Bacteriological Assessment of Awarawa Tomatoes (*Lycopersicon esculentum*) Consumed in Awka, South Eastern Nigeria

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ABSTRACT

Tomatoes are among the indispensible ingredients in foods worldwide. They are rich in nutrients that promote healthy living of man. Unfortunately they are fragile when ripe and their storage is difficult. Most of the fruits spoil before reaching final consumers and when spoilt, they are sold at cheap cost. This study looked into the bacteria associated with the semi-spoilt tomatoes consumed in Awka, South East Nigeria. Standard procedures were used to isolate, characterize and identify bacteria from the samples. Bacteria counts ranged from 5.84-8.64 X 10^3cfu/ml, and the six bacteria isolates were identified as *Bacillus* sp, *Pseudomonas* sp, *Escherichia coli*, *Klebsiella* sp, *Enterobacter* sp and *Proteus* sp. The organisms occurred as mixed cultures in the awarawa tomatoes samples and at varying frequencies, with *E. coli* and *Pseudomonas* sp having lowest frequency of 7% while *Klebsiella* sp had the highest frequency of 40%. These isolated bacteria are pathogenic and can pose adverse health problems to consumers. Therefore it is not advisable to consume these semi-spoilt tomatoes to avoid food poisoning and other related problems.

Key words: Tomatoes, Semi-spoilt tomatoes, Post-harvest spoilage, Bacteria.

INTRODUCTION

Tomato (*Lycopersicon esculentum)* is a perishable vegetable widely cultivated and consumed worldwide (Agrios, 2005; Valadez *et al.*, 2012). It has long been used as food both in cooked as well as raw form due to its richness in nutrients, vitamins, dietary fibres, and phytochemicals (Freeman and Reimers, 2011; Mariga 2012). The deep red coloration of riped tomato fruits is due to the presence of lycopene, a form of β-carotenoid pigment which forms the precursor of Vitamin A and hence is of great nutritional value. It is rich in water and its composition is about 95% (Valadez *et al.*, 2012).

Fig 1: Fresh, hard tomato fruits

Fig 2: Awarawa tomato fruits

Today with an increased number of ailments especially sight and vision, these fruits rich in carotenoid pigment forms of prime importance to minimize the problems related to vision. It is known to be a very profitable crop that provides high returns for small scale farmers in most developing countries (Wogu *et al.*,

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2008; Wogu and Ofuaes, 2014). Due to its nutritive value, taste, affordability, and accessibility, there has been an increase in demand by consumers (Behravesh *et al.,* 2012).

Tomato is grown in many countries including Nigeria. It is consumed by almost every family either cooked or raw. But there is a huge loss of tomato across the globe due to microbial spoilage. Since it is composed mostly of water, microbes grow best using it as its substrate. Microbial deterioration of tomato fruits causes reduction in market values and nutritional qualities, and at times rendered the fruits unfit for consumption.

About one third of the tomatoes harvested tend to spoil before reaching the consumers, this loss has been attributed to a number of factors which includes; mechanical breakage, bruises, physiological factors and also damages caused by pathological agents. Market values of the tomato are mainly reduced by the above factors (Erinle *et al.,* 2007) and the tomatoes will turn to Awarawa tomatoes.

Awarawa tomatoes are those semi spoilt tomatoes that had leaked off their water content as well as their mesocarp and nutrients. Sellers especially retailers sort out this semi spoilt tomatoes and sell them at cheap cost to customers. Low incomes as well as rich people do patronize them because they are cheap. The semi spoilt tomatoes can harbor some microorganisms due to the way they are exposed in the market (Akinyele and Akimkunmi, 2012). Some of the bruises that cause tomatoes to lose their contents can occur during the harvesting period, post-harvesting, handling, storage, transportation, and processing by customers (Baiyewu *et al.,* 2007; Barth *et al.,* 2009; Yeboah, 2011). Microbial infestation of these awarawa tomatoes could be considered to be harmful if such contaminated tomatoes are consumed when improperly cooked (Danlande *et al.,* 2008; Valadez *et al.,* 2012).

Some studies have been carried out on bacteria associated with tomatoes and tomato products in some countries. A study carried out by Ajayi (2013) in the United State has revealed that *Clostridium* sp ., *Staphylococcus* sp., and *Bacillus* sp. were predominant bacteria isolated from both canned and rawtomatoes. In India, a study carried out on tomato puree revealed the presence of *Klebsiella* sp., *Proteus mirabilis, Vibrio* sp., *and Pseudomonas* sp. (Garg *et al.,* 2013). In Nigeria, Wogu and Ofuase (2014) isolated *Bacillus subtils, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis,* and *Staphylococcus aureus* from spoilt tomatoes in Benin City. A similar study also revealed high levels of *Staphylococcus* sp. (22.5%), *Bacillus* sp. (20%), and *Escherichia coli* (15%) in Lagos State, Nigeria (Ogundipe *et al.,* 2012).

This present research was therefore carried out to assess the presence of bacteria in awarawa tomatoes consumed in Awka, Anambra State, Nigeria.

MATERIALS AND METHODS

Sample collection

Ten samples of semi-spoilt tomatoes, commonly called “Awarawa” were randomly collected from ten different retailers at Eke Awka Market using sterile polythene bags and labeled A-J. They were taken to the Department of Applied Microbiology and Brewing Laboratory for analysis. Reagents, chemicals, culture media and other materials used for the work were obtained from the laboratory and were of analytical grade.

Media Preparation

Nutrient agar (NA) was used for the enumeration and isolation of the bacterial isolates. The media was prepared according to Manufacturer’s instructions. After preparation the media was incubated over night to test for its sterility. Contaminated media that showed some growth colonies were discarded while sterile ones which did not show any growth were used for culturing.

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Sample preparation

The awarawa tomatoes were blended with a sterile Monilex Blender to make a paste and ten folds serial dilution was carried out to obtain tomato paste solution. One milliliter of the Awarawa tomato paste solution was transferred into 9ml of distilled water contained in a test tube and mixed properly using a sterile string rod. Serial dilution three (10^{-3}) of each sample was cultured. Before culturing each of the prepared media were dissolved by gentle heating using a water bath.

Bacterial isolation

The method of Chees brough (2010) was used. Using pour plate method, one milliliter (1ml) of the serially diluted sample (10^{-3}) was dispensed into a sterile petri dish and then about 20ml of NA was poured into the Petri dish for each sample and rocked gently to achieve homogeneity. They were allowed to solidify, then turned upside down and incubated at room temperature for 24hr. The colonies that developed were counted. After counting, the isolates were sub-cultured to get pure colonies that were preserved in slants before characterization and identification.

Bacterial Characterization and Identification

The method of Chees brough (2010) was used to carry out the characterization and identification of the isolates. The following tests were performed:

Gram's staining

An evenly spread smear of the isolate was made on a clean, dry slide using a sterile normal saline and the smears were allowed to air-dry in a safe place. The smears were fixed by passing three times over Bunsen flame and stained by Gram technique.

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as *Staphylococci*, from non- catalase producing bacteria such as *Streptococci*. A 3 ml of 3% hydrogen peroxide was introduced into a test tube, a loop full of the test organism was introduced in the hydrogen peroxide solution. Immediate bubbling in the solution was checked.

Coagulase test

This test is used to differentiate *Staphylococcus aureus* which produce the enzyme coagulase, from *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* which do not produce coagulase. A drop of physiological saline was placed on a clean grease free slide. A colony of the test organism was emulsified in the drop to make a thick suspension. A drop of plasma was added to the suspension and gently mixed. Clumping of the organisms within 10 seconds was observed.

Urease test

Testing for urease activity is important in differentiating Enterobacteria. The test organisms were cultured on a medium containing urea and phenol red indicator. Using a sterile straight wire, tubes containing the medium were inoculated with test organisms and incubated overnight at 37^{o}C. If the strain was urease-producing the media become alkaline and change the colour of indicator to redish-pink.

Citrate Utilization Test

This test is one of several techniques used to assist in identification of Enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of

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nitrogen. The test organism was cultured on slope media containing sodium citrate and then incubated overnight at 37^{O}C. Growth in media was shown by turbidity and change in colour of indicator (bromothymol blue) from light green to blue.

Indole Test

Testing for indole production is important in identification of Enterobacteria. This test is based on the ability of organisms to break down the amino acid tryptophan with the release of indole by producing tryptophanase enzyme. The test organism was cultured on peptone water containing tryptophan or tryptone water and then incubated overnight at 37^{O}C, indole production was detected by kovac's reagent which contains 4(p)-dimethyl amino benzaldehyde, this react with indole to produce red colour ring.

Motility Test

This test is used to differentiate motile bacteria from non-motile. The test organism was inoculated on semi solid medium and incubated overnight at 37^{O}C and a result shown as a moving of bacteria away from line of inoculation.

Sugar fermentation test

Peptone water was used as the growth medium. It was prepared according to manufacturer's instructions and 1% Bromocresol purple was added as indicator. The mixture was mixed thoroughly and dispensed in 8 ml volume in sets of clean test tubes arranged in racks and labeled. Durham tubes were introduced into the test tubes in inverted position. The tubes were plugged with cotton wool and sterilized by autoclaving at 121°C for 15 minutes. Separate conical flask containing 1% of different sugar solutions: Maltose, glucose, sucrose, Lactose and fructose were prepared and autoclaved at 115°C for 10 minutes. Five milliliter aliquots of the sterilized sugars were inoculated aseptically into those test tubes already prepared. The set of test tubes were then inoculated with 24h culture of the test organisms and incubated at 28$\pm$2°C for 24hr and checked for acid and gas production.

RESULTS AND DISCUSSION

Results of the study showed that a high bacterial load was harbored by the awarawa tomatoes. Mean bacterial count was high ranging from 5.84 – 8.64 X 10^3cfu/ml as shown in Table 1. The high mean bacterial count in the tomatoes may be as a result of the way these tomatoes were exposed in the market, flies perch on them anyhow and transmit bacteria on them. Also bacteria from the air and dust can contribute to the high load of bacteria (Wogu and Ofuase, 2014). The high count of 8.64×10^3cfu/ml agrees with the findings of Ogundipe *et al*., (2012) who carried similar research in Lagos State, Nigeria.

Fifteen different bacterial isolates were obtained as mixed cultures in the tomatoes samples as presents in Table 2. Some of the organisms like *E,coli* are indicators of direct or indirect feacal contamination (Ogundipe *et al*., 2012). The isolates from this study was the same as those found by other researchers like; Ogundipe *et al*., (2012) who worked on tomatoes in Lagos Nigeria, Ajayi (2013) who worked on tomato puree in the United States of America, Wogu and Ofuase (2014) worked on spoilt tomatoes in Benin City, Nigeria.

The frequency occurrence of the isolates as presented in Figure 3 showed that *Escherichis coli* and *Pseudomonas sp* had the least occurrence (7%) while *Klebsiella sp* had the highest occurrence (40%). The high frequency occurrence of *Klebsiella* sp in this work (40%) disagree with the findings of Ugwu *et al*., (2014) who reported the same organism with the frequency of 8.6%. Wogu and Ofuase (2014) also reported similar frequency of the same organism from similar work in Benin City, Nigeria. The high frequency may be as a result of environmental factors, handling as well as exposure of the fruits in the market.

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The presence of *Enterobacter* sp, *Proteus sp* and *Bacillus* sp in this study is in conformity with Ogundipe *et al*., (2012), that isolated similar bacteria with percentages of 12.5%, 2.5% and 20.0%, respectively, from semi spoilt tomatoes in Lagos State, Nigeria. According to Bartz *et al*., (2009), certain species of *Pseudomonas* and *Bacillus* from the farm can cause soft rot of harvested tomatoes. Some *Bacillus* sp. can cause food poisoning, resulting into different kinds of intoxication including; nausea, vomiting, and abdominal cramps (Magercroy *et al.,* 2019). They can also cause anthracis (Mariga, 2012). Oladipo *et al*., (2010) reported that bacteria strains such as *Bacillus* sp, *Proteus* sp., *Pseudomonas* sp can spoil tomato juices and that their presence may pose risks to consumer health and should not be taken for granted. Therefore from the findings of this study, it is not advisable to consume spoilt or semi-spoilt tomatoes no matter what reason. Spoilage of this fruit can be prevented by finding ways of packaging the product for transportation to avoid much transit spoilage that used to occur when using baskets.

Table 1: Total bacterial counts from the awarawa tomatoes

Sample	Total bacterial count ($X10^3$cfu/ml)
A	6.24
B	6.16
C	6.68
D	8.64
E	6.45
F	7.33
G	7.91
H	7.82
I	6.33
J	5.84

Table 2: Gram and biochemical characteristics of the isolated bacteria

Isolates	BIOCHEMICAL TESTS							Probable organisms
	Gram Staining	Catalase Test	Coagulase Test	Urease Test	Citrate Ultilization Test	Oxidase Test	Motility Test	
1	+ve Rod	+	–	–	+	–	–	Bacillus sp
2	-ve Rod	+	–	–	+	–	–	Enterobacter sp
3	-ve Rod	+	–	–	–	–	+	Escherichia coli
4	-ve Rod	+	–	+	+	–	–	Klebsiella sp
5	+ve Rod	+	–	–	+	–	–	Bacillus sp
6	-ve Rod	+	–	–	+	+	–	Pseudomonas sp
7	-ve Rod	+	–	+	+	–	–	Klebsiella sp
8	-ve Rod	+	–	+	+	–	–	Klebsiella sp
9	-ve Rod	+	–	+	+	–	+	Proteus sp
10	-ve Rod	+	–	–	+	–		Enterobacter sp
11	-ve Rod	+	–	+	+	–	–	Klebsiella sp
12	-ve Rod	+	–	+	+	–	–	Klebsiella sp

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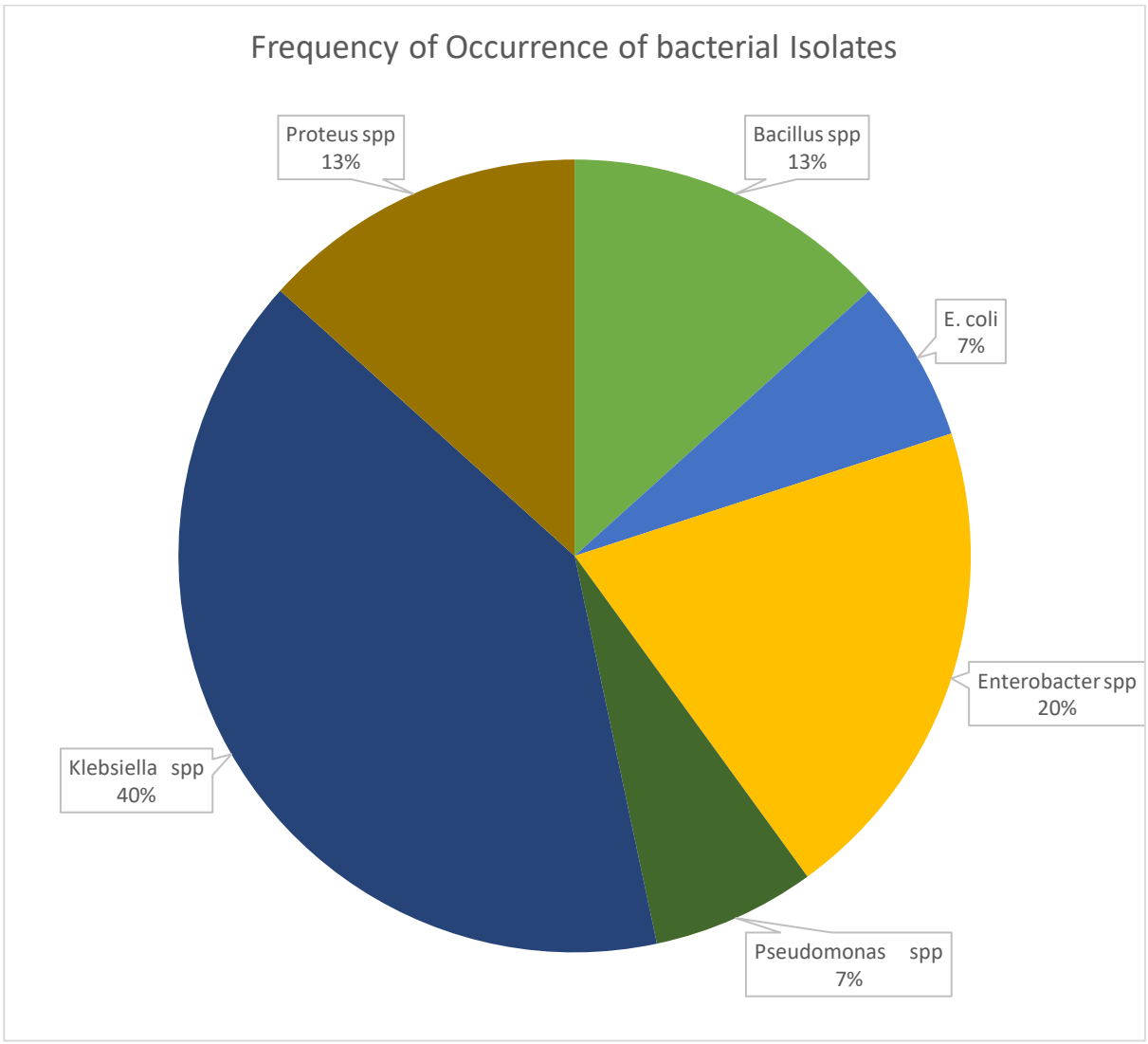
13	-ve Rod	+	–	–	+	–		Enterobacter sp
14	-ve Rod	+	–	+	+	–	+	Proteus sp
15	-ve Rod	+	–	+	+	–	–	Klebsiella sp

KEY:

+ = Positive Reaction

-= Negative Reaction

Fig 3: Frequency occurrence of the isolates from the awarawa tomatoes.



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