
MICROBIOLOGICAL QUALITY OF READY -TO-EAT SLICED WATERMELON (*Citrullus Lanatus*) SOLD IN LOKOJA MARKETS AND THE BACTERIAL ISOLATES ANTIBIOGRAM

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ABSTRACT

Microbiological assessment of two hundred and forty samples of ready-to-eat sliced watermelon fruits sold in Lokoja markets, Kogi State was carried out to determine their microbiological quality. Potato Dextrose Agar and Nutrient Agar were used for culturing and isolation of microorganisms from sliced watermelon using pour plate technique. Bacterial isolates were identified by biochemical test while fungal isolates were identified based on their colonial and microscopic morphology. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Proteus* sp are the bacteria isolated while the fungi isolated are: *Aspergillus niger*, *Alternaria* sp, *Mucor* sp and *Rhizopus* sp. The bacterial load was in the range of $2.97-6.60 \times 10^5$ while the fungal load was in the range of $3.33-4.92 \times 10^4$. Results of the antibiotic sensitivity test showed that the Gram positive isolate; *S.aureus* was most sensitive to pefloxacin while the Gram negative isolates were most sensitive to ciprofloxacin. The result of the microbiological assessment of sliced watermelon sold in Lokoja markets suggest that they are of poor microbiological quality as such it is recommended that sliced watermelon fruits are processed, packed and sold under hygienic condition.

Keywords: *Microbial load, Percentage of occurrence, Bacterial Isolates, Fungal Isolates and Biochemical test.*

1.0. INTRODUCTION

Watermelon (*Citrullus lanatus*) is a plant species of the family Cucurbitaceae. The watermelon plant is a vining plant with thin grooved extended branches that produce spherical, light to dark green fruit that has red core (Abu-Nasser and Abu-Naser, 2018). It produces solitary yellow flowers and deeply lobed pinnate leaves. Watermelons are predominantly grown in the Northern part of Nigeria and sold to other parts of the country

where they are bought by fruit vendors and sold either whole or in slices. This nutrient rich watermelon is a fruit of choice because of its numerous health benefit and great thirst quenching ability (Szalay, 2017). Microbial contamination of fruits at any point of their production, distribution, processing or preparation can cause food spoilage and human infections by pathogenic bacteria, viruses or parasites (Harding, *et al.*, 2017). Watermelon

grows on the grounds and comes in contact with pathogens that remain on their rind and eventually contaminate the inner part of the watermelon when they are cut open thereby increasing the risk consumers are exposed to (Federal Institute for Risk Assessment, 2013).

The sale of sliced watermelon is a very common business in Nigeria which leaves much to be desired considering the handling process of watermelon from farm to fork which is further compounded by the fact that watermelon is being processed for sale by vendors who do not have the knowledge of how to prevent microbial contamination. This calls for concern because food borne outbreaks linked to fruits and vegetables is on the increase worldwide (Elias, 2018).

The importance of carrying out the microbiological analysis of watermelon fruit cannot be over emphasized because microbiologically safe watermelon fruit are essential for maximizing the health benefits obtainable from adequate consumption of watermelon. The microbiological quality of sliced watermelon sold in Lokoja market is being investigated so as to find out how safe for consumption they are because of the prevailing circumstance of poor hygiene practice by the fruit vendors.

2.0 MATERIALS AND METHODS

2.1 Study Population/Site

Lokoja is situated at 7.8° North Latitude, 6.74° East Longitude and 55 meters elevation above the sea level. It is the capital of Kogi State and a confluence city in North Central part of Nigeria. The study population included sixteen vending points from four markets (Old market, New market, Phase one market and Adankolo market).

2.2 Sample Collection

Two hundred forty samples of sliced watermelon were purchased from sixteen vending points from the month of June to

September, 2016 and taken to Federal University Lokoja laboratory for microbiological analysis.

2.3 Microbiological Analysis

Twenty five grams (25g) of five slices of homogenized watermelon were weighed aseptically and transferred into 225ml of the sterile diluent, 0.1% peptone water to form the stock solution. 1ml of the inoculums from the stock solution was serially diluted in a set of test tubes containing 9ml of 0.1% peptone water from which further serial dilutions were carried out using the method of Daniel *et al.*, 2014 with slight modification. Pour plate technique was used for culturing and isolation of the microorganisms using nutrient agar and potatoes dextrose agar for the isolation of bacteria and fungi respectively at an incubation temperature of 37°C for 48 hour for bacterial growth and 28°C for 2-7 days for fungal growth. The colonies formed were counted and expressed as colony forming unit per gram (cfu/g). The bacteria isolated were identified using biochemical test: indole, simmon citrate, motility, methyl red, voges proskauer, catalase, oxidase and sugar fermentation test while the fungal isolates were identified based on their standard cultural and morphological characteristics as described by Barnett (1960).

2.4 Antibiotic Susceptibility Test

Antibiotic susceptibility test was carried out by using disc diffusion method on Mueller–Hinton agar according to National Committee for Clinical Laboratory Standards. Commercially available antimicrobial discs were used and plates were incubated at 35°C. Zones of inhibition were interpreted as resistant, intermediate and sensitive using the interpretation chart of zone sizes of Kirby-Bauer sensitivity test method as described by Cheesbrough (2006).

2.5 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the microbial load of sliced watermelons from the four markets using SPSS 20.

3.0 RESULT AND DISCUSSION

3.1 Result

3.1.1 Mean Microbial Load of Bacterial and Fungal Isolates

The mean microbial load of fungi and bacteria are presented on Table 1. New market had the highest bacterial load of $6.60 \pm 1.69 \times 10^5$ cfu/g while phase one market had the least bacterial load of $2.97 \pm 0.72 \times 10^5$ cfu/g. New market also had the highest fungal load of $4.92 \pm 0.92 \times 10^4$ cfu/g while Adankolo market had the least fungal load of $3.33 \pm 0.14 \times 10^4$ cfu/g as shown in Table 1.

3.1.2 Percentage of Occurrence of Bacterial and Fungal Isolates

S.aureus (34.2%) is the most occurring bact. followed by *E. coli* (31.3%), *P. aeruginosa* (16.6%), *K. aerogene* (10.4%) and *Proteus sp* (7.6%) as the least occurring bacteria as seen in table 2. *Aspergillus niger* (34.5%) is the occurring fungi followed by *Rhizopus sp* (28.9%), *Mucor sp* (24.4%) and *Alternaria sp* (12.2%) as the least occurring fungi as seen in Table 2.

3.1.3 Antimicrobial Sensitivity Test

The Gram positive bacteria *S.aureus* was most sensitive to pefloxacin and least sensitive to septrin as shown in Table 3. The Gram negative bacteria: *Klebsiella spp*, *Proteus spp*, *E.coli* and *P.aeruginosa* were most sensitive to ciprofloxacin and were not sensitive to vancomycin as shown in Table 3.

Table 1 Mean microbial load of bacteria and fungi isolates from sliced watermelon collected from four Lokoja markets.

Market Location	Bacteria Load x10 (cfu/g)	Fungi Load x10 ⁴ (cfu/g)
New Market	6.60±1.69 ^a	4.92±0.29 ^b

Old Market	4.88±1.32 ^a	3.65±0.39 ^a
Phase Market	2.97±0.72 ^a	3.92±0.37 ^a
Adankolo Market	4.43±1.20 ^a	3.33±0.14 ^a

Values with same alphabets in a column do not differ significantly at p>0.05, cfu/g-colony forming unit

Table 2: Percentage of Occurrence of Bacterial and Fungal Isolates

Bacterial isolates	Percentage of occurrence	Fungal isolates	Percentage of occurrence
<i>S.aureus</i>	34.2	<i>Mucor sp</i>	24.4
<i>E.coli</i>	31.3	<i>Rhizopus sp</i>	28.9
<i>Proteus sp</i>	7.50	<i>Apergillus niger</i>	34.5
<i>P.aeruginosa</i>	16.6	<i>Alternaria sp</i>	12.2
<i>k.aerogenes</i>	10.4		

Table 3. Antibiotic susceptibility profile of the Gram positive isolate (*S.aureus*)

Antibiotics	Disc concentration (µg)	Zones of inhibition (mm)
Seprtrin	30	5.00
Chloramphenicol	30	9.00
Ciprofloxacin	10	19.00
Sparfloaxcin	10	16.00
Pefloxacin	30	21.00
Tarivid	10	17.00
Gentamycin	10	10.00
Streptomycin	30	10.00
Amoxicillin	30	12.00
Augmentin	30	11.00

Zone of inhibition of: ≥ 18 mm (sensitive), 13 - 17 mm (intermediate), < 13 mm (resistant).

Table 4. Antibiotics susceptibility profile of Gram Negative isolates.

Antibiotics	Disc Conc. (µg)	Zone of inhibition (mm)			
		<i>E.C</i>	<i>K. A</i>	<i>P. A</i>	<i>P.S</i>
Erythromycin	15	6.00	11.00	9.00	16.00
Gentamycin	10	19.00	23.50	18.00	12.00
Ampiclox	30	3.00	9.00	6.00	19.00
Chloramphenicol	30	27.00	10.00	7.00	9.00
Ciprofloxacin	30	20.00	26.00	23.00	21.00

Amoxicillin	10	5.00	10.00	5.00	9.00
Ampicillin	10	5.00	9.00	5.00	7.00
Septtrin	30	3.00	8.00	4.00	8.00
Tetracycline	30	4.00	7.00	3.00	5.00
Vancomycin	30	0.00	0.00	0.00	0.00

* Zones of inhibition of: ≥ 18 mm (sensitive), 13 – 17 mm (intermediate), < 12 mm (resistant).

E.C-*E.coli* K.A- *K. aerogenes* P.A-*P.aeruginosa* P.S-*Proteus .species*

3.2 Discussion

Food including fruits is important and beneficial to the body but can be a source for transmission of diseases that can lead to death when they are contaminated with harmful microorganisms (Alum *et al.*, 2016). Medical literature reveals that microbiological contamination of food products is the most common form of food contamination that causes illness (Scallan *et al.*, 2011).

All the watermelon fruits samples were found to be contaminated with bacteria and fungi which is an indication that the food hygiene practice of the fruit vendors is poor. The microorganisms isolated in this research reflect different aspects of food hygiene and safety of sliced watermelon sold by fruit vendors in Lokoja markets because the microbial safety and quality of food are determined by the kind and number of microorganisms that occur in them (Deak, 2003). For instance, the isolation of unsatisfactory level of *S.aureus* from sliced watermelon is an indication that the sliced watermelons are being processed (cut and sliced) with bare hands and displayed for sell at high temperature which promotes the contamination and proliferation of *S.aureus* in sliced watermelon as asserted by Center for Food Safety (2006) that an unsatisfactory level of *S.aureus* indicate that time/temperature abuse of food is

likely to have occurred following improper handling of food with bare hands. The isolation of *E.coli* from sliced watermelon in this research provides direct evidence of faecal contamination; probably resulting from poor personal hygiene (hands not being washed thoroughly after using the toilet) during cutting, slicing and packaging of sliced watermelon (Centre for food safety, 2006). It is no surprise that there is a high number of the coliform group, *E.coli* because watermelon has a high water activity which according to Ferrati *et al.*, (2005) products with high water activity posses good amount of unbound water molecule that support the growth and survival of microorganisms. The isolation of *P.aeruginosa* is an indication of contamination from water, soil, sewage and faeces more so that *P.aeruginosa* can multiply in water environments and on the surface of suitable organic material in contact with water (Centre for Food, 2014)

The isolation of *P.aeruginosa*, *Proteus sp*, *klebsiella aerogenes*, *S.aureus*, *E.coli*, *A.niger*, *Rhizopus spp*, *Alternaria spp*, and *Mucor spp* from sliced watermelon can be linked to a number of factors such as improper handling, use of contaminated water for washing of the fruits, flies infestation, and use of improperly washed utensils like: knives and trays. Other sources of contaminations are from the dust containing microbes and their spores. Watermelons not being washed before they are cut up favours contamination by microorganisms like: *Alternaria spp*, *Pseudomonas spp* and *k.aerogenes* that greatly abound in the soil. The presence of *k.aerogenes* and *Alternaria sp* can be

due to contamination from the soil, unclean water and dust in the environment. Handling of soiled notes and currency by street vendors might also act as vector for the transmission of pseudomonas in sliced watermelon samples (Barro *et al.*, 2006).

According to Nwanchukwu *et al.* (2008), fungi contaminants on sliced watermelon are from water and the environment (spores in the air). Mailafia *et al.*, (2017) implicated fungi as contaminations of fresh fruits and isolated 38% of *A.niger* from watermelon fruits. The presence of fungi isolates might be due to the open space prone to flies visitation and airborne spores contamination that watermelon is processed and sold since the sliced water melon are left exposed before they are tied up in a polythene bag.

Almost all of the bacteria and fungi isolated in this study have been isolated by various researchers in some other part of the Nigeria from sliced watermelon except for the fungi *Alternaria*. Daniel *et al.*, (2014) isolated *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Mucor spp* and *Aspergillus spp* in Bida, Niger State. Nwanchukwu *et al.* in 2008 isolated *E. coli*, *Proteus mirabilis*, *S.aureus*, *Rhizopus stolonifer* and *Mucor spp* in Umuahia, Abia State. Oranusi and Olorunfemi, 2011 isolated *S.aureus*, *E.coli*, *E. aerogenes*, *Proteus spp* and *A. niger* in Ota, Ogun State. Daniyan *et al.*, 2011 isolated *S.aureus*, *E.coli*, *E. aerogenes*, *Mucor spp* and *A. niger* in Minna, Niger State, Afolabi and Oyebode in 2014 isolated: *E. coli*, *Pseudomonas spp*, *S. aureus*, *Proteus mirabilis*, *Mucor spp*. and *R. Stolonifer*. Eni *et al.*, in 2010 isolated: *S. aureus*. Ijah *et al.*, in 2015 isolated: *S.*

aureus, *Pseudomonas*, and *A. niger*; Chukwu *et al.*, in 2009 isolated: *E. coli*, *S. aureus*, *Proteus spp*, *E. aerogenes* in Kano State.

The isolation of microorganisms from ready-to-eat sliced watermelon by different researchers from different part of Nigeria is an indication that consumers should be weary of buying and consuming sliced watermelon since most of them are contaminated with microorganisms that can cause various diseases; commonly diarrhea.

According to World Health Organization (2015), 2.2 million people worldwide die every year from eating contaminated food and water. Similar microbes may isolated because sliced watermelon are sold in Nigeria by vendors with little knowledge of hygiene as such they have the same poor processing technique.

The sliced watermelons sampled had a higher bacterial load of $1.0 - 9.9 \times 10^5$ (cfu/g) than its fungi load of $2.0 - 5.0 \times 10^4$ (cfu/g) because of the high water activity and high pH (alkalinity) of watermelon which is in accordance with the assertion of Ndife *et al.*, (2013) who stated that food acid dictates the dominant microflora in food to a large extent (fungi dominate in acidic food while bacteria dominate in alkaline food). As such since watermelon is alkaline, bacteria will thrive in it more than fungi that are acid loving. More so that bacteria will readily inhabit a fruit with high water content than fungi. The bacteria load ($1.0 - 9.9 \times 10^5$ cfu/g) obtained from the sliced water melon sold in Lokoja market is similar to the bacteria load of $3.0 - 9.3 \times 10^5$ (cfu/ml) obtained by Adetesan *et al.*, (2013) and Daniyan *et al.*, (2011) with a bacterial load of $3.5 \times 10^4 - 9 \times 10^5$ while the mean fungi load of $2.0 - 5.0 \times 10^4$ cfu/g

in this study is similar to the fungi load of $0.5 - 3.1 \times 10^4$ cfu/g obtained by Afolabi and Oyeboode (2014) from sliced watermelon

Afolabi and Oyedele (2004) deduced that the presence of *Mucor* spp and *Rhizopus stolonifera* might be due to dusty and dirty environment.

The result of the antimicrobial sensitivity of the only Gram positive bacterial isolate, *S. aureus* corresponds with the report of Srinu *et al.*, (2012) that reported that *S. aureus* was sensitive to the antibiotic; ciprofloxacin and Adetesan *et al.*,(2013) reported that *S. aureus* was resistant to the antibiotic; amoxicillin. The antibiotic sensitivity of Gram negative isolates correspond to that of Marwa *et al.*, (2012) and Adetesan *et al.*, (2013) that isolated, *Proteus* spp and *E.coli* (from sliced watermelon) that are sensitive to ciprofloxacin. Adetesan *et al.*, (2013) isolated *E.coli* that are sensitive to chloramphenicol; *E.coli* and *Proteus* spp that are resistant to amoxicillin which is similar to the result obtained in this research.

All the watermelon samples collected from the various markets are considered unsatisfactory going by their microbial load and the criteria for fruits and vegetables (fresh) stated in microbiological guidelines for ready-to-eat food recommended by Centre for Food Safety (2014) that the colony forming unit per gramme (cfu/g) of *E.coli* should be less than 100 (<100) in every one gramme (1g) of fruit sample and should be less than 1000 (<1000) for *S.aureus*.

4.0 Conclusion

The result of this study showed that the microbiological quality of all the sliced watermelon samples examined from the

four markets in Lokoja are unsatisfactory as such fruit vendors and consumers are advised to follow good hygiene practice such as washing, peeling, slicing, handling and cutting fruits using clean and sanitized utensils and surfaces and finally storing cut fruits at 4°C or below until served or sold.

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