MICROBIOLOGICAL EVALUATION OF GREEN VEGETABLES AND FRUITS SOLD IN ADO EKITI KINGS MARKET (OJA OBA)

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ABSTRACT

Vegetables are good to be consumed fresh because they are sources of various components of food that support a healthy living, but evidence showed that they encourage the growth of microbes that cause spoilage of foods and equally cause adverse effect on the health of human beings (the consumers). Microbial analysis was carried out on ten different fruits and vegetables, such as Amaranthus cruentus, Lettuce (Lactuca sativa) , Senecio biafrae, Corchorus oliforus, Celosia argentea, Cuccumber(Cucumis sativus), Talinum triangulare, Venonia amygdalina, Cabbage (Brassica oleracea) and Moringa oleifera. Celusia argentae had the highest count of *Salmonella* $5.6x10^4$ and the least count of *E.coli* $2.1x10^4$, cucumber had counts of *Salmonella* $5.4x10^4$ and the least count of *E. coli* $1.8 x10^4$ Six bacteria that belong to different genera namely *Staphylococcus aureus* , *Salmonella sp., Bacillus subtilis, Escherisia coli, Pseudomonas aeroginosa and Erwinia sp.*were isolated, while some fungi sp were also isolated and identified.

Keyword: Fresh vegetable, Microbial analysis, Consumers, Bacteria, fungi.

INTRODUCTION:

In recent years, vegetables and fruits consumption has increased drastically due to their nutritional values and their contributions to health, they are important part of a healthy diet (Vandekinderen *et al*., 2008). These fruits and vegetables are full of variety of vitamins and minerals, because of these vitamins and minerals, fruits and vegetables are mandatorily added to every healthy diet (Hackett, 2012; Prentice and Jebb, 2003). Fruits that are produced in Nigeria include Pawpaw, Pineapples, Citrus fruits (Oranges, Grapes, Lemon Tangerin), Cucumber, Mango, Guava. While vegetables include Amaranthus, Ugu , Bitter leaf, Water leaf, Tomato, Pepper, Onion, Cabbage Lettuce, even Moringa (Ibeawuchi *et al*., 2015). Fresh vegetables and fruits are considered an important parts of a healthy and nutritious diets. It was observed that plant- based foods are not containing enteric organisms that may pose adverse effects on human. (Cock and Voss, 2004). Foods of high energy density may cause the consumption of excess calories, thereby promoting weight gain which can lead to obesity, but eating much fruits and vegetables may lower the energy density. Consumption of more satisfying portion of vegetables and fruits maintains the energy level and encourage weight management (Rolls *et al*,. 2004; Cock and Voss, 2004).. It has been reported that taking 100% fruit juice does not contribute to weight gain (Clemens *et al*., 2015). Although, the consumption of fruits and vegetables has some benefits, however, these fruits and vegetables harbor varieties of microorganisms that can cause infections to the consumers. Due to the increase in the consumption of fresh and semi processed fruits and vegetables, the number of outbreak of food borne diseases or illnesses associated with vegetables increased (Beuchat, 2002; Johnston *et al.,* 2005). Several factors contributed to the microbial contamination of fruits and vegetables. Consumption of vegetables has been reported to lower the incidence of cardiovascular diseases and obesity inclusive (Pem and Jeewon, 2015). Fruits and vegetables can be contaminated with parasitic pathogens throughout the process from planting to consumption (Adenusi *et al*., 2015) These may be certain pre and post harvest practices which is not only the use of contaminated water through irrigation, untreated animal dung or manure, inappropriate fertilizers and during harvesting and packaging by workers that lack good hygienic practices (Johnston *et al*., 2005). Previous studies on fruits and vegetables indicated that fruits and vegetables can be contaminated due to contact with soil and manure, irrigation water , or it may also be due to birds or animal fecal matters (Berger *et al.,* 2010), Various bacterial pathogens have been found to

be pectinolytic species *Erwinia* , *Pseudomonas, Clostridium* , *Salmonella, Shigella, E. coli 0157:H7, Listeria monocytogenes* and *Campylobacter sp.* Certain fungi that spoil vegetables are *Aspergillus* , *Fusarium* and even *Penicillium* species. They appear in filamentous fungi that grow on vegetables, and their growth may result in production of toxins known as mycotoxins (Opere *et al.,* 2020), at any of several points from farm through the time of consumption. High incidence of human intestinal parasites has been discovered in places that consume raw fruits and vegetables, especially where the vegetables were cultivated on farms or areas treated with human and animal sewage, storage temperature. Some of these vegetables are consumed without further treatment or processing, the microbes in them could be a risk factor to the consumers health. Very serious health issue can develop due to the presence of pathogenic microorganisms in the vegetables used to prepare foods. This can lead to food poisoning and equally lead to instant death to the unlucky ones(WHO, 2003).It has been established that fruits and vegetables are beneficial to human growth, about 400g of fruits and vegetables is recommended in dietary guideline (Agudo, 2005). Green vegetables are good source of nutrients that are needed for the growth of man and even animal, they contribute sufficient protein, vitamins, fibers, minerals and some other nutrients that are in short supply in our diets (Mohammed and Sharif, 2011). A survey based on epidemiology indicated a positive result in relation to a diet rich in fruits and vegetables and reduced incidence of diseases including cancer, cardiovascular diseases, muscular degeneration, aging etc (Michel *et al*., 2000). A wide range of micronutrients and non nutrients are found in fruits and vegetables. They include dietary fiber, minerals (calcium, Potassium and magnesium), vitamins (A, C and E), Phytochemicals (polyphenolic compounds and carotenoids and organosulphur compounds). About 5000 or more phytochemicals are found in fruits and vegetables , though majority of them are not known (Dhellot *et al.,* 2006; Liu, 2013; Haddad *et al.,* 2003 ; Ali *et al*., 2002; Ibeawuchi *et al*., 2015). The aim of this study is to carryout microbial evaluation of different vegetables that are sold in Oja Oba Market in Ado Ekiti to ensure their safety for consumption.

MATERIALS AND METHODS

MATERIALS:

Ten samples of green vegetables were purchased randomly from oja Oba and oja Bisi Markets in Ado Ekiti Ekiti State. The green vegetables include Amaranthus cruentus, Lettuce, Senecio biafrae, Cerchorus oliforus, Celosia argentae, Cuccumber, Talinum triangulare, Venonia amygdalina, Cabbage and Moringa oleifera. These vegetables were labeled and packed after purchase in separate sterile nylon bags in ice packs at a temperature between 6-12 ^{o}C and were transported to the Food microbiology laboratory of Federal Polytechnic Ado Ekiti for immediate analysis

METHODS:

ISOLATION OF MICROORGANISMS

1 gram of each of the vegetables was weighed and crushed aseptically, and 9ml of sterile ringer solution was added in a test tube and Serial dilution was performed on the mixture .One milliliter of 10 $^{-5}$ dilution factor was pipetted into two sterile petri dishes respectively. Different types of culture media were used , such as Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), Potato Dextrose Agar (PDA). These culture media were prepared as directed by the manufacturers (Shalini, 2010). After sterilizing the culture media by autoclaving at 121 ^{O}C, the molten agar (45 ^{O}C) was introduced into the petri dishes with diluted samples. The mixture in the test tubes were mixed gently and the agar was allowed to solidify, after which the plates were incubated in the incubator at 37 ^{O}C for 24 hours. The petri dishes that have Potato Dextrose Agar (PDA) was incubated at 27 ^{O}C for 72 hours. After incubation, colonies that developed on the agar were observed and the colony forming unit (cfu) calculated to obtain total viable count of the isolates. The pure culture of the isolates were obtained by carrying out sub culturing on freshly prepared agar.

TOTAL AEROBIC MESOPHILIC COUNT:

Total aerobic mesophilic count of the samples was performed by using Plate Count Agar (PCA). After serial dilution, 1ml of the dilution level of 10 $^{-1}$ - 10 $^{-6}$ was taken and put on the standard plate count agar and incubate at 37 ^{O}C for 24 hours. The plates that have from 30 to 300 colonies were picked ad the colonies counted as cfu/ml. (Shalini, 2010).

TOTAL COLIFORM COUNT:

1 ml of the solution from 10^{-1} to 10^{-5} dilution level was pipetted into two sterile petri dishes and sterile molten Eosin Methylene Blue (EMB) Agar was poured into thepetri dishes. Total Coliform from the vegetables were counted on the EMB agar after incubation for 24 hours at 37 ^{O}C . Greenish metallic sheen coloured coloies were counted as coliforms (Birhanu *et al*., 2022).

IDENTIFICATION OF ISOLATES:

Pure cultures of fungal and bacterial isolates were identified using morphological and biochemical characteristics which include shape, arrangement, size, colour, motility, grams reaction and others such as coagulase test, Catalase test, fermentation test, indole test. Berge's determinative manual was used to get the names of the bacterial and fungal isolates (Thomas *et al.*, 2016).

RESULTS AND DISCUSSION

One of the important parameters involved in food quality control is to ensure safety of the consumers. Microbiological characteristics are as well important properties that relate to safety of foods we consume. Based on the tests carried out, all the vegetables examined in this study were found to be contaminated by one type of microorganism or the other. The presence of microorganisms in the fruits and vegetables can be attributed to food-borne diseases, or microbes responsible for the reduction of shelf-life of fruits and vegetables (Franco and Landgraf 2005; and Faour Klingbeil *et al*., 2016). Most of these fruits and vegetables are contaminated through waste water that were used in irrigation that might be from sewage water used to water the vegetable garden or water used of manure for the fertilization of the farm where vegetables were grown.

Table 1 shows bacterial and fungal counts which include aerobic mesophilic bacteria, coliform, staphylococci, salmonella, yeast, and mould. The count for these organisms were determined, High counts of aerobic mesophilic bacteria were observed in 8 of the examined samples in this study. Celusia argentae had the highest count of aerobic mesophilic bacteria with $4.3x10^6$ and Moringa oleifera had the least count $1.1x10^4$. High counts of Enterobacteriaceae can be found in raw vegetables because some genera are part of soil microbiota (Johnston *et al*., 2005). *Escherisia coli* count was high in Cerchorus oliforus with $2.7x10^4$ and least in Moringa with $1.2x10^4$, also showed high fungal count on cucumber, $4.2\ x10^4$ of yeast and $3.2\ x\ 10^4$ of mold on cucumber and the least count of fungal $1.8\ x\ 10^4$ of yeast and mold on both Amaranthus and Lettuce.

Table 1: Microbial Count (cfu/g) of the vegetable samples

Sample	*Erwinia*	*E. coli*	*Staph*.	*Salmo*	*Pseud*	Yeast	Mould
Amaranthus cruentus	$3.2x10^4$	$2.1x10^4$	$3.7x10^4$	$4{,}5x10^4$	$2.4x\ 10^4$	$1.8x10^4$	$2.2x10^4$
Lettuce (Latuca sativa)	$3.0x10^4$	$2.4x10^4$	$5.1x10^4$	$5.0x10^4$	$2.6x10^4$	$2.4x10^4$	$1.8x10^4$
Senecio biafrae	$3.0x10^4$	$2.3x10^4$	$3.3x10^4$	$4.5x10^4$	$3.1x10^4$	$3.2x10^4$	$3.0x10^4$
Cerchorus oliforus	$3.2x10^4$	$2.7x10^4$	$2.7x\ 10^4$	$3.8x10^4$	$3.5x10^4$	$4.0x10^4$	$2.6x10^4$
Celusia argentae	$4.3x10^4$	$2.1x10^5$	$3.5x10^4$	$5.6x10^4$	$3.7x10^4$	$3.7\ x10^4$	$3.3x10^4$
Cuccumber(C. sativa)	$2.5x10^{64}$	$1.8x10^4$	$5.0x10^4$	$5.4x10^4$	$4.2x10^4$	$4.2\ x10^4$	$3\ 2x10^6$
Talinum triangulare	$3.5x10^4$	$2.0x10^4$	$4.6x10^4$	$4.3x10^4$	4.5x104	$3{,}8x10^4$	$1.9x10^4$
Venonia amygdalina	$3.4x10^4$	$2.5x10^4$	$3.8x10^4$	$4.0x10^4$	$3.7x10^4$	$3.9x10^4$	$2.4x10^4$
Cabbage(B. oleracea)	$4.0x10^4$	$2.2x10^4$	$2.6x10^4$	$3.7x10^4$	$3.8x10^4$	$3.4x10^4$	$3.7x10^4$
Moringa oleifera	$1.1x10^4$	$1.2x10^4$	$3.0x10^4$	$2.6x10^4$	$2.3x10^4$	$2.2x10^4$	$2.4x10^4$

Table 2 shows the morphological characteristics of the isolates , the appearance of some colonies are smooth and some are rough on the agar surface. Some of the isolates were circular and some were irregular in shape, some had raised or flat surface under elevation, in size some are large, some are moderate and some are small. Under margin, some have undulate, some have entire, some have rhizoid. Some colonies of the isolates appear white in colour, some appeared yellow, there are some that have green colour and cream colour.

Table 2: Morphological Characteristics of isolated Bacteria.

Unknown Isolates	Shape	Margin	Elevation	Size	Appearance	Colour
Va	Irregular	Undulate	Flat	Moderate	Rough	White
Vb	Circular	Entire	Convex	Large	Smooth	Yellow
Vc	Circular	Entire	Raised	Small	Smooth	Cream
Vd	Circular	Entire	Raised	Moderate	Dull	White
Ve	Irregular	Rhizoid	Flat	Moderate	Smooth	Yellow
Vf	Irregular	Rhizoid	Flat	Moderate	Rough	Cream
Vg	Circular	Lobate	Raised	Moderate	Smooth	Green
Vh	Irregular	Undulate	Flat	Moderate	Smooth	Yellow
Vi	Circular	Entire	Flat	Small	Shiny	Cream
Vj	Circular	Entire	Raised	Large	Smooth	White

Table 3 showed the biochemical test and the suspected isolates include *E. coli* , *Staphylococcus aureus*, *Bacillus subtils*, *Salmonella* sp. *Pseudomonas aeroginosa* and *Erwinia sp.*

Tabble 3: Biochemical Characteristics and Fermentative Tests and suspected Isolates

Isolates	Cata	Coag	Citr	Oxid	Indo	MR	Vp	Mot	Grams	Glu	Fru	Suc	Malt	Suspected isolates
a	+ve	-ve	+ve	-ve	-ve	-ve	+ve	–ve	-ve	+ve	+ve	-ve	+ve	*E. coli*
b	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	*S. aureus*
c	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	*B. subtilis*
d	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	*Salmonella*
e	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	*P. aeroginosa*
a	+ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	*E.coli*
b	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	*S. aureus*
e	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	*P.aeroginosa*
f	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	*Erwinia*
f	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*Erwinia*

The frequency and percentage of bacteria occurrence on Table 4,*showed E. coli* appeared significantly in two major vegetables and constituted 20% , *Staphylococcus aureus* also appeared in two major vegetables and constituted 20% , *Bacillus subtilis* appeared in only one vegetable and constituted 10% , *Salmonella* appeared in only one vegetable and had 10% while *Pseudomonas aeroginosa* and *Erwinia sp.* Appeared in two vegetables each and constituted 20% each.

Table 4: Frequency of Bacteria occurrence

Isolates	Occurrence	% Occurrence
Eschericia coli	2	20
Staphylococcus aureus	2	20
Bacillus subtilis	1	10
Pseudomonas aeroginosa	2	20
Salmonella sp.	1	10
Erwinia sp.	2	20

DISCUSSION

Vegetables are consumed by most people because of their satisfactory quality and rich mineral contents. Gastrointeritis has been a major problem of consuming vegetables in developing countries. There have been plenty concerns over handling and sanitary quality of vegetables and these exposed vegetables to possible microbial contaminations that can pose health risks to the consumers. From the results in table 1, it can be deduced that all the samples of vegetables were contaminated or infected with one microorganism or the other. The highest bacterial count of $5.1x10^4$ was on lettuce and the least count of $1.1x10^4$ was on moringa. The highest fungal count $4.2 x10^4$ was on Cucumber and the least fungal count 1.8×10^4 was on lettuce and amaranthus. The microorganisms that are present in these vegetables as shown in this study may be a reflection of the unhygienic handling of facilities (Adekanle *et al*., 2015). *Escherisia coli* were isolated from amaranthus and cucumber vegetables. Aerobic microorganisms isolated from vegetables that were analyzed and studied in this work were in agreement with the report of Beuchat (2002). The resemblance of the results may be due to handling , processing and storing of samples at temperature different from their usual keeping temperature. The result attributes the facts that fresh vegetables could be contaminated with pathogenic microorganisms and will act as medium of transmission of diseases or infections. This is in agreement with Baishakhi Biswas *et al.*, 2020. Isolation of *Escherishia coli* and other enteric organisms such as *Pseudomonas* from vegetables is an indication that fecal contamination occurred on the vegetables samples. High count of *Enterobacteriacaea* can be found on raw vegetables because some genera are part of soil microbiota (Johnston *et al.,* 2005). The prevalence of Coliform(*E. coli*) in Amaranthus was 2.1×10^4 cfu/ml, Lettuce was 2.4×10^4 cfu/ml., Senecio biafrae had 2.3×10^4 cfu/ml., Cerchorus oliforus had count of $2.7x10^4$ cfu/ml., Celusia argentae had $2.1x10^4$ cfu/ml. Cuccumber had count of 1.8×10^4 cfu/ml., Talinum trangulare had 2.0×10^4 cfu/ml., Venonia amygdalina had 2.5×10^4 cfu/ml., Cabbage had 2.2×10^4 cfu/ml., and Moringa oleifera had 1.2×10^4 cfu/ml. The high count of microorganisms on vegetables could be as a result of various stages of vulnerability in the process line of vegetables, such as the use of contaminated water to wash the vegetables before use, and most importantly, the exposure to the contaminated market environmental condition. The perishable conditions may be due to the moisture content of the vegetables which may assist in the growth of microorganisms such as bacteria (Opere *et al.,* 2020). Celusia agentae recorded the highest *Salmonella* sp.in this study. This is in agreement with previous work (Uzeh, *et al.,* 2009; Goja, *et al.,* 2013).

CONCLUSION:

The result of this research showed that fresh vegetables that are not handled hygienically are reservoir of both bacterial and fungal microorganisms that are known to cause infection or disease to consumers.

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