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MICROORGANISMS ASSOCIATED WITH THE SPOILAGE OF TOMATOES (*Lycopersicon esculentum*), SOLD IN NIGERIAN MARKET

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ABSTRACT

Tomato is a widely consumed fruit, eaten in both raw and processed forms. It is rich in vitamins which are vital nutrients in man. This study investigates the microbes associated with the deterioration of fresh tomatoes, (*Lycopersicon esculentum*) in Nigeria, Market. A total of sixteen tomato samples were obtained from eight different retail outlets and transported to Bingham University Pharmaceutical Microbiology laboratory. Pour plate method was used to isolate bacteria and fungi from the tomato samples. The bacterial count ranged from 2.9 x 10^5 to 9.8 x 10^5 cfu/g. Bacteria isolated and identified were *Staphylococcus aureus*, *Escherichia coli, Klebsiella sp* and *Salmonella sp*. The most prevalent bacterial isolate was E. coli with 37.5% and was found in some samples and *salmonella sp* was the lest prevalence with 12.5%. The fungi count ranged from 1.4 x 10^5 to 2.2 x 10^5 cfu/g. The fungal isolates were *Saccharomyces Cerevisiae*, *Penicillium sp* and *Mucor sp* with the most prevalent being *Saccharomyces cerevisiae*. The

Introduction

Tomato is a widely consumed fruit eaten in both raw and processed forms. It has the botanical name *Lycopersicon esculentum* and belongs to the plant family Solanaceae (Wogu and ofuase, 2014). It is rich in vitamins such as vitamin B, C, and E, carbohydrates such as fructose and glucose; and trace elements like iron, copper, zinc, and dietary fibre, which are all vital nutrients in man. The high water content of tomatoes makes it more susceptible to spoilage by the action of microorganisms (Obunkwu *et al*, 2018). In northern Nigeria, freshly harvested tomato fruits are stored, conveyed and marketed in wooden boxes and baskets. These baskets are

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presence of these fungi, as well as the bacteria isolates, which are capable of causing food poisoning, raises concern over public health risks that may be associated with the consumption of spoilt fresh tomato. Proper handling, transportation and thorough washing with clean or chlorinated water will reduce the risk of tomato spoilage associated with microbes

KEYWORDS: Tomatoes, Spoilage, Associated Microbes, Food Poisoning, Bacteria

Often reused severally which foster propagation of both microorganisms that are in their reproductive and dormant state. Pathogenic inoculums on these wooden boxes and baskets can initiate spoilage upon contact with healthy tomato fruits resulting in losses, which translate to a waste of the farmers' resources, a reduction in their income and ultimately their welfare. These pathogenic inoculums could also originate from infected farm tools, or during transportation. It is estimated that ripe tomato fruits contain approximately 94% water, 4.3% carbohydrates, 1% protein, 0.1% fat, 0.6% fibre and vitamins. The nutrients support the growth of microorganisms such as fungi and bacteria, which produce enzymes that degrade the nutrients. Tomato fruits contain a lot of water which makes them more susceptible to spoilage by microorganisms. Also, the high-water content makes storage and transportation of this vegetable difficult. The microorganisms reduce not only the nutritional value but also the market value of tomato fruits. In recent years, the incidence of diseases in tomato fruits has been a cause for global concern and intensive research has been undertaken to comprehend the measures which can be taken to effect some radical control. The parameters during quality control include various factors such as time of harvesting, temperature and moisture during storage, selection of agricultural products prior processing, decontamination conditions, addition of chemicals and final product storage. It has been observed that the high cost of fresh ripened tomato fruits sold in local markets has tended to lure the unwary public to patronize spoilt tomato fruits because they are relatively cheaper. But however, these spoilt tomatoes contain microorganisms that can cause food borne diseases. The microbial deterioration on tomato fruits causes reduction in its market values and nutritional qualities. The tomato fruits are rendered unsafe for consumption due to contaminations with mycotoxins that produces aflatoxins in human, following inhalation or ingestion and thus resulting to food poisoning (Bello *et al.,* 2016).

MATERIALS AND METHOD

Bacteriological Media

Nutrient agar, MacConkey agar (Titan Biotech.Ltd,India). Potato dextrose agar. Urease agar (Guangdong Hunakai, ltd; China) Peptone water Citrate agar, TSI agar, Peptone water ((Himedia, Mumbai India)

Others:

Kovacs Reagent, Test tubes, Petri dishes, Microscopic Glass Slides, Safranine, Distilled water, Crystal Violet, Gloves, Alcohol, Hydrogen peroxide, Iodine, Refrigerator, 5ml of blood, hydrogen peroxide, lactophenol cotton blue.

Samples Collections and Transportation.

The materials for the test were obtained from within Masaka which is a town in Nasarawa central Nigeria. It is a district of Karu Local Government Area. Its coordinates include Latitude 9°0′18″N and Longitude 7°40′25″E. The laboratory tests were carried out in Bingham University Microbiology Laboratory.

Eight samples of Tomatoes (*Lycopersicon esculentum*) with spoilage signs were purchased from eight different retail stands in Nigerian Market. The tomatoes were placed and transported separately in sterile Ziploc bags to Bingham University Pharmaceutical Microbiology Laboratory.

Isolation of Bacteria from Bio deteriorated Tomato Samples

The pour-plate method was adopted. Using standard Microbiological technique (serial dilution), the aliquot was made by adding 25g of the tomato sample into 225ml of sterile water. A serial dilution of up to (10^{-4}) of the aliquot was carried out using sterile test tubes. Precisely, l ml of the aliquot was pipetted and mixed in another 9ml of sterile distilled water in a test-tube. The test-tube was shaken vigorously to homogenize. The exponential dilution continued to the fourth factor (10^{-4}). The 1ml of the third and fourth factor was aseptically transferred and plated in duplicate sets using sterile nutrient agar and MacConkey agar. The poured plates were allowed to set and were incubated at 37°C for 24hours. Discrete colonies that developed after incubation were counted and enumerated as colony forming unit (CFU/g) after multiplying with the dilution factor. Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The subculture plates were incubated at 37°C for 24 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C.

Isolation of Fungi from Bio deteriorated Tomato Samples

The pour-plate method was employed for the isolation of fungi on potato dextrose agar (PDA). After the serial dilution, 1ml of the diluents from the 4th test-tube were aseptically transferred to sterile Petri dishes and about 18 ml of sterile PDA were poured into the plate and was allowed to solidify and incubated at room temperature (28±2°C) for 3days for each sample respectively. Colonies that developed after incubation were counted and enumerated as colony forming unit (CFU/g) after multiplying with the

dilution factor. Colonies from the primary plates were aseptically picked with a sterile inoculation needle and transferred onto a freshly prepared sterile PDA using streak plate method and were incubated for 3 days at 28°C.

Characterization and Identification of Bacterial Isolates from Biodeteriorated Tomatoes.

The characterization of bacteria isolates from tomatoes were based on Grams staining and selected biochemical tests which include catalase test, indole production test , citrate, coagulase test, urease test , Triple Sugar Iron Agar (TSI) test.

Gram staining process

Clean, grease free slides were taken and arranged on a metal board. A loopful of each of the samples were taken and smeared of each of the clean, grease free glass slides and they were left to air dry, after that the slides were heat fixed. Then after Crystal Violet was poured on all the glass slides and kept for1 minute and then rinsed with water. Then gram's iodine was poured on all glass slides and left for 1 minute then rinsed with water. Then 95% alcohol was poured on all glass slides for about 10-20 seconds and rinsed with water. safranin was then for about 1 minute and rinsed with water. All the glass slides were then left to air dry before they were immersed with microscopic oil then observed under the

Microscope.

Biochemical Tests

Catalase test: This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacteria isolates smeared on a slide. The production of gas bubble indicates indicates presence of catalase enzyme.

Coagulase test: Coagulases are enzymes that clot blood plasma and they produced by *Staphylococcus aureus.* The enzyme protease converts fibrinogen to fibrin resulting to blood clotting. The Slide method was used. Here, clean slide was divided into two sections, to one section the test organism was smeared on it using a sterile wire loop while a drop of distilled water was added to the other section serving as control. Then human plasma was added to both sections and the slide was rocked gently for some minutes. A clumping/agglutination of the plasma indicates the presence of coagulase.

Urease test: The bacteria isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48hours. Red-pink colour indicates presence of Urease cultures.

Indole test: This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is used to

differentiate Gram-negative *Bacilli* especially those of the *Enterobacteriaceae*. Peptone water was prepared and about 10 ml of it was dispensed in test tubes using a sterile graduated cylinder. Then, fresh sterile loops were used to pick a well-isolated colony of bacteria and inoculated into the test tubes, thereafter, the tubes were incubated at 37°C for 48 hours. After incubation period, 0.5 ml of Kovac's Indole Reagent was added to the inoculated test tubes. A red ring in the surface layer within 10 minutes indicates an indole positive reaction.

Citrate utilization test: This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. It was carried out by inoculating the test organism in test tube containing Simon's citrate medium and incubated at 37°C for 24-48 hours. A deep blue colour indicates a positive result.

Triple Sugar Iron Agar Test: Triple Sugar Iron Agar Medium is recommended for identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production. 64.42 grams (the equivalent weight of dehydrated medium per litre) was suspended in 1000 ml purified/ distilled water. It was heated to boiling to dissolve the medium completely. It was then sterilized in the autoclave by maintaining at 10lbs pressure (115°C) for 15 minutes. It was mixed well and distributed into test tubes. Allow the TSI Agar to warm to room temperature before use. An isolated, pure colony is inoculated on the agar. The tubes are then incubated aerobically at 35-37ºC for 18-24 hours. Acid slant / acid butt - dextrose and sucrose fermented or dextrose and lactose fermented or all the three sugars, dextrose, lactose and sucrose fermented. Alkaline /acid butt- dextrose fermentation only

Alkaline butt/ alkaline butt – absence of fermentation results

Bubbles or cracks present - gas production Black precipitate present - H_2S gas production

Characterization and Identification of Fungal Isolates.

Identification and classification of the fungal isolates were based on macroscopic and microscopic examination.

Macroscopic examination: It was carried out by observing the colonial characteristics especially the colour formation of both the front and reverse sides of the plates.

Microscopic Examination: Lactophenol cotton blue solution was used. A drop of the solution was placed on a clean grease-free slide. A fragment of the fungi isolate was emulsified in the solution after which the slide was covered with a cover slip, avoiding bubbles. The slide was thereafter viewed under the microscope.

Fungal growth on plate culture was observed; surface, spore, underside colour, structure of hyphae and details of sporulating structure.

RESULTS

Table 1. Total Viable Bacteria Count of Spoilt Fresh Tomatoes

Sample	Total Bacteriological count (CFU/g)
A1	3.8×10^5
A2	6.2×10^5
B1	9.8×10^5
B2	7.1×10^5
C1	7.4×10^5
C2	5.7×10^5
D1	2.9×10^5
D2	8.0×10^5

Keys:A- 1st sample from 1st retail stand. **B**- 2nd sample from 2ndretail stand, **C**- 3rd sample from 3rd retail stand. **D**- 4th sample from 4th retail stand

Total Viable Fungal Count of the Spoilt Fresh Tomatoes

Sample	CFU/g x 10^5
A1	1.4
B1	2.3
C1	1.8
D1	2.2

Gram Staining Results

Sample	Result
A1	Negative
A2	Negative
B1	Negative
B2	Negative
C1	Negative
C2	Negative
D1	Positive
D2	Positive

Sample	Catalase test	Coagulase Test	Indole test	Triple Sugar Iron Agar test	Urease test	Citrate test	Organism
A1	+	-	-	+ (Acid/Acid)	+	+	*Klebsiella Spp*
A2	+	-	-	+ (Alkaline/Acid)	-	+	*Salmonella sp*

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B1	+	-	-	+(Acid/Acid)	+	+	*Klebsiella Spp*
B2	+	-	+	-	-	-	*Escherichia Coli*
C1	+	-	+	-	-	-	*Escherichia Coli*
C2	+	-	+	-	-	-	*Escherichia Col*
D1	+	+	-	-	+	-	*Staphylococcus aureus*
D2	+	+	-	-	+	-	*Staphylococcus aureus*

Biochemical Results

Keys- + positive, - negative ; 1st sample from 1st retail stand ,**B**- 2nd sample from 2nd retail stand, **C**- 3rd sample from 3rd retail stand , **D**- 4th sample from 4th retail stand

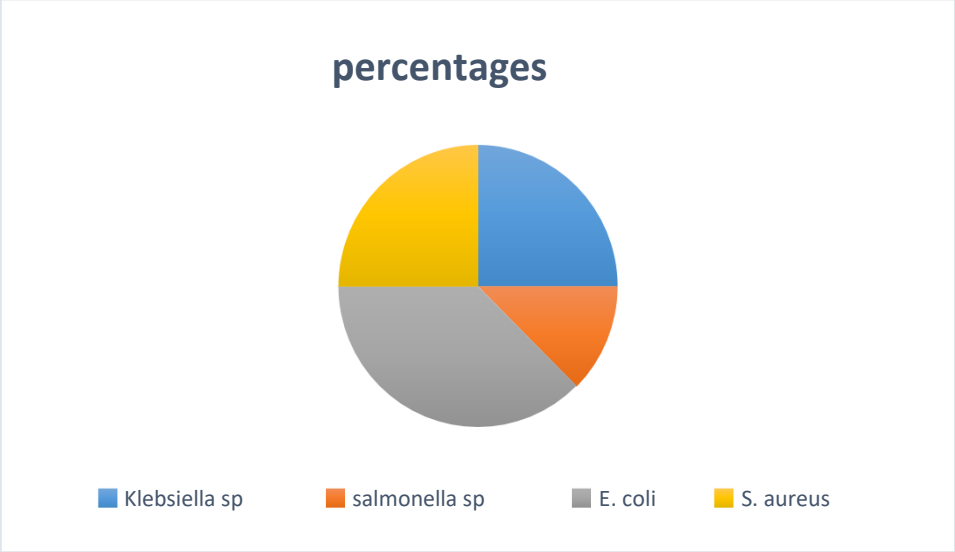

Fig 1. The % occurrence of bacterial isolates in samples from various markets.

Table 6. Identification of Fungal Isolates

Sample	Macroscopic Examination	Microscopic Examination	Probable Organism
A	It has a creamy colour and it is smooth and dull looking.	Small budding seen, blastophores which are oval.	*Saccharomyces cerevisiae*

B	It has a creamy colour, smooth, moist and dull looking.	There is multilateral budding and absence of hyphae. Blastophores are seen.	*Saccharomyces cerevisiae*
C	It has a whitish in colour and it has a cream colour in reverse. It is flat and wooly in texture.	It has a chain of single celled conidia. They end in clusters of flask shapes known as phialides.	*Penicillium sp*
D	It has a white colour and is pale white on reverse.	There is presence of hyphae and the sporangiophores are long.	*Mucor sp*

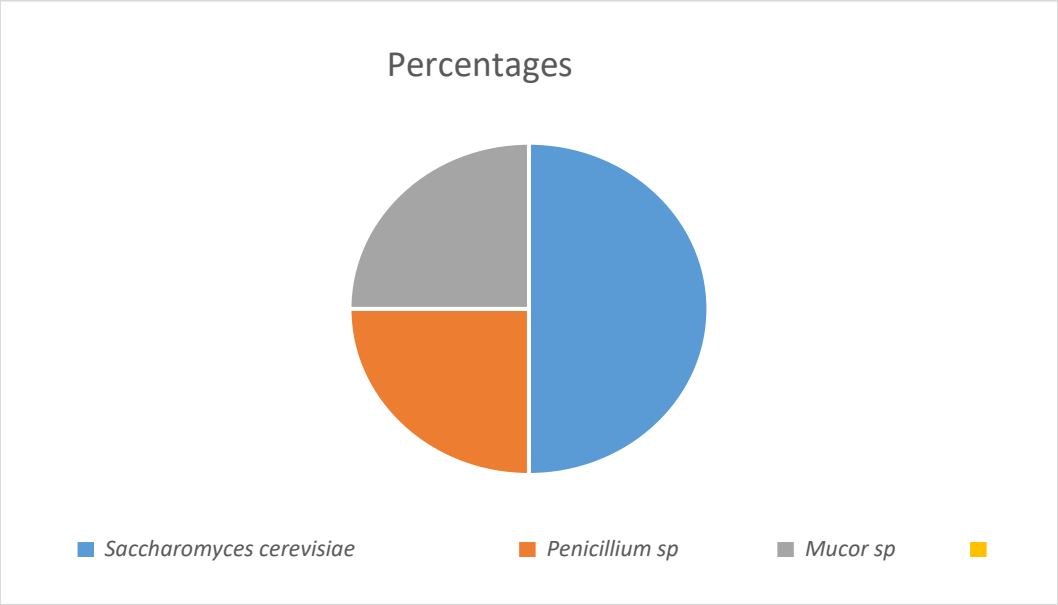

Fig 2. The % occurrence of Fungal isolates in samples from various markets

DISCUSSION

From the results of this study, different genera of bacteria and fungi were identified as being associated with the spoilage of tomato fruits. Some gram negative and gram-positive bacteria were gotten, which include gram positive bacteria like: *staphylococcus aureus* and gram-negative bacteria *like Salmonella spp, Escherichia coli and Klebsiella spp. Escherichia coli making up to 37.5%* which is the most prevalent. The resultsof the tests were determined by the different biochemical tests, which were carried out and include the catalase test, coagulase test, indole test, triple sugar iron test, urease and

citrate test. The bacteria isolated in this study is similar to that of Ogundipe *et al.* (2012) and Wogu and Ofuase (2014) who also isolated these bacteria as organisms associated with tomato spoilage. The fungi which were obtained include *saccharomyces cerevisiae, Penicillium sp and Mucor sp with Saccharomyces cerevisiae* being the most prevalent with 50%. The fungi were identified by their morphological and microscopic characteristics. Since tomatoes fruits are sometimes eaten raw, the presences of these microorganisms on tomatoes have been reported to cause human diseases such as meningitis, gastroenteritis, diarrhoea in man following ingestion (Beuchat, 2006). The major sources of contamination of tomato, by microorganisms, could be because of poor handling practices in the tomato production chain, storage condition, distribution marketing practices and transportation (Beuchat, 1996). The effect of these microorganism has the potential of causing substantial market losses to both the farmers and traders of tomato fruits across the country. Besides causing huge economic losses, some fungal species could produce toxic metabolites in the affected site of the fruit, constituting a potential health hazard for humans (Tournas, 2005). Additionally, vegetables have often served as vehicles for pathogenic bacteria, viruses and parasites and were implicated in many food- borne illness outbreaks. Therefore, in order to slow down vegetable spoilage and minimize the associated adverse health effect, great cautions should be taken to follow strict hygiene, good agricultural practices (GAPs) and good manufacturing practice (GMPs) during cultivation, harvesting, storage, transportation and marketing (Tournas, 2005).

Extending the shelf life of tomato fruits by controlling its ripening when it is to be transported over a long distance can be employed. This effectively controls fungi that can only deteriorate ripe tomato fruit (Cienciae, 2006). Appropriate temperatures and relative humidity should be employed in tomato fruit storage. Mechanically- injured and fungal- infested fruits should not be packaged with healthy fruits, so as to prevent mass deterioration of tomato fruits. Bacteria contaminants can be controlled by disinfecting all farm equipment such as picking basins, baskets, crates, buckets, e.t.c. that are used in tomato fruit harvesting, so as to effectively prevent cross contamination by spoilage microorganisms from fruits, vegetables and containers (Abdullahi and Choji, 2009; FAO, 2010). All other control measures could also be employed.

CONCLUSION

Tomato fruit is the world's most popular home garden fruit and the second most consumed vegetables after potato in the world. Tomato fruit is highly used in stew and sauce making in Nigeria. As most Nigerians cannot do without taking stew daily in their meals and diets, the presence of potentially pathogenic organism in the fruits could lead to great economic and health hazard. Several genera of bacteria, such as *staphylococcus aureus, Salmonella spp, Escherichia coli and klebsiella spp* and fungi species which are; *cerevisiae, Penicillium sp and Mucor sp* have been identified in this research as being

associated with the spoilage of tomato fruits. Therefore, concerted efforts should be made by the relevant health workers to discourage or stop the display and sale of spoilt tomato fruits in local markets. Effective control of bacteria and fungi plant pathogens, as well as contaminants in tomato fruit will bring about a great reduction in its deteriorations, prevent economic losses, improve the quality of tomato fruits delivered to consumer and also prevent the health hazards accompanied with the consumption of contaminated fruits and vegetables in Nigeria.

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