in fruits have a lower risk of developing cancer and Cardiovascular disease with Chronic conditions such as cataracts, asthma and bronchitis. Currently, the most common fresh cut fruits in the tropical region are pineapple, melon, water melon, apple, pear and grapes, besides their attractive colors, tastes and aromas, tropical fruits have significant amount of bioactive compound with antioxidant capacity. Ready to eat fruits can be bought directly from street vendors or hawkers or at local markets and eaten immediately, that is without necessarily having to cut, peel or rinse them before consumption as they have already been prepared by vendors. One effective way of limiting microbial growth is increasing the acidity of a particular food by adding an acidic substance. Acids attack cell walls, cell membrane, metabolic enzyme, protein synthesis systems and the genetic material of micro – organisms (Angela *et al.*, 2010),

Pineapples (*Ananas comosus*) are a composite of many flowers whose individual fruitlets fuse together around a central core. Each fruitlet can be identified by an "eye", the rough spiny marking on the pineapple's surface. They have a wide cylindrical shape, a scaly green, brown on yellow skin and a regal crown of spiny, blue-green leaves and fibrous yellow flesh. The area closer to the base of the fruit has more sugar content and therefore a sweeter taste and a more tender texture than the upper part. Pineapples have exceptional juiciness and a vibrant tropical flavour that balances the tastes of sweet and tart. They are second only to bananas as America's favourite tropical fruits. Although the season for pineapple runs from March through June, they are available year-round in local markets. Thailand, Philippines, Brazil and China are the main producers of pineapple in the world supplying nearly 50% of the total world output. Other important producers include India, Nigeria, Kenya, Indonesia, Mexico and Costa Rica. Pineapple production is widespread in Ghana and is largely grown in the central and western regions of Ghana. In Ghana tropical fruits produced during bumper harvest are consumed fresh, sold at relatively cheap prices or allowed to go waste due to inadequate processing facilities (Yeboah and Kun Ze, 2004). Much of the fresh pineapple fruits go to the industry for slices and juice market. In Ghana, the volume of pineapple export in 2005 was 46,694 tonnes as against 71, 804 tonnes in 2004. This marks a percent change of -34.97%. Pineapple export in 2005 had a value of $12,784,300 ($12.7 million) as against $22,068,600 ($22 million) in 2004 representing a percent change of – 42.07%. These earnings from pineapples stand out as markedly high when compared to its closest competitor, banana (Wardy *et al.*, 2009).

MATERIALS AND METHODS

SAMPLE COLLECTION

Five samples each were purchased from four selected markets namely Majalisa, Chechenia, Kawo and Saturday markets (25pineapple and 25watermelon) in Kaduna metropolis and were transported to the Zoology Laboratory, Kaduna State University in a sterile universal containers for analysis. Commercially available dehydrated media were prepared following manufacturer's instructions.

SAMPLE PREPARATION

ENUMERATION OF BACTERIA

Twenty five grams of each sample was dissolved in 225ml of 0.1% peptone water to form the stock solution. 1ml of each stock sample was transferred aseptically into 9ml of 0.1% peptone water. Tenfold dilutions were then prepared using a sterile pipette i.e. 1ml of 10^2, 10^5, 10^6, dilution were aseptically transferred into sterile plate count agar. With the aid of sterile glass rod, the inoculum was spread on the surface of the culture media used. The inoculated plates were incubated at 37^0C for 48hours. The numbers of bacterial colonies counted, after the incubation periods were recorded as colony forming units' ml (cfu/ml).

GRAM STAINING REACTION

A thin film of each of the isolates was prepared by picking a bit of the growth from the plant into a drop of sterile distilled water on a glass slide using a sterile wire loop. After smearing, it was allowed to air dry before heat fixing. The crystal violet stain was then used to flood the smeared slide and allowed to remain for 10 seconds before the stain was washed off and the remaining stain mixed with iodine solution which was used to flood the slide to mordant for 10 seconds. After this, the side was rinsed with a gentle steam of distilled water. Alcohol

acetone solution was used to decolorize the slide after which was counter stained with safranine for 10 seconds and finally washed off with distilled water and allowed to air dry. The stained smear was later examined under the microscope using oil immersion objective. The purple or red appearance of the isolates indicated gram reaction positive (+ve) or negative (-ve) respectively.

CHARACTERIZATION OF ISOLATED BACTERIAL FLORA

Colonies to be identified were picked from standard plate count medium and maintained on plates of the same medium. A series of biochemical tests were carried out to characterize and identify each isolate.

Catalase Test

A drop of 3% hydrogen peroxide (H_2O_2) was placed on a glass slide and a bit of colony from the plate was taken with wire loop and emulsified with the hydrogen peroxide on the slide. A positive test was indicated by bubbling and frothing.

Coagulase Test

A clean slide was divided into 2 parts with grease pencil and a drop of physiological saline placed on each part under a specific condition. A colony of bacteria was picked and emulsified in each drop of saline and mixed with human plasma using a sterile wire loop. The slide was held up and titled back and front for 1 minute. A positive test was indicated by clumping and agglutination.

Motility Test

The motility medium was incubated by making fine stab with a wire loop containing isolate to depth of 1-2cm short of the bottom of the tube. The inoculated medium was then incubated

at 35^{0}C for 24-48 hours for motile organism the line of inoculation was not defined and the rest of the medium was somewhat cloudy. For non-motile organism, growth were restricted to the line of inoculation which became sharply defined, the rest of the medium remaining clear.

Indole Test

The isolate was grown in 5ml peptone water for 24hours. After the three drops of kovac'sindole reagent were added and shaken gently. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth within 1 minute while in the negative reaction, the indole reagent retained its yellow colour.

Voges Pros Kauer Test

The isolate was grown in 5ml of 5MRVP broth and incubated for 40-72 hours at 37^{0}C. After this, 1ml of the broth was transferred into sterile serological tubes. To the small quantity 5 drops of 40% potassium hydroxide (KOH) was added followed by 5 drops of 5% naphtol in ethanol. The tube was shaken and placed in a sloping position. The development of red colours starting from the liquid air interface within 1 hour.Indicated a voges pros kauer test. Absence of red colour indicated negative result.

Simmon's Cirate Agar Test

The isolate was inoculated into a Simmon citrate agar slant in a bottle and incubated for 24-72hours at 37^{0}C.The development of a deep blue colour indicated positive reaction.

Oxidase Test

A piece of fitter paper was moistened with a few drops of a 1% solution of oxidase reagent (Tetramethyl 1-p- phenylaminediaminedihydrochloride). A bit of the isolate was obtained with a sterile wire loop and smeared on the moistened portion of the filter paper. The

development of intense purpose color within 30 seconds indicated positive result; failure of development of an intense purple color within 30 seconds indicated negative test.

Isolation of Fungi

A sterile pipette was used to transfer of each of the fruit sample into the prepared Saboraud's dextrose agar (SDA) the sample was spreads out thinly and evenly in the SDA using a sterile bent glass rod, sterilized by dipping in alcohol. The spread sample was then incubated at room temperature (27-37^{0}C) for 24 hours before identification.

Identification of Yeast and Mould

The isolated yeasts and mould were identified by their colonial and microscopic characteristics, a wet mount preparation observing in lactophenol in cotton blue.

RESULT

All the fruits obtained in the selected markets were contaminated. Table 1 shows the total bacterial count of pineapple and watermelon found in different markets of Kaduna metropolis shows that the total bacteria count ranges from 4.1 x 10^5 - 8.6 x 10^5 and the mean of 1.7 x l0^5 - 9.5 x 10^5 cfu/g. The results also show that the total bacterial count for pineapple found in different markets of Kaduna metropolis ranges from 2.4 x 10^5 - 7.1 x 10^5 and the mean ranges of 1.4 x 10^5 – 9.9 x 10^5. Table 2 showed the total fungal count of watermelon in cfu/g, which ranges from 1.0 xl0^5 - 8.09 x 10^5 and the mean ranges from 1. 1 xl0^5 -2.0 x 10^5. Also the total fungal count of pineapple in cfu/g, which ranges from 5.3 x 10^6 – 6.3 x 10^5 and the mean ranges from 1.0 x 10^5 - 9.2 x 10^5 was shown in table 2.Table 3 shows the biochemical tests result for bacterial isolates and their colonial morphology. The bacterial isolated from fruits include *Bacillus* spp, *Staphylococcus* spp, *Escherichia coli.* The highest frequency that occurs in watermelon is *Staphylococcus* spp, (20%), followed by *Escherichia coli* (14%) and

Bacillus spp (6%), the highest frequency that occur in pineapple is *Staphylococcus* spp (19%), followed by *Escherichia coli* (13%) and *Bacillus* spp (8%).Table 4 shows the frequency of occurrence of fungal isolates in spoilt watermelon and pineapple fruits. The fungal isolates include *Aspergillus* spp, *Mucor, Penicillium, Rhizopus*, *Fusarium* and Yeast. In watermelon sample *Aspergillus* spp had the highest frequency of occurrence followed by *Mucor a*nd *Fusaruim* and Yeast the lowest. In pineapple same *Aspergillus* spp has the highest frequency, followed by mucor , Yeast and *Penicillum* lowest frequency.Table 4 shows the different fungi isolates identified in different market of Kaduna .*Aspergillus* spp and *Mucor* were isolated in all the four markets, *Penicillum* was only isolated in Chechenia and Kawo markets.Yeast and *Rhizopus* were isolated in Kawo, Majalisa ands Saturday markets. *Fusarium* was not observed in Kawo market.

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TABLE1: Total bacterial counts of watermelon and pineapple sold in some market within Kaduna metropolis

Market Location	Watermelon Total Bacteria	Watermelon Bacterial Range	Watermelon Mean	Pineapple Total Bacteria	Pineapple Bacterial Range	Pineapple Mean
Chechenia market	8.6×10^5	1.76×10^5 - 2.30×10^5	1.7×10^5	2.4×10^5	1.92×10^5 -3.05×10^5	4.8×10^5
Kawo market	7.9×10^5	1.90×10^5-3.05×10^5	1.5×10^5	4.9×10^5	2.00×10^5 -2.50×10^5	1.4×10^5
Majalisa market	4.7×10^5	1.96×10^5-2.67×10^5	8.2×10^5	3.1×10^5	2.0×10^5-3.50×10^5	6.2×10^5
Saturday market	4.1×10^5	1.95×10^5-3.10×10^5	9.5×10^5	7.1×10^5	1.74×10^5-2.65×10^5	9.9×10^5

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TABLE 2: Total fungal counts of watermelon and pineapple sold in some market within Kaduna metropolis

Market Location	Watermelon Total Fungi	Watermelon Fungal Range	Watermelon Mean	Pineapple Total Fungi	Pineapple Fungal Range	Pineapple Mean
Chechenia market	5.9×10^5	2.00×10^5 -3.45 x 10^5	1.1×10^5	6.3×10^5	2.02×10^4 - 3.02×10^5	1.2×10^5
Kawo market	8.09×10^5	2.19×10^5 -2.95 x 10^5	1.6×10^5	4.4×10^5	2.04×10^5- 3.09×10^5	1.0×10^5
Majalisa market	5.7×10^5	1.95×10^5-3.00 x 10^5	1.1×10^5	4.6×10^5	1.62×10^5- 2.99×10^5	9.2×10^5
Saturday market	1.0×10^5	2.08×10^5-3.01 x 10^5	2.0×10^5	5.3×10^5	1.92×10^5- 2.82×10^5	1.0×10^5

TABLE 3: BIOCHEMICAL TEST OF BACTERIAL ISOLATE

Cultural name	Gram's Xtics	Catalase reaction	coagulase	motility	citrate	methylred	VP	indole
Round raised smooth pink colony	**Gram -ve rod**	+	-	+	-	-	+	+
E. coli								
Creamy raised round colonies	**Gram +ve**	+	+	-	-	-	+	-
***Staphylococcus* Sp, with smooth edges**							**cocci**	
Entirely round Creamy *Bacillus Sp* Colonies	**Gram +ve rod**	-	-	+	-	+	-	-

Key = - ve = negative, + ve = positive.

TABLE 4: Frequency of Occurrence of the Various Fungal Isolates

LOCATION	SAMPLE	ASPERGILLUS SPP.	MUCOR SPP.	RHIZOPUS SPP.	PENICILIUMSPP	FUSARIUM	YEAST
MAJALISA MARKET	WATERMELON	5(27.77)	4(22.22)	3(16.66)	4(22.22)	0(0)	2(11.11)
	PINEAPPLE	6(42.85)	4(28.57)	2(14.28)	0(0)	2(14.28)	0(0)
SATURDAY MARKET	WATERMELON	2(25)	1(12.5)	2(25)	2(25)	1(12.5)	0(0)
	PINEAPPLE	2(20)	0(0)	2(20)	2(20)	2(20)	2(20)
KAWO MARKET	WATERMELON	1(12.5)	1(12.5)	3(37.5)	1(12.5)	2(25)	0(0)
	PINEAPPLE	3 (37.5)	1(12.5)	3(37.5)	1(12.5)	0(0)	0(0)
CHECHENIA MARKET	WATERMELON	2(16.67)	2(16.67)	2(16.67)	2(16.67)	2(16.67)	2(16.67)
	PINEAPPLE	1(25)	1(25)	0(0)	1(25)	0(0)	1(25)

Percentage occurrence is given in parenthesis

DISCUSSION

All the fruits obtained in the selected markets were contaminated with microbes. The microorganisms present in the fruit are as a result of the sanitry quality of the cultivation water, transportation, harvesting, storage and processing of the fruits for consumption (Beuchat,1996; Ray and Bhunia,2007). The high contamination of the sample fruit observed may be as a result of the processing procedure and the long period of storage of the produce kept before usage.All the bacteria isolated in the study were previously isolated from fruits in other studies elsewhere (Dunn *et al.*,1995; Adebolu and Ifesan 2001; Uzeh *et al.*, 2009). The result of bacteria and fungal counts shows that samples collected from the markets were high , this could be as the result of transfer to produce and cross contamination between the produce during prewashing

with the same wash water by the vendor.This could be attributed to unhygienic conditions and practice as well as the extent of exposure to dust under which they are displayed. Also improper storage conditions can encourage growth of pathogen on produce. Most of the organism isolated in the study might have been introduced into these fruits from fecal polluted water used for washing utensils (e.g. knives, trays, and pans), wrapping materials and the exposure of the product to low temperature. It may also be a result of the failure of food handlers to observe basic sanitary rules. Bacteria are indicators of some degree of potentially hazardous contamination. Among the genera of bacteria isolated in the study, *Staphylococcus* spp was predominant in both the fruits samples. The contamination could be as a result of discharge into the atmosphere through sneezing or coughing or even to the manner in which the fruits are hawked and sold that continually predisposes them to contamination. *E coli* were also isolated in this study. *E. coli* count in fruits is widely used and accepted as indicators of fecal contamination (Abbas and Mohammed, 1986 and Prescott, 1999). *Staphylococcus epidermis* might have been introduced from handlers being a normal flora on the skin of human. These organisms are known to be associated with food poisoning or food infection. Also outbreak of food borne diseases such as consumption of contaminated fruits other genera isolated from the tested sample includes *Bacillus spp.* The presence of this organism in fruits can be due to ecological and environmental influence since their survival in the atmosphere depends on a number of factor such as nature of microorganism, susceptibility to changes, resistance to new physical environment and their ability to form resistant strains. Dust particles become airborne at intervals during period of human activities in market home and enclosed environments.

The environmental factors such as temperature, humidity and harmattan wind, favour the spread of spores and whole organisms or fragments from one locality to another. Wind creates dust from soil which carries microorganism that inhabits the soil onto food sample during processing.

The commonest genera of fungi that were isolated and identified included *Aspergillus* spp, *Mucor*, *Penicillum* spp, *Rhizopus*, *Fusarium* spp, and Yeast. Poor handling, inadequate transportation system, poor packaging and also congestion of the fruits in containers or bags during transit could be a pore puncture on the fruits thereby making it easier for fungi and other microorganisms to penetrate the biological barriers that is the outer covering.

Fungi especially mould secrete mycotoxin which cause serious intoxication in man and contamination of organism could be attributed to poor hygienic, poor storage condition.

Environment to which fruits are normally exposed makes products come in contact with large number of different types of microorganism. Some fungi produce toxins that are carcinogenic. The most thoroughly studied of these carcinogenic toxins are produced by species of *Aspergillus* which are called aflatoxins. Ingestion of aflatoxins in moldy food has been implicated in development of liver cancer. (Nester, 2004).

CONCLUSION

Microorganism associated with the spoilage of pineapple and watermelon include *Staphylococcus* spp, *Bacillus* spp, *E. coli*, *Aspergillus*, *Mucor*, *Rhizopus*, *Penicillium* spp, *Fusarium* spp, Yeast, all were documented in this study as the common bacteria and fungi flora of fruits. Because these microflora are widely distributed in the soil, air, water, and the spore formers are so resistant, their control should be of great economic concern to the food processing industry. To prevent the occurrence of possible health hazard due to contaminated fruit, measures have to adopt in order to minimize fruits contamination of these fungi. The spray of

chemical to the soil will help to prevent the spread of soil fungi which constitutes the major source of contamination.

Proper storage facilities should be employed. An improved means of transportation should be facilitated this can be achieved by proper loading of fruits to areas they are sold. And at the market, the frequent removal of spoiled fruits from the healthy ones will help in checking the horizontal contamination. In addition the removal of over-ripped fruits also will help in checking the rate of contamination and subsequent spoilage, since over-ripped fruits are pore to quick spoilage than unripe one.

Fruits should not been spread on bare floors of storage rooms, the floors should be disinfected and similarly, the carpet or any suitable materials on which the fruits are spread. The practice of sprinkling water on the harvested fruits should contain disinfectants.

Since fruits are highly nutritional foods consumed by all groups of people ranging from infants to the elderly in Nigeria, its microbiological quality should be enhanced. This could be done by improving the processing procedure so as to help to reduce contamination.

RECOMMENDATIONS

Public awareness of the danger these contaminated fruit can caused to the health status of the consumer should be created due to unhygienic handling of the product. Proper practices, cleanliness, proper handling and washing of fruit before sale should be followed and highly stressed .Consumers should thoroughly wash fruits before consumption particular when given to children .Fruits handlers and vendors should be educated on proper sanitary way of processing the product in order to reduce microbial contamination and poisoning. Conferences and workshops to address the control of human infection associated with consumption of contaminated raw fruits and vegetable should be organized.

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