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Microbiological and Proximate Properties of Healthy and Diseased/spoilt (Broken) Tomatoes (*Lycopersicum esculentum L.*) Sold in Open Markets in Benin City: Public Health Implications

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Abstract: The study was carried out to evaluate the microbiological and proximate properties of healthy and diseased/broken tomatoes sold in open markets in Benin City. Samples were obtained and evaluated using standard microbiological and analytical techniques for isolation and enumeration of bacteria. 16sRNA molecular technique was used for identification of bacteria isolates. Proximate properties (moisture, ash, crude protein, fibre and carbohydrate content) of the fruits were evaluated using standard protocols. The phenotypic virulence properties of the isolates, antibacterial susceptibility and multiple antibiotic resistance index of the isolates were also evaluated. Results revealed that the bacterial counts (log10 cfu/g) for diseased/broken tomatoes ranged from 3.92±0.27 (Osa market) - 4.85±0.12 (Uselu market). The counts for healthy fruit samples were in the range of 1.08±0.05 (Oba market) - 1.91±0.15 (Aduwawa market). Relative to the diseased/broken tomato samples, there was a minimum of a 2-log reduction in the fresh/healthy samples. All the fresh/healthy tomato samples obtained from the respective markets were found to contain moisture contents with Oba market samples having the least contents (44.59 %) while New Benin market samples had the highest (42.29 %) moisture contents. It was evident that there was obviously no significant difference ($p>0.05$) in all tested parameters except for moisture and crude protein contents. The bacterial isolates from the samples include *Serratia marcescens, Leclercia adecarboxylata, Bacillus subtilis, Pectobacterium carotovorum* and *Salmonella enterica*. The isolates showed resistance to some antibiotics and their MAR index was found to be greater than 0.2.

1. Introduction

Tomatoes (*Lycopersicum esculentum* L.) is cultivated worldwide (Agrios, 2005), as well as for its famous fruits, known to contain several minerals and essential vitamins as John *et al*. (2010) opined. The fruit of *L. esculentum* is known to contain "lycopene", an excellent antioxidant, which can help in reducing the risk of developing cancerous cells such as breast and prostate cancers (Osemwegie *et al*., 2010). Tomato is a significant fruit vegetable, which is extensively used in making salads, stews and soups (Emoghene and Futughe, 2016). The fruits (tomato) are also used in making fruits drinks and sauces. In Nigeria, the Northern parts is widely credited for the production of tomato; nevertheless, it is however consumed in all parts of the country (Kutama *et al*., 2007). Today, it is a necessary ingredient in many Nigerian dishes; hence it is perennially in high demand. Etebu and Enaregha (2013) posited that a substantial portion of tomato fruits transported from the Northern parts to Yenagoa

metropolis (in Southern Nigeria) were found to suffer from several postharvest diseases. Precisely, it was reported that tomato fruits devoid of any symptom of disease or disorder (which arrived from the North) were about 25.09% only. A further statistic by Adeoye *et al*. (2009) opined that on the average, postharvest losses of tomato accruing to retailers and wholesalers were in the range of 50-70%. Due to several issues surrounding transportation, loading, and lack of storage facilities or preservation methods amongst others, the number of broken tomatoes in our local market is on the increase and several consumers do prefer these broken tomatoes because it is cheap, compared to healthy (unbroken or fresh) tomatoes. Some of the possible reasons for the abundance of broken tomatoes in our open markets include, physical damage during harvesting, transportation, and rough handling which can cause the tomatoes to bump into each other or other surfaces, leading to bruises and cracks. Others include poor storage conditions (such as high temperature and humidity), and overripening (harvest of fruits long after ripening) can also predispose fruits to damage during handling. More importantly, Varietal characteristics, pest and diseases of tomato fruits can also contribute to the relative abundance of broken tomatoes in the markets. Amongst the bacterial diseases of tomatoes, soft rot is of utmost importance as it destroys the fruit vegetable, resulting in huge losses in yield and a greater loss in market value than any known bacterial disease (Etebu and Enaregha, 2013; Akbar *et al*., 2014). A report by Adamu *et al*. (2017) opined that yield loss in tomato fruit caused by soft rot bacteria under insufficient storage conditions may reach up to 100%, resulting in huge impacts on the farmers, vendors as well as the final consumers. It is argued that tomatoes regardless of the state (diseased/broken or healthy), contain same proximate properties which may not be statistically significant when compared to one another. It is also hypothesized that broken tomatoes may have lower nutritional content compared to healthy tomatoes due to damage and loss of nutrients during handling, storage and by action of microbial activity in the fruits. While the healthy or unbroken tomatoes might have a higher level of moisture content compared to broken tomatoes due to loss of water in the fruits while in storage or in the markets. However, both healthy and broken tomatoes are low in calories and high in fiber, making them a healthy addition to a balanced diet. But given the amount or density of bacterial population in the broken tomato fruits, how safe or healthy can it be when consumed without proper cooking condition?

This study was carried out to evaluate the microbiological and proximate properties of healthy and broken tomatoes sold in open markets in Benin City and evaluate the corresponding public health implications of bacteria isolates from broken/diseased tomato fruits.

2. Methodology

2.1 Isolation and Enumeration of Bacteria from Healthy and Broken Tomato Fruits

Diseased, broken or spoilt tomatoes samples showing typical soft rot symptoms were obtained from random locations (markets) in Benin City. Then, fresh or apparently healthy tomato fruits were also selected and used in the study. The tomato fruits (diseased and healthy) were analyzed using standard microbiological techniques (Abeer and Fawzi, 2013; Ogofure and Igbinosa, 2021). For the healthy fruit's samples, a four-fold dilution was done by dissolving 10 g of aseptically cut tomato fruit into 30 ml of saline water. Meanwhile, for the soft rotted samples, a ten-fold stock was prepared before serial dilution was carried out for the isolation and enumeration of bacterial isolates. The stock was prepared by taking 10 g of the soft rotted samples into 90 ml of saline water.

$$Dilution\ factor = \frac{final\ volume}{aliquot\ volume} \qquad (1)$$

$$where: final\ volume = aliquot\ volume + diluent\ volume$$

After serial dilution was carried out for the broken samples, 1 ml aliquot was pour plated in triplicate using nutrient agar. The plates were incubated at room temperature 28 ± 2 ^{0}C for 24 h. Bacterial colonies were counted using a colony counter and results were recorded. Enumeration of the isolates was done using the formula given in equation 2 as described by Willey *et al*. (2008).

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (2)$$

2.2 Identification of Bacterial Isolates

Bacterial isolates were subjected to phenotypic and molecular technique of identification. The isolates were purified and the biochemical characterization performed for the isolates include, indole, oxidase, urease, triple sugar iron agar tests, catalase, oxidase, citrate, urease, Gram and KOH reactions, to mention a few.

2.3 Extraction of Genomic Bacterial DNA

Genomic bacterial DNA was extracted using boiling method described by Chakravorty *et al*. (2007) and Igbinosa *et al*. (2023). Briefly, 2 ml of 24 h. broth culture was transferred to Eppendorf tube and centrifuged at high speed (10,000 xg) for 5 min. The supernatant was discarded after which 200 µL of sterile distilled water (SDW) was added to the pellets and vortexed using a vortex mixer for 1 min before heating at 100 ^{o}C for 15 mins in a heating block. Lastly, it was further thereafter centrifuge at high speed for 2 minutes. The supernatant from the second spin (the pure DNA) was used for further analysis such as PCR amplification of the DNA.

2.4 Polymerase chain reaction (PCR)

The cocktail, used for the preparation of PCR procedure consists of a colourless reaction containing mixtures of 10µl of 5xGoTaq, 1µl of dNTPs mix (10mM), 3µl $MgCl_2$ (25mM), 1µl of 10pmol each 27F-5'-AGAGTTTGATCMTGGCTCAG-3' and - 1540R, 5′- TACGGYTACCTTGTTACGACT-3′ primers as well as 0.3 units of Taq DNA polymerase, which was made up to 42 µl containing 8µl of DNA template (Taq DNA polymerase was from Promega, USA) and SDW (sterile distilled water). PCR experiment was performed using a PCR System, GeneAmp 9700 thermal cycler (made in USA by Applied Biosystem Inc.). The profile for PCR used, consists of initial denaturation for 5 minutes at 94°C; closely or briefly accompanied by a 30 cycle, of 94°C for 30 s, 50°C for a minute and 72°C for a minute and 30 seconds; and a final step of termination for 10 minutes at 72°C. After the aforementioned processes, the product was kept in the refrigerator at 4 ^{o}C until ready for use.

2.5 Integrity of Amplified DNA

The integrity of the amplified DNA fragment (1.5mb) was confirmed on agarose gel (1.5 %). 1x TAE buffer was used in the preparation of 1.5% agarose gel mixture boiled for 5 minutes in a microwave. It was thereafter left to cool to 60°C (a molten state) before staining with 3 µl of ethidium bromide (of 0.5 g/ml concentration), which transmits energy as a visible orange light after absorption of invisible UV light. The molten agarose was poured into the casting tray slots after insertion of a comb. The gel was left to stand for 20 minutes to allow for solidification to form the wells. Into the gel tank, the 1 XTAE buffer was poured into the tank of the gel to submerge it. A loading dye of 2 µl quantity (10X blue gel) was added to 4µl of each product of PCR before they were loaded into the wells. The function of the loading dye was to give color and density to the samples in order to make loading into the wells easy as well as monitoring the progress of the gel. For a 45 minutes time frame, and at 120V the gel

was electrophoresed, then visualized by ultraviolet trans-illumination before it was thereafter photographed. The amplified PCR product sizes were assessed by comparing their mobility with that of the molecular weight ladder (of 100bp), which were evaluated together in the gel with the experimental samples.

2.6 Purifying the Amplified DNA Product

The purification of amplified DNA fragments of bacteria was carried out to remove the PCR reagents in the samples. Briefly, 95 % ethanol (240 µl) and 3M sodium acetate (7.6 µl) were placed in a sterile Eppendorf tube to which was added to each 40µl of the amplified PCR product, which was thoroughly mixed using a vortexer and stored for at least 30 minutes at -20 °C. It was then centrifuge at 13,000 g for 10 minutes at 4°C and the supernatant was briefly removed by inverting tubes once on thrash. The pellets were thereafter washed via the addition of 150 µl of 70% ethanol. This was mixed again before another round of centrifugation at 7500 g and 4°C for 15 minutes. This time around, all supernatants are also removed via the inversion of tubes on tissue paper and were left to dry for about 10-15 minutes. Thereafter, SDW (20 µl) was added to the resuspend the mixture and stored at -20 ^{0}C prior to sequencing. 1.5% agarose gel was used to check the integrity of the purified fragment, which was ran for an hour on a voltage of 110V. The confirmation of the integrity was carried out as previously described using a nano-drop of model 2000 from thermo scientific.

2.7 Sequencing, Blasting and Deposition of Sequences in Gene bank

Sequencing of the amplified bacterial DNA fragments was carried out using the Genetic Analyzer (model 3130xl) sequencer from the Applied Biosystems. The instructions on the manufacturer's manual were strictly followed while the BigDye terminator was the manufacturer of the sequencing kit. Following sequencing of the amplified bacteria DNA, blasting was carried out on the sequences using the national centre for biotechnology information (NCBI) blast website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Bacterium with the highest homology chosen and the sequences were deposited in the GenBank (https://submit.ncbi.nlm.nih.gov/subs/genbank/) to obtain ascension numbers for the isolated bacteria.

2.8 Proximate Analysis of Healthy and Diseased/Broken Tomatoes

The protein, fat, carbohydrate, ash, moisture and fiber contents of diseased and healthy tomato fruit samples were evaluated and determined using the method described by Association of Official Analytical Chemists (2000).

2.8.1 Moisture Contents: The moisture contents were evaluated via taking the initial weight of a clean crucible (W_0), and that of a tomato fruit in the crucible (W_1). The weighed portion of the tomato fruit was oven-dried (Uniscope SM9023 Laboratory Oven Surgifriend Medigals, England) at 105^{0}C for 3 h., allowed to cool in a desiccator and weighed (W_2). Using the equation below, the weight loss due to moisture was evaluated:

$$\%\, moisture = \frac{W_1 - W_2}{W_1 - W_0} x \frac{100}{1} \qquad (3)$$

W_0 = Initial weight of empty crucible
W_1 = Initial weight of sample before drying + the crucible
W_2 = Weight of sample after drying + the crucible

***2.8.2* Crude Protein**: Determination of crude protein was carried out via the multiplication of nitrogen content by a factor 6.25 (on the assumption that there is 16% nitrogen content is found in most protein). Experimentally, the crude protein content was evaluated using kjeldahl method involving a 3-step process of digestion, distillation and titration. Briefly, 2g of sample was added to a solution (in a kjeldahl flask) containing 0.5g of copper sulphate and 25ml of concentrated sulphuric acid, 5g of sodium sulphate, and a speck of selenium tablet. In a fume cupboard, heating was slowly applied (for prevention of undue frothing) and the digestion continued for 45 min until a clear to pale-green digestate was obtained and allowed to cool completely before rapid addition of distilled water (1 litre). Following digestion, the flask was rinsed and the liquid was added to the bulk. Distillation was carried out using the Markham distillation apparatus. Briefly, 10 ml of the digestate was added to the steamed-up Markham apparatus with the aid of a funnel and boiled. Briefly, 10ml of NaOH was added (to conserve the ammonia) to the measuring cylinder and slowly distilled into 50ml of boric acid (2%) containing methyl red indicator. The formed solution (alkaline ammonium borate) was directly titrated with 0.1 N HCl. The volume of acid which was the formed titre was recorded and the formulas below was used to evaluate percentage nitrogen and crude protein.

$$\%\ nitrogen\ = \frac{14\ x\ volume\ of\ acid\ x\ weight\ of\ sample}{1000\ x\ 100} x\ \frac{100}{1} \qquad (4)$$

$$\%\ crude\ protein\ = \frac{\%\ nitrogen}{1} x\ \frac{6.25}{1} \qquad (5)$$

2.8.3 Ash Content: the ash content was determined after weighing an empty clean crucible (W_0), and 5g of the sample was placed on another crucible and re-weighed (W_1). The sample and crucible was placed in a furnace (Gallenkamp Muffle Furnace) and ashed at 660 ^oC for 6 h., then allowed to cool in a desiccator and re-weighed (W_2). The equation below was used to evaluate the ash content of diseased and healthy tomato samples.

$$\%\ \boldsymbol{ash}\ = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_1 - Wo}} \boldsymbol{x}\ \frac{\boldsymbol{100}}{\boldsymbol{1}} \qquad (\boldsymbol{6})$$

W_0 = Initial weight (g) of the empty crucible
W_1 = Initial weight (g) fresh sample + crucible
W_2 = Weight (g) of ashed sample + crucible

2.8.4 Crude Fibre: Briefly, petroleum ether was used to extract 5g of sample, stirred and allowed to settle. Decantation was done for the stirred samples and the process was repeated thrice and, a portion (of the fibre extract) was removed into a beaker, before adding a solution of 200ml of pre-heated H_2SO_4 (1.25%) and boiled gently for a 30 min. Hot water was poured into the Whatman filter fitted Buckner flask funnel to pre-heat the funnel and the mixture of the boiled acid sample was briefly filtered under sufficient suction while still hot via the funnel. The residue was severally re-washed using hot water (until it appears neutral to litmus paper) before it was transferred into the beaker. This was then followed by the addition of 200ml of pre-heated Na_2SO_4 (1.25%), which was boiled for 30 minutes. Again, this was filtered under suction and washed twice with hot water and ethanol respectively. At a temperature of 65^oC for 24 hours, the residue was dried and weighed, before it was briefly transferred into a crucible and placed at 400-600oC for 4 hours, in a muffle furnace. This was then weighed after cooling.

$$\%\ \boldsymbol{Crude\ fibre} = \frac{\boldsymbol{dry\ weight\ of\ residue\ before\ ashing - wt\ after\ ashing}}{\boldsymbol{weight\ of\ sample}} \boldsymbol{x}\ \frac{\mathbf{100}}{\mathbf{1}}$$

2.8.5 Crude Fat Content: In a pre-weighed filter paper (W_0), 1g of sample was weighed (W_1). Then wrapped and loaded in a Soxhlet extractor mounted on a Kedjahl apparatus improvised as a heating mantle. 200 ml of petroleum ether was added and extracted for 5 hours, oven-dried, cooled and weighed again (W_2). The percentage of crude fat was obtained using the formula delineated below:

$$\%\ \boldsymbol{Crude\ Fat} = \frac{\boldsymbol{weight\ of\ crucible - tare\ weight\ of\ crucible}}{\boldsymbol{weight\ of\ sample}} \boldsymbol{x}\ \frac{\mathbf{100}}{\mathbf{1}}$$

2.8.6. Carbohydrate (CHO) Content:
The CHO was calculated by "difference" method as shown below :

$$\%\ \boldsymbol{CHO} = \mathbf{100} - (\%\ \boldsymbol{moisture} + \%\ \boldsymbol{crude\ fibre} + \%\ \boldsymbol{ash} + \%\ \boldsymbol{crude\ protein})$$

2.9 Standardization of Bacterial Culture
The bacteria isolates obtained from the samples were purified and standardized using the method delineated by Bauer *et al*. (1996) where 0.5 ml of $BaCl_2$ was added to 99.5 ml of 1% H_2SO_4. The corresponding turbidity (referred as 0.5 McFarland standard) was used as control for the turbidity of the prepared bacterial suspension.

2.10 Phenotypic virulence of Bacteria Isolates
The isolated bacteria isolate from the samples were tested for the presence of certain phenotypic virulence properties such as possession of DNAse, Gelatinase, Liapse and production of Beta-Hemolysin. For the analyses of the above, a modified method of Georgescu *et al*. (2016) and Shruthi *et al*. (2012) were employed and briefly, bacteria isolates were cultured on DNAse agar, Spirit Blue agar (with the addition of a lipase substrate), blood agar and gelatinase medium. The blood agar was evaluated for the presence of beta hemolysis (clear zones around bacteria colonies), clearing zones were also observed for DNAse and Spirit-blue agar while liquefaction of gelatin on ice was observed for cultures to confirm positive cases for the respective phenotypic virulence properties.

2.11 Antibiotic Susceptibility Testing
The bacterial isolates were subjected to standard antibacterial susceptibility testing (AST) using Kirby-Bauer disk diffusion method (Bauer *et al*., 1966) as modified by Ogofure *et al*., (2022). The standard discs were produced by Oxoid (UK), which was used to execute the disc diffusion method and for this assay, a fully grown bacterial standardized culture (from 18-24 h.) was inoculated on Mueller Hinton agar. The inoculum corresponding to 1.5 x 10^8 cells/ml McFarland standard was streaked on the agar using a sterile loop before introduction of antibiotic discs (added with care) to the plates. The susceptibility results were recorded after incubation for a 24-hour period at 37 ºC. Following the standard established in 2017 by Clinical Laboratory Standards Institute (CLSI). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on 2017 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S). The antibiotic discs used in the study with their corresponding codes and concentrations include CIP; Ciprofloxacin (5µg), AMC; Amoxicillin/clavulanic acid (20/10 µg), E; Erythromycin (15 µg), CRO; Ceftriaxone (30 µg), RL; Sulfamethoxazole (1.25/23.75 µg), VA; Vancomycin (30 µg), MEM; Meropenem (10 µg), CN; Gentamicin (10 µg).

2.12 MAR (Multiple Antibiotic Resistance) Index

In this study, the MAR index was determined by employing the methods delineated by Chitanand *et al*. (2010). The MAR index was obtained using the formula:

$$MAR\ index = \frac{y}{nx}$$

$where\ y = number\ of\ resistance\ scored,$
$n = number\ of\ isolates\ and$
$x = total\ number\ of\ antibioitics$

According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source or that the bacteria were of public health significance.

2.13 Statistical Analysis

Data were subjected to analysis of variance (ANOVA), using the statistical package for social scientist (version 21) and Microsoft excel 2019. Means of the proximate parameters were compared using unpaired Students t test for disease and healthy tomato fruits at probability level of 95% ($p<0.05$). Microbial or bacterial log reduction calculation or estimation (between diseased/spoilt and healthy samples) was also employed in this study using the following equations below:

$$Log\ Reduction\ (LR) = log_{10}\left(\frac{A}{B}\right)\ or\ LR = log_{10}(A) - log_{10}(B)$$

where A is the number of organisms in spoilt/diseased samples and B is the amount in healthy samples. For percentage bacteria reduction, the following mathematical formular was employed:

$$P = (1 - 10^{-L})\ x\ 100$$

$where\ P = percentage\ microbial\ reduction, L = \log reduction$

3. Results and Discussion

3.1 Heterotrophic Bacteria Counts of Healthy and Disease/Broken Tomatoes

The heterotrophic bacterial counts of healthy and broken/diseased tomatoes revealed that counts for broken/diseased tomatoes were higher compared to counts for fresh/healthy tomatoes. The bacterial counts (log_{10} cfu/g) for diseased/broken tomatoes (**Figure 1**) ranged from 3.92±0.27 (Osa market) - 4.85±0.12 (Uselu market). The counts for healthy fruit samples were in the range of 1.08±0.05 (Oba market) - 1.91±0.15 (Aduwawa market). Relative to the diseased or broken tomato samples, there was a minimum of a 2-log reduction in the healthy samples with log reduction values ranging from 2.65 (New Benin market) – 3.72 (Oba market). The corresponding log reductions obtained in the healthy fruits relative to the broken or diseased tomatoes revealed that there was at least a 99% decrease in bacteria population in the healthy fruits relative to the broken samples. This connotes that about 99% of bacteria found in diseased/broken tomato fruits were sourced from the external environments as a function of a plethora of activities bordering around postharvest practices, transportation, storage and pests. Regardless, the bacteria count obtained for the healthy fruits were found to be less than the log of 2 and this was found to be within acceptable limits. Tomato, as a fruit and a vegetable contain high moisture and this has an effect on its ability to be stored for long duration as well as having an impact on its shelf life (Ghosh, 2009; Alaoui *et al.* 2007). The nature of the fruit/also makes its transportation difficult as well and thus, for the several ways by which it can be consumed, the presence of bacterial contamination is a constant menace to consumers especially those who consume them in their fresh/raw state (**Figure 2**).

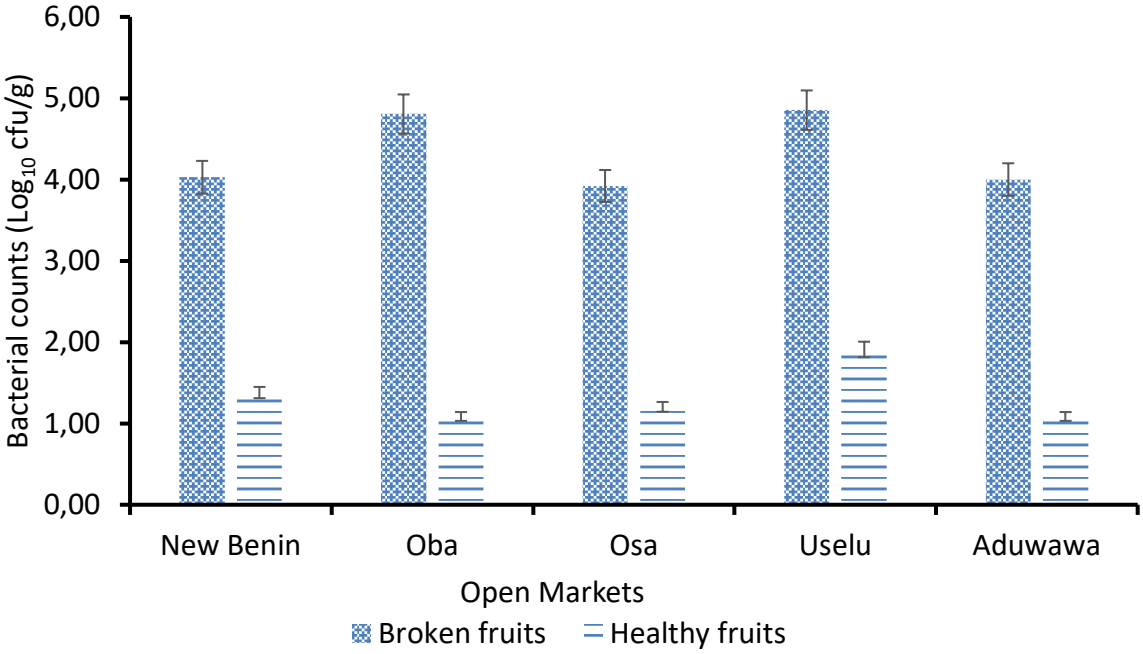

Figure 1. Bacterial count of broken and healthy (fresh) tomato samples from open markets. Legend: values are Mean ± S.E of triplicate determination

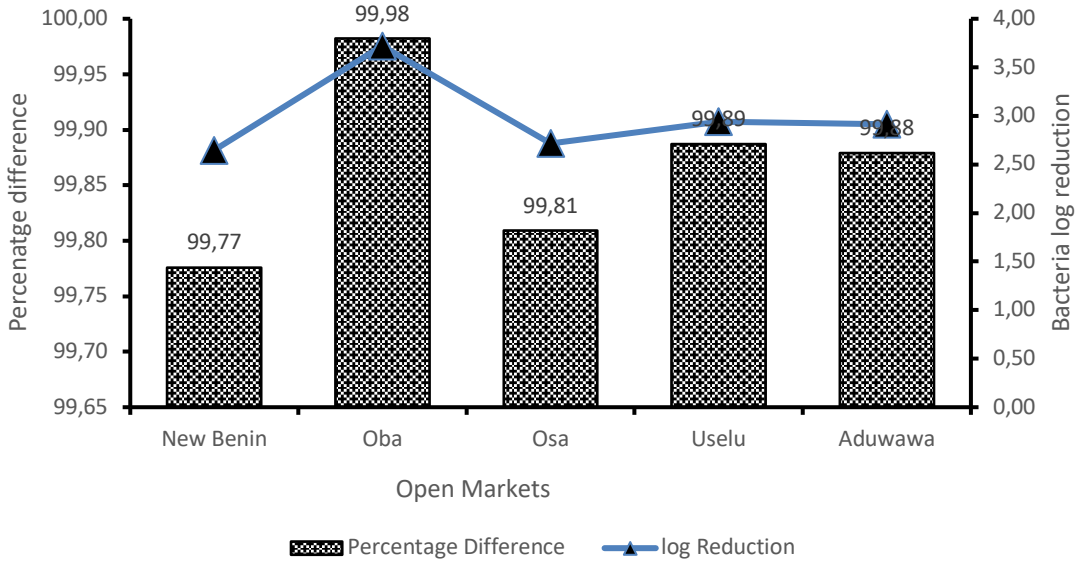

Figure 2. Bacterial log reduction and percentage reduction of healthy samples relative to broken samples

More so, the presence of microorganisms in their numbers on tomato fruit contributes to the shelf life, economic loss to farmers and sellers alike (Agbabiaka *et al*., 2015) and the safety (positive or negative) of consumers (Guo *et al*., 2001; Ghosh, 2009). The presence of bacterial population in diseased and healthy tomato fruits obtained in this study was consistent with several reports in literatures where bacterial population have been associated with spoilage and disease of tomato fruits with proof following isolation and characterization (Guo *et al*., 2001; Agbabiaka *et al*., 2015). The values obtained in this study for diseased/broken tomato fruit samples were lower than the reported value of $\log_{10}$ 6.60 cfu/g by Agbabiaka *et al*. (2015) in Ibadan, and also lower than the value of $\log_{10}$ 6.52 cfu/g reported by Ogundipe *et al*. (2012) for tomato samples classified as soft and slightly damaged tomato in Lagos state Nigeria. The high bacterial density observed for broken/diseased tomato samples could be attributed to the nature, and quality of the fruit since the integrity of the plant cell wall has been

breached, thereby causing the bacteria (including saprophytes, spoilage or pathogens) to have easily colonized the tomato fruits.

The low bacterial population in the fresh healthy fruits could be attributed to the fact that the integrity of fruit is still intact. By nature, tomato fruit is covered by pectin layer which offers protection from invading microbes or saprophytes in the external environment. This layer of protection must be breached before pathogens, spoilage bacteria or other microbes can gain entrance to cause deterioration or degradation of the fruits. Most plant pathogenic bacteria are able to colonize tomato fruit once there are openings on it and this explains why there might be more bacterial population in diseased or spoilt tomato samples compared to healthy or fresh samples. More so, the phenomenon of microbial endophytism which is defined as the stable occurrence of bacterial or microbial population inside a healthy plant (Muresu *et al*., 2010) that may not necessarily be undergoing spoilage or disease condition could be responsible for the isolation of bacterial population (howbeit in low density) from healthy tomato fruits in this study. More so, the ubiquitous nature of bacteria in the field as well as during transit and storage might have also influenced the presence of bacteria on apparently healthy tomato fruits in this study. The result obtained in this study with regards to the presence of bacteria in healthy fruits was consistent with the report of Ogundipe *et al*. (2012) who revealed that bacterial count in healthy tomato fruits can be as high as log10 3.0 which is lesser than the range of values reported in this study. Guo *et al*. (2001) also posited that fresh tomato fruits can be home to a plethora of bacteria which can survive on the plant from inoculation at flowering and early stages of fruits development to fruit ripening stage.

3.2 Proximate Properties of Healthy and Diseased/Broken

The proximate composition of healthy or fresh samples of Lycopersicum esculentum (tomato) is shown in **Table 1.** All the fresh and healthy tomato samples obtained from the respective markets were found to contain moisture contents with Oba market samples having the least contents (44.59 %) while New Benin market samples had the highest (42.29 %) moisture contents. Tomato sample with the highest carbohydrate content was obtained from Oba market (37.65 %) while the least was found in New Benin market (16.88 %).

Table 1. Proximate composition of diseased or broken samples of *Lycopersicum esculentum* (tomato)

Parameters (%)	New Benin market	Uselu market	Oba market	Osa market
Moisture	39.82	42.29	44.59	37.87
Lipids	1.50	0.50	0.50	2.00
Crude fibre	11.56	10.99	10.55	11.31
Crude protein	5.25	5.95	5.60	4.38
Ash	1.08	1.55	1.13	1.41
Carbohydrates	40.79	39.12	37.65	43.03

The variation in the moisture contents of the fruits could be due to varietal differences in the fruits obtained and evaluated. It is noteworthy that while samples evaluated in the study were either ready to eat samples or broken samples which consumers purchase at cheap give-away prices, the samples were not segregated into different varieties as different varieties may have been in the mix of broken samples. Regardless, this study focused on the microbiological and proximate evaluation of tomato fruits sold in open markets in Benin City. The result of the comparative evaluation of the proximate composition of healthy and fresh as well as diseased or broken samples of tomato is shown in **Table 3**. It was evident that there was obviously no significant difference ($p>0.05$) in all tested parameters except for moisture and crude protein contents.

Table 2. Proximate composition of good/healthy samples of *Lycopersicum esculentum* (tomato)

Parameters (%)	New Benin market	Uselu market	Oba market	Osa market
Moisture	49.67	45.87	44.59	47.85
Lipids	2.50	4.00	0.50	2.50
Crude fibre	19.64	6.06	10.55	9.73
Crude protein	8.93	8.58	5.60	7.35
Ash	2.38	2.25	1.13	2.30
Carbohydrates	16.88	33.24	37.65	30.27

Table 3. Comparative evaluation of the proximate composition of healthy and diseased tomato samples

Parameters (%)	Healthy tomato	Diseased tomato	*p-value*
Moisture	46.99±1.11	41.14±1.46	0.019
Lipids	2.37±0.71	1.12±0.37	0.174
Crude fibre	11.49±2.89	11.10±0.21	0.897
Crude protein	7.61±0.75	5.29±0.33	0.031
Ash	2.01±0.29	1.29±0.11	0.063
Carbohydrates	29.51±4.47	40.14±1.15	0.061

Diseased and healthy tomato fruits showed similarity in their proximate composition, as there was only a difference in the moisture and crude protein contents of the diseased and healthy samples. It is noteworthy that the difference in the diseased samples could be because of several factors, which include the variety of tomatoes that are diseased, the type and number of microorganisms that are present in the diseased samples as well as the level of infection or the type of degradation being perpetuated by the microbes, which colonizes the tomato fruits. The proximate composition of broken/diseased tomato is good news for those who cannot afford the healthy or fresh tomatoes. It also contains all the necessary parameters, which can be found in healthy tomato samples. This finding was consistent with the report obtained in the work of Ogofure and Emoghene (2016) who opined that the presence of disease in a plant sample could have little or no impact in the proximate properties of the plant. Several other reports of diseased and healthy fruits have revealed similar proximate composition but the only crux is about the fact that toxins and other microbial products could build up in the diseased samples which obviously would be absent from healthy fruit samples. More so, the abundant presence of microbes in broken samples could give a clear call for concerns of clinical significance.

3.3 Identification of Bacterial Isolates, Phenotypic Virulence and Antibacterial Susceptibility

The molecular characterization of bacterial isolates (**Plate 1., Table 4, and 5**) from diseased/broken tomatoes as well as healthy fruits revealed that the bacteria in the samples were *Serratia marcescens, Leclercia adecarboxylata, Bacillus subtilis, Pectobacterium carotovorum* and *Salmonella enterica*. These were identified using 16sRNA of the bacteria genome. The isolates following their deposition in gene bank were given accession numbers by which they can be referenced. They were also found to have 99.9% similarity to *Serratia marcescens, Leclercia adecarboxylata, Pectobacterium carotovorum*, *Salmonella enterica* and *Bacillus subtilis.*

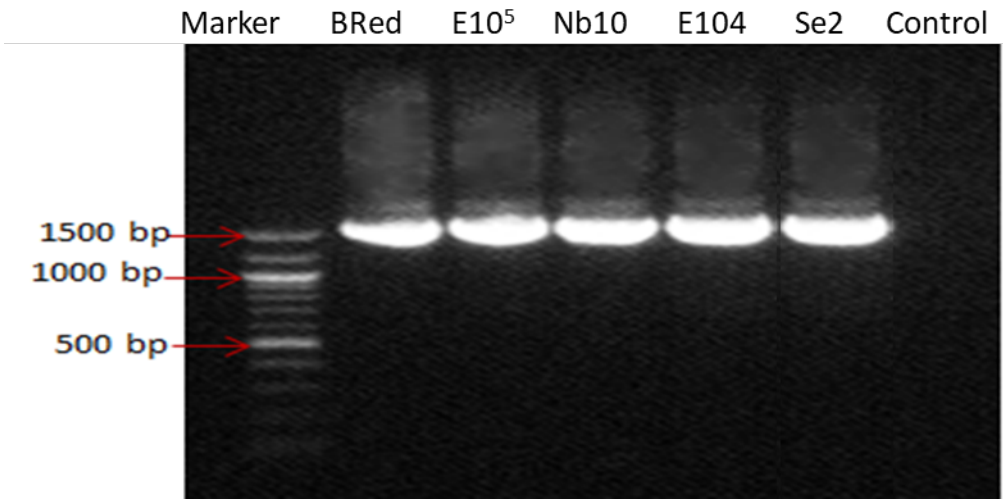

Plate 1. Agarose gel electrophoresis of 16S rRNA of bacterial isolates

Table 4. Molecular characterization of bacterial isolates from tomato fruits

Code	Closest Similarity	Query cover (%)	Identity (%)	Accession number
BCA Red	*Serratia marcescens*	99	100.00	MK813904.1
PCA $E10^5$ (1)	*Leclercia adecarboxylata*	99	100.00	MK814570.1
MSA $NB10^3$ (5)	*Pectobacterium carotovorum*	99	100.00	MK814568.1
SSA $E10^4$ (1)	*Salmonella enterica*	99	100.00	MK814569.1
BCA SE2	*Bacillus subtilis*	99	100.00	Mk875780.1

Table 5. Distribution of bacteria isolates from broken tomatoes from the sampling locations

	Open Markets				
Isolates	New Benin	Oba	Osa	Uselu	Aduwawa
Serratia marcescens	+	+	+	+	-
Leclercia adecarboxylata	-	+	+	+	+
Pectobacterium carotovorum	+	+	+	+	+
Salmonella enterica	+	-	+	-	+
Bacillus subtilis	+	+	+	+	+

Key: + = Present, - = absent

Table 6. phenotypic virulence properties of bacterial isolates from broken tomatoes

Isolates	Hemolysin	DNAse	Gelatinase	Lipase
Serratia marcescens	β 4(100)	4(100)	0(0)	4(100)
Bacillus subtilis	β 2(50)	0(0)	4(100)	4(100)
Pectobacterium carotovorum	β 0(0)	0(0)	0(0)	0(0)
Leclercia adecarboxylata	β (0)	0(0)	0(0)	0(0)
Salmonella enterica	β 0(0)	0(0)	0(0)	4(100)

The result obtained in this study was consistent with the reports of Ogundipe *et al*. (2012) and Agbabiaka *et al*. (2015) who opined that *Salmonella, Escherichia, Bacillus* and *Pseudomonas* were isolated from tomato fruits. Furthermore, Guo *et al*. (2001) reported the presence of a plethora of bacterial isolates

amongst which were also listed in this study. There have also been reports where contamination from plants have been confirmed but the roles of these bacteria have not been fully elucidated. It can be safe to assume that apart from some of the bacterial species appearing as saprophytes/or pathogens in the tomato fruit samples, their presence could also have a potential impact on the health of consumers especially for highly contaminated samples consumed in their raw state. Some of the isolated bacteria from analyzed tomato fruits have been reported to be a public health threat world over. *Leclercia adecarboxylata* (commonly confused with *Escherichia coli*) has been reported to cause opportunistic infection in humans especially in immunocompromised individuals (Willey *et al*. 2008; Karmakar *et al*., 2018; Jajere *et al*., 2019). Amongst all isolates obtained in the study, only *P. carotovorum* has not been copiously implicated to be a public health problem. Nonetheless, *Salmonella* and *Serratia* have made name for themselves for being a public health problem in Nigeria and beyond. More so, the fact that these isolates are ubiquitous in nature is a testament of their ability to survive a plethora of environmental conditions and their roles, and function in the environment deserves to be studied extensively. Consistent with the findings in this study, Barak and Liang (2008) reported that the presence of *S. enterica* within the phyllosphere of tomato plant could pose a consequent risk to consumers as well as production of tomatoes in areas prone to bacteria diseases.

All bacterial isolates were found to be susceptible to gentamicin (100%). *Leclercia* showed resistance to meropenem and augmentin. *Salmonella* was also found to be resistant to erythromycin, ceftriaxone and augmentin. S. marcescens and *P. carotovorum* were also found to be resistant to augmentin and ceftriaxone.

Table 7. Antibiotic susceptibility (%) of isolated bacteria from spoilt tomato samples

Isolates (n)	Antibiotic susceptibility of isolates (%)							
	CIP	CRO	RL	E	VA	MEM	AMC	CN
S. enterica (4)	(75)	(50)	(0)	(25)	(-)	(100)	(50)	(100)
Serratia marcescens (4)	(100)	(0)	(-)	(0)	(-)	(50)	(50)	(100)
P. carotovorum (4)	(100)	(0)	(-)	(100)	(-*)	(50)	(50)	(100)
L. adecarboxylata (4)	(50)	(0)	(-)	(0)	(-)	(50)	(50)	(100)
B. subtilis (4)	(100)	(25)	(-)	(0)	(50)	(100)	(50)	(100)

Legend: CIP; Ciprofloxacin (5µg), CRO; Ceftriaxone (30 µg), RL; Sulfamethoxazole (1.25/23.75 µg), E; Erythromycin (15 µg), VA; Vancomycin (30 µg), MEM; Meropenem (10 µg), AMC; Amoxicillin/clavulanic acid (20/10 µg), CN; Gentamicin (10 µg), (-) no guidelines in the CLSI (2020) manual were found CLSI (2020) guidelines were consulted.

The phenomenon of multidrug resistance of the isolated bacteria has been an age long battle in cases where they have been reported to be of clinical significance. As with most literature regarding *Serratia* species, the vast majority of antimicrobial resistance that has been described for this genus has occurred in *S. marcescens*. The fact that *S. marcescens* was a very resistant organism was recognized in early published cases (Wheat *et al*., 1951). As for the case of *Salmonella*, the issue has been over flogged with recourse to its resistance to virtually every class of antibiotics (Byarugaba, 2010; Jelalu *et al*., 2015; Britto *et al*., 2018; Igbinosa *et al*., 2023) as well as its consequent effect on the economy in terms of cost of control. *Leclercia adecarboxylata* has also been evaluated for its role in infection (Stone *et al*., 2015) but resistance to commonly used antibacterial agents have not been the case with this pathogen.

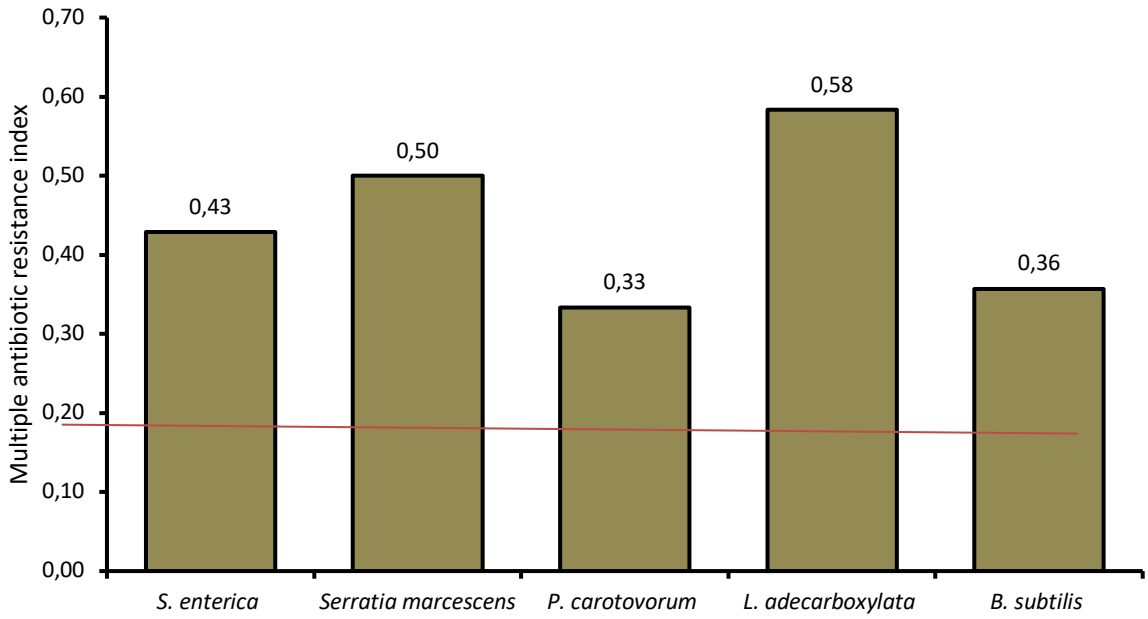

Figure 3. Multiple antibiotic resistance index of bacterial isolates from broken tomatoes

Although there are a few reports of multidrug resistant strains of *Leclercia*. *Pectobacterium* has also been implicated rarely in immunocompromised patients and resistance to most antibacterial agents have not been reported in literatures. However, all the isolated bacteria in this study were found to be multiresistant. All isolates were found to be of public health importance because their respective MAR index was greater than 0.2. In fact, their MAR index underscores that the pathogens have acquired resistance to some of the antibiotics in our locality, which could be a function of prior antibiotic exposure, or they must have acquired this resistance from the environment in terms of bacteria cell-to-cell communication or interactions (Igbinosa *et al*., 2016; Ogofure *et al.,* 2018; Ogofure and Igbinosa, 2021). Regardless of whatever pattern or route by which pathogen obtain resistance, it does not bode well for the community because their status as public health menace has been confirmed.

Conclusion

This study has laid emphasis on the microbiological and proximate properties of healthy/fresh and diseased/broken tomatoes sold in open markets in Benin City. While it is correct that there may be no significant difference in the proximate parameters of fresh and broken tomatoes, however the safety of broken tomatoes should be of immense concern as it can be a source of bacteria pathogens of public health concern in the community.

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