EVALUATION OF MICROBIOLOGICAL AND PROXIMATE PROPERTIES OF FRESH AND BROKEN TOMATOES (*Lycopersicum esculentum*)

BY

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BENIN CITY

JULY, 2021.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY FACULTY OF LIFE SCIENCES UNIVERSITY OF BENIN, BENIN CITY. IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN MICROBIOLOGY.

JULY, 2021.

CERTIFICATION

We certify that this project work was carried out by **Anita Ehinomhen OGUN (MISS)** in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) Degree in Microbiology.

__________________________ ____________________

Dr. A.G Ogofure **DATE**

(Project Supervisor)

__________________________ ____________________

Professor Solomon E. Omonigho **DATE**

(Head of Department)

DEDICATION

This project is dedicated to the Almighty God, my helper and my source, and also to my lovely parents

ACKOWLEDGMENTS

My profound gratitude goes to God Almighty for his tender mercies and faithfulness that never cease.

My sincere gratitude goes to my ever supportive supervisor Dr. A.G Ogofure for his patience, guidance and excellent supervision throughout the duration of my project work.

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ABSTRACT

This study was carried out to evaluate the microbiological and proximate analysis of diseased and healthy tomato fruits *(Lycopersicum esculentum)*. Standard microbiological and analytical methods were used to determine the total heterotrophic bacterial and fungal count of healthy and diseased tomato samples obtained from two locations in Benin City. The isolated bacteria were characterized and identified using cultural and biochemical means from the samples. The proximate parameters such as carbohydrates, proteins, lipids, fibres and ash contents were evaluated using standard methods. The results revealed that the bacterial count ranged from 7.13 ±0.11 x 10^4cfu/g in Uselu market to 4.25 ±0.96 x 10^4cfu/g in Osa market. There was however, a reduction in the fungal count compared to bacterial count. The mean fungal count ranged between 0.04 ±0.01 x 10^4cfu/g to 0.55 ±0.12 x 10^4cfu/g. However, the number of heterotrophic bacteria and total fungal count was drastically reduced in the good tomato samples analyzed in the study. It was shown that *Pseudomonas aeruginosa, E. coli, Bacillus subtilis* and *Salmonella enteritidis* were the identified bacterial isolates from tomato samples in the study. It was evident that healthy samples of *Lycopersicum esculentum* has higher values of moisture content (46.99±1.11) compared to diseased samples (41.14±1.46). More so, there were also higher values for other proximate parameters such as lipids (2.37±0.71), crude fibre (11.49±2.89), crude protein (7.61±0.75) and ash (2.01±0.29) compared to diseased samples with respective values of 1.12±0.37 (lipids), 11.10±0.21 (crude fibre), 5.29±0.33 (crude protein) and 1.29±0.11 (ash). Although, there was no significant difference ($p>0.05$) in certain of the proximate parameters such as lipids, crude fibre, ash and carbohydrates.

CHAPTER ONE

1.0 INTRODUCTION

Fruits and vegetables are vital sources of nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development (Mbajiuka and Enya, 2014). Fruits and vegetables however, have serious challenges to their existence. These include changes in climatic condition, pests and microbial attack. Over the years, there has been an increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Akinyele and Akinkunmi, 2012). Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents are associated with greater predisposition to microbial spoilage (Mbajiuka and Enya, 2014). The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animal. This is due to their production of mycotoxins which are capable of producing aflatoxin in man, following ingestion or inhalation. Early intervention measures during crop development and harvesting through the use of good agricultural practices provided dramatic reductions in the yield loss due to deterioration at all subsequent steps in the food (Barth *et al*, 2009). Fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with these whole fruit surface and those from adjacent infected fruits (Baiyewu *et al*, 2007). In developing countries, post-harvest deterioration are often more severe due to inadequate storage and transportation facilities (Mbajiuka and Enya, 2014). One of the most common fruits affected by deterioration and spoilage in Africa and Nigeria especially is the *Lycopersicum esculantum* (tomato). Etebu and Enaregha (2013) posited that a significant proportion of tomato fruits that are conveyed from Northern Nigeria into Yenagoa metropolis (in Southern Nigeria) were observed to suffer from various postharvest diseases and disorders.

Specifically, only about 25.09% of tomato fruits that arrived Yenagoa metropolis were devoid of any form of postharvest disease symptoms or physiological disorder. Opadokun (1987) revealed that whilst 21% of harvested tomato was lost to rot in the field, an additional 20% were also lost to poor storage system due to post harvest disease. Adeoye and colleagues (2009) advanced that postharvest tomato losses accruing to the wholesalers and retailers were 50-70% on the average.

1.1 AIM AND OBJECTIVES

This study was carried out to evaluate the microbiological and Proximate analysis of diseased and healthy tomato fruits *(Lycopersicum esculentum)*

The objectives of the research include to:

1. evaluate the total heterotrophic bacterial and fungal count of spoilt or diseased and healthy tomatoes
2. isolate and characterize bacteria species from diseased or spoilt and healthy tomato samples
3. evaluate the proximate properties of diseased and healthy tomato fruits conduct an *in vitro* pathogenicity test of isolated bacteria from diseased or spoilt *Lycopersicum esculentum*
4. comparative evaluation of diseased and healthy tomato fruits

CHAPTER TWO

2.0 LIETRATURE REVIEW

The increase in the yearnings and cravings all year round by consumers for healthy diets has necessitated the consumption of fresh vegetables which has now became climacteric in nature up until recently were considered to be seasonal (Wakil and Oyinlola, 2011). In the last few years, minimally processed as well as pre-prepared vegetables have gained popularity amongst consumers. These preserved vegetables in a natural packaging system, combine their healthy and fresh-like characteristics along with a minimal preparation time at both the catering and consumer levels (Watada *et al*., 1996; Ahvenainen, 1996). The appeal and demand by consumers for these vegetables are a function of the fact that consumers perceive these products to be tasty, healthy, fresh and convenient (Sloan 2000; Buck *et al*., 2003; Mehrotra, 2004; Pivarnik *et al*., 2005). Vegetables usually have a shelf-life of about 7-14 days when kept at a temperature of 5 °C (refrigeration temperature). This temperature thus limit the growth, physiological and biochemical changes of endogenous and spoilage bacteria present in the produce (Garcia-Gimeno and Zurera-Cosano, 1997; Heard, 2002). Wakil and Oyinlola (2011) reported that vegetables have a high water activity (>0.99) because of their high moisture content. Apart from the water activity of vegetables, its intracellular pH, which usually in the range of 4.9-6.5 is another intrinsic factor that encourage microbial growth (Lund, 1992). Several available literatures had revealed little info on the diversity of pectinolytic bacteria on fruits and vegetables in a quantitative manner. Although several species of bacteria like*, Erwinia, Pseudomonas, Pantoea, Burkholderia, Serratia, Stenotrophomonas* and *Enterobacter* are often isolated but, their population density/burden are rarely quantified (Freire and Robbs, 2000; Hamilton-Miller and Shah, 2001).

2.1 Tomato Cultivation History

Osemwegie *et al*. (2010) described the plant "tomato" (*Lycopersicum esculentum*) as a widely grown as well as popular plant all over the globe. Enrique and Eduardo (2006) posited that the fruit is ranked globally as the second (2nd) most important vegetable, with respect to its contribution to the diet, based on the amount of minerals and vitamins it contains. The plant, which was introduced to Europe (in the 16th century) is a native of South America. The plant became popular and recognized by Botanists in Europe. Green herbaceous plants with fruits (green in colour) in the islands of Peru are known to be the progenitors of tomatoes as revealed by genetic evidence (Smith, 1994). One of the species to tomato known with the botanical name "*Solanum lycopersicum*" is cultivated and consumed by Mesoamerican (Etebu *et al*., 2013a). In the year 1521, a Spanish explorer, Cortes by name was credited to be the first person, who transported the little fruits (yellow tomato) to Europe after the conquest of the city of Tenochtitlan (Aztec), which is now known as City of Mexico. Nonetheless, it was assumed that Christopher Columbus, who worked for the Spanish monarch in the year 1493, might have taken the fruit back to Europe. In European literature, the earliest mention and discussion of tomato appeared in 1541 by an Italian botanist and physician named Pietro Andrea Mathiolian Halian who named tomato as "gold apple" or "pomod'oro" (Smith 1994). Tomatl (a Nahuatl word) is the root word for ''tomato'' from which it was derived. Literally, Tomatl, means 'the swelling fruit' from its original root word. However, *Lycopersicum esculentum* Mill. is tomato's Latin name which according to Cutler, (1998) is synonymous to *Solanum lycopersicum.*

Not until the 1590s, the plant was not cultivated in England. An herbal surgeon by the name John Gerard, who in 1597 published Gerard's Herbal, was one of the earliest cultivators of tomato. Smith (1994) posited/opined that Gerard's Herbal in England, is obviously one of the first report about tomato. Likewise, Tindall (1988) stated that countries in western Africa may have received and started the cultivation of tomato from Portuguese traders and from slaves who became free.

2.2 Cultivation, Transportation and Losses of Tomato in Nigeria

About tomato, second only to Egypt, Nigeria is the major producer of tomato with an annual production of about 6 million metric tonnes. She occupies the 13th position in the world prior to the year 1990 (Erinle, 1989). The major places/areas where tomato are being produced in Nigeria fall between latitudes 7.5 °N and 13 °N and according to Villareal, (1980), who also opined that tomato usually have an optimal range of temperature between 25 °C and 34 °C. These places include some States in the North and Middle Belts which include, Kaduna, Sokoto, Kano, Plateau, Benue, Borno, Bauchi and a few South-western states such as Oyo and Kwara (Olanrewaju and Swamp, 1980). There are serious constraints facing tomato cultivation in the South-South region of the country especially in states like Delta and Bayelsa, because they are located in the region of tropical rainforest with low topography. Another constraint according to Etebu *et al*. (2013b) is because these states experience more rainfall period than sunshine and too much water does not support the growth of tomato (which is a heat-loving crop). Farmers, in the Southern part of the country, as a result, cultivate the crop for the purpose of consumption (subsistence farming) and not for commercial purpose.

Etebu *et al*. (2013a) opined that compared to South America, Europe, Asia and North America, the relatively low tomato production in Nigeria is a function of several factors amongst them are environmental constraints, social and biotic factors. A prominent factor amongst these constraints are due to diseases and pests, which have been found to reduce the quality, yield and market value of marketable fruits. In the tropics, several pests are directly associated with yield losses and damage to tomato fruits, while they have also been credited as important vectors of numerous diseases according to Messiaen (1992) and Umeh and Oyedun (1995). On the basis of Agrios (2005) estimate, the worldwide postharvest losses of tomato can be as high as 40 %, but in developing countries such as Nigeria, postharvest losses are usually higher as a result of dearth of methods to prevent decay and improper handling procedures (Prigojin, *et al*, 2005). Several authors such as Stinson *et al*. (1981); Philips, (1984); and Moss, (2002) have revealed that the nature of tomato fruits in that they have a higher moisture content low pH, as well as high nutrient composition, makes the fruit susceptible to attack by phytopathogens such as fungal species, which may not only induce disease but also render the fruit to become non-viable via the production of mycotoxin. Sommer (1985) opined that phytopathogenic fungal species with a broad host range resulting to destructive as well as economically important losses to fruits and vegetables during the period of transportation and storage thus leading to a in the reduction of market value of the fruit are the most important pathogens. For tomato, the extent of damage due to postharvest phytopathogens as well as spoilage fungi largely dependent on the variety of tomato fruits. To drive home the aforementioned point, Adeoye *et al.* (2009) carried out a study in Oyo state and reported that postharvest spoilage of tomato up to 44% was attributed to a microbe, taking into account the variety of tomato, but the same microbe could only account for about 14-23% of spoilage in other tomato varieties.

2.3 CAUSES OF CROP LOSSES/SPOILAGE OF TOMATOES

As the saying goes, "pre-harvest production practices usually affect the postharvest return in most fruits and vegetables". Crop losses attributed to soil-borne phytopathogens such as *Phytophthora capsici* is well-documented in several lietratures as found in the report of Ewin and Riberio (1996) and Hausbeck and Lamour (2004). In tomato, *P. capsici* is known to cause late blight, which results to wilting according to Hausbeck and Lamour (2004). *Alternaria solani, Septoria lycopersici,* and *Fusarium oxysporum* are other soil-borne phytopathogenic fungi which have been shown to cause devastating losses to tomato as they have been found to cause early blight, Septoria leaf spot and *Fusarium* wilt respectively. Olayemi *et al.* (2010) posited that in Nigeria, postharvest losses of tomato during transportation and storage period has been projected to be as high as 20%. Wilson *et al.* (1991) reported that the decay in tomato fruits is a function of the changing physiological state of the tomato fruit as well as the increase in the number and growth of microbes associated with the spoilt fruit during the process of packaging, improper handling, transportation as well as storage. Transportation and storage of *L. esculentum* from the Northern region of the country where production is mostly carried out are usually in traditional weaved baskets which are often used till the baskets become contaminated with fungal spores, which might have infected fruits previously carried in such contaminated baskets according to report by Kutama *et al.* (2007). Snowdon, (1992) revealed that baskets and wooden boxes (used for storage or transportation) containing pathogenic inocula can induce an infection when they come in contact with fruits, which are healthy and susceptible. This could result to eventual crop losses to the farmer and the vendor. More so, farming equipment or farm implement has been attributed to harbour pathogenic inocula responsible for the several postharvest diseases of tomato during storage.

Reasons For Broken/Spoilage of Tomatoes: Poor Transportation

As aforementioned, postharvest tomato losses can be incurred during transportation of produce from farm to the markets. Nigeria has two main transportation modes to convey fresh produce and they are: the road and railway system. Nevertheless, there are complaints by transporters about the unusual delays and non-availability with respect to the railway system. Thus, most farmers and food handlers regularly employ the use the road system for either long or short journeys according to the 2010 and 2007 respective reports of Olayemi *et al.* and Idah *et al.* Diverse vehicular types are employed for the conveyance of the fruits from the North to the Southern part of Nigeria. The last two aforementioned workers have described the major types of vehicles for transportation of food produce. Most transporters use the 911 lorry for it superior ventilation and largely due to its capacity. The 911 lorry could take up to 250 and 300 baskets of tomato (which is between 7.5 to 9.0 tonnes) according to Etebu *et al*. (2013b). Sometimes, when needed the most, vehicles could be unavailable, and this is one of the problems facing transportation of produce and desperate traders or farmers could employ alternatives to avoiding the loss of produce, by using other kinds of vehicles including passenger buses.

More so, the desperation of farmers and/or traders who convey harvested tomato from the North to the South are sometimes obvious enough as they are found to fasten tomato consignments onto other articulated vehicles and worse still fuel tankers as reported by Idah *et al.* (2007) and Olayemi *et al.* (2010). Etebu *et al*. (2013a) added that these desperate and weird actions by marketers of the produce expose them to injuries during accidental fall-off, which often result to losses that may not be usually quantified but are sometimes heavy on the part of the marketers. More often than not, the baskets used for conveyance of tomato are sometimes arranged in layers of woody planks (of about 5 to 6 layers) with leaves between each layer inside the vehicle.

Damaged/Spoilt/Broken Tomatoes

Damaged tomato fruits mainly consist of compressed, water soaked, bruised as well as rotten fruits as a function of described method of transportation. Particularly, Idah *et al.* (2007) reported that about 13.89% on the average of fresh produce of the fruit “tomato” was found to be impaired in the process of transportation. This implies that for every tomato consignment containing 7.5 tonnes of tomato fruits, about 1.04 tonnes (which represents 13.89 %) of the produce would become bad or destroyed. In monetary terms, if on the average, 1 kg of fresh tomato is sold for two hundred naira (N200.00), the loss could amount to N200,000.00 or thereabout, when such fruits are destroyed completely and this is actually a big challenge for marketers (Idah *et al.,* 2007). One should bear in mind that the above assessment was carried out in Ilorin which is one of the major producers of tomato in the North. There is every probability that there would be a substantial increase in losses (spoilt tomatoes) if the produce are to be transported to the Southern part of the country as Bayelsa, Edo and Delta states.

In Yenagoa, Bayelsa State, a survey of markets showed that tomato produce displayed for sale were poorly arranged with little or no facilities as reported by Etebu *et al.* (2013). Tomato fruits were reportedly washed with dirty water or rather the trader care less about the hygiene of the environment because the produce is ready for sale. Spoilt and decaying fruits are indiscriminately left around packing shops and this condition may expose fruits to pathogenic organisms as well as other environmental factors that may lead to speedy deterioration of the farm produce. It has been revealed that the highest economic postharvest losses of tomato is mechanical damage which is followed by pathological damage. Meanwhile, the least but also substantial damage to tomato produce is physiological damage according to Adeoye *et al*. (2009). Furthermore, it was also reported that at the market level, retailers suffer more postharvest losses than wholesalers.

Bacterial Soft Rots Diseases of Fruits and Vegetables

Inevitably, whenever fleshy plant tissues in storage or in field undergo rot, you can be sure that bacteria are always present. This is evident by the foul smell given off as a function of volatile substance released by the bacteria during disintegration of plant tissues. The tissues undergoing rottenness become soft, watery with a slimy mass of cellular debris. Bacterial cells are regularly discharged from the cracks in the tissues. In several soft rot cases, the bacteria involved could be saprophytes or parasites and not necessarily a pathogen (Agrios, 2005). They could be present in the soft rotten fruits because of the avenue already created by the pathogen or prevailing environmental conditions.

Globally, bacterial soft rot occur with a corresponding serious consequence in terms of losses to crops from the field, to during storage (Agrios, 2005). Bacterial soft rot has been reported to be more devastating in terms of reduction of yield and market value than any other known bacterial diseases. Most vegetables and fresh fruits are usually susceptible to soft rot due to bacteria, which usually in the space of few hours may develop during storage as well as marketing. The disease may reduce the amounts and quality of crop available for sale as well as the market value of the produce. However, the preventative measures against bacterial soft rot are pretty expensive to adopt.

2.4 Ecology of the Soft Rot Bacteria (*Erwinia*)

Globally, amongst the most destructive vegetable diseases, soft rot due to *Erwinia* popularly called *Erwinia* soft rot is one of its most devastating and arguably the most destructive disease. According to Opara and Asuquo (2016), the disease occur wherever fleshy tissues of ornamentals as well as vegetables are found. Bacterial soft rot pathogens are not only found in infected plants but can take up habitation in soil, various water bodies and guts of insects (Perombelon 2002).

2.5 Morphology of *Erwinia* species

The bacterium is a rod-shaped, Gram-negative non-sporulating and peritrichously flagellated bacterium, which lives can aggregates into pairs and chains or possibly lives alone. It is facultatively anaerobic, oxidase negative as well as it shows a positive result to catalase test according to Pérombelon (2002) and Agrios, (2006). *E. carotovora* the typical standard species implicated to cause soft rot, extracellularly produce a plethora of plant cell wall degrading enzymes (PCWDEs) like the pectic enzymes (responsible for degradation of pectin), hemicellulases, arabinases, cyanoses, cellulase (responsible for the breakdown of cellulose) and proteases. As a mesophile, *Erwinia* thrives best in temperature range between 27^0C - 30^0C (Perombelon, 2002). The cultural characteristics of the bacterium on most cultures are round, smooth, slightly raised, glistering, greyish-white to creamy-white with proper visibility on culture plates (nutrient agar) after about 24 hours especially for colonies of *E. carotovora* pv. *carotovora* and pv. *atroseptica*. Species such as *E. chrysanthemi* according to Gupta and Thind (2006) appear macroscopically on most media as round, smooth, flat to slightly raised, with margins becoming undulate to feathery, greyish-white to creamy –white colonies.

2.6 Modes of Transmission of *Erwinia*

Opara and Asuquo (2016) reiterated that *Erwinia* can be disseminated in a variety of ways and one of such is via plant-to-plant infection. The caullosphere of most infected tubers have some form of inoculum, which are capable of initiating upon injury, the infection ensue and quickly rots the entire tuber. Often, this spreads wild and infect several other tubers. For tubers like potato, during washing in the wash tanks, the inoculum of bacterium on the surface of the tubers can gain entry into the lenticels with the aid of hydrostatic pressure in the water tanks.

E. carotovorum can also survive in the gut of insect for hours and these insects' serves as vectors or carriers of the pathogen from one plant to another where they induce disease condition in healthy plant. Using insect as vectors, soft rot bacteria are easily transmitted from one plant to another even when the feeds are miles apart. Agrios, (1998) reported that most plant pathogens (viruses, fungi, nematodes, bacteria, and protozoa) can be transmitted by insects. Insects transmission occur in three basic ways according to Agrios (2005);

1. Passive transmission: this involves movement via an infected plant part, which has plant pathogen(s) on its surface. Sticky spores or pathogen propagules may cling to the insects while it moves about, from whence they can be transferred to begin a new infection in other plants parts or plants.
2. During the course of feeding, pathogen propagules of viruses, bacteria, protozoa, fungi and nematodes can be transferred from diseased plants to other plant parts or new plants.
3. Specific pathogen propagules such as nematodes, phytoplasmas, protozoa, viruses, and fastidious bacteria (xylem-and phloem-inhabiting bacteria) can be transmitted via the process of sucking or ingesting the propagules along with the sap of the plant, when the insects feed.

Consequently, the insects act as vectors for the pathogen, which circulates in the insect (without or with further pathogen multiplication) until it reaches the salivary glands from whence it can be injected into new susceptible plants (Agrios, 2005). The relationship between insects and bacterial soft rot has been evaluated in the research of Leach (1926) who was credited to be the first to make a report in this regard. Leach (1926) revealed that *Hylemyia cili-crura*, a seed-corn maggot, plays an important role in the development and spread of blackleg of potato in both field and under storage. Following the study by Leach (1926), there exist a symbiotic relationship between *Hylemyia cili-crura* and the phytopathogen of potato blackleg. The eggs of the seed corn maggot, may be contaminated with the phytopathogenic bacteria while being deposited in the soil and the inoculum of the phytopathogen may be introduced into pieces of planted potato by the young maggots. These pathogens and other microorganisms are present in the internal portion of the pupal and larval stages as well as on the surface of the eggs (Leach, 1930; 1933). In a related study, Leach (1927), found that two insects (*Scaptomyza graminum* and *Elachiptera costata*) are common agents of inoculation of celery heart rot also caused by *Erwinia carotovora* the cause of blackleg and seed-piece decay in the potato. Bacterial pathogens as well as spores of other agents can also be transferred via aerosolized droplets during periods of rainfalls on either diseased plants or any other thing that is contaminated with *Erwinia*, an aerosol can be created where the bacteria is airborne in water. Around 50% can only survive as an aerosol for 5- 10 minutes, but this is long enough to travel many miles in a brisk wind. It has also been reported that *E. carotovorum* can be isolated or found in water as it has been isolated or recovered from "surface waters, reservoirs, streams, ditches, lakes, rivers and the sea (Perombelon, 2002).

2.7 Host Range of *Erwinia*

Numerous literatures published in 2014; 2007; 2006; 2002 and even 2003 by Garba *et al*., Howard and David, Agrios, Perombelon and Toth *et al*. respectively had reported that *Erwinia* and other phytobacteria implicated in soft rot have a wide assortment of host range, from several agricultural and scientifically significant crop species such as tomato, green peppers, African violets, carrot, onion, leafy greens, squash, potato and other cucurbits. Apeyuan (2000) opined that the soft rot bacteria are common with plants, which have fleshly storage tissues such as tubers, tomato, root, stalk or leaves like lettuce and cabbage, and succulent stem. Wakil and Oyinola (2011) reported that *Erwinia* species can also induce rot on stored tubers as well as on the aforementioned plants species such as tomatoes, onions and pepper.

Arsenijevic (1978) cited by Opara and Asuquo (2016) reported that other plants such as pepper, pineapple, cabbage, cactus, tomato and cauliflower have been found to be infected by soft rot. More so, Tsuyama (1978) also cited by Opara and Asuquo (2016) revealed that tobacco plant has been found to be susceptible to the bacterium hence confirming the pathogenicity of the bacterium. In the same literature text by Opara and Asuquo (2016), it was cited that Robert and Blanchette (1994) revealed that the lignified walls of the world's most durable timber (*Eusideroxoylon zwageri*), which has known resistance to white and brown rot fungi, can be degraded by *Erwinia*. The phytopathogen have also been reported to infect the fruits of eggplant, carrot, sweet potato, apple, garlic, olive, lemon, apricot, onion, radish, squash, as well as turnip (Ismail *et al*., 2012). The host range of *Erwinia* include several plant genera ranging from all families of fruits, ornamentals, root and tuber crop, vegetables, as well as nearly as about 64 plant species (Opara and Asuquo, 2016). Rajeh and Khlaif (2000) cited by Opara and Asuquo (2016) revealed that *E. carotovorum* was implicated as the major cause of soft rot in a survey of eighty seven bacterial soft rot isolates recovered from several vegetable crops such as, pepper, potato, spinach, cabbage, lettuce, cauliflower, tomato, onion and sweet melon. Agrios (2006) reported that *E. carotovorum* has been implicated as the cause of severe stem end decay as well as devastating postharvest soft rot, which give considerable losses to pepper. More so, it was also revealed that *Erwinia* is a pathogen of economic importance, which can cause a devastating postharvest loss in stored fruits and vegetables via disintegration/decay of plant tissues.

2.8 Disease Symptoms Caused By the Species *Erwinia*

Lumb *et al*. (1986), opined that the symptoms of the foliar parts of plants are common with infection by *Erwinia* and related species. Foliage symptoms are usually wilting at the top of the leaves with successive withering around the margins and by extension, the entirety of the leaves. Foliage symptoms sometimes from the lower leaves do spread to the stem, and eventually the whole plant may dry out in extreme cases (Opara and Asuquo, 2016). Powelson and Franc (2001), described certain symptoms of bacterial soft rot on tubers which ranged from some vascular discoloration to total deterioration or spoilage of the tuber. Necrotic lesions develop first in the lenticels, at the attachment site of the stolon, or it could also be in the injury/wounds created on the tuber surface. Affected tuber tissues appear as cream- to tan-coloured and then becomes granular and soft. Elphinestone and John (2010) added that the decayed tissue usually develop pigments (brown to black in colour) at the margins.

2.9 Pathology of the Soft Rot *Erwinia*

E. carotovora usually results to eventual death of the plant via the disintegration or degradation of the fleshy succulent plant organs like the tubers, roots, thick leaves and stem cuttings through the creation of osmotically fragile cell (Opara and Asuquo, 2016). This degradation is possible via the ability to produce PCWDEs (Plant cell wall-degrading enzymes) as correctly reported in 2004 by Bell *et al*. PCWDEs include cellulase and extracellular pectic enzymes, which break down the cellulose and pectin respectively. Amadioha (2012) and Oladoye *et al*. (2013) in detail, evaluated enzymes, which were able to induce hydrolysis of the middle lamella of certain tissues in the host plant following development of soft rot. They reported that extra-cellular enzymes are produced by spoilage organisms and these enzymes, which include cellulase, amylases, polygalacturanases (PG), zylanases as well as PMEs (pectin-methyl esterases) are able to degrade the components of the cell walls of susceptible plants. The enzymes are also able to induce dissolution of the middle lamella, (which functions as the bond between the adjacent cell walls thereby loosening the cells with a corresponding discharge of water and foul odor. The by-products released due to the growth of bacteria similarly set ex-osmosis of liquid, which contains salts and sugar to the inter-cellular spaces from within the cells, where it functions as food source for further growth of bacteria. The continuance of this process explains the loss of consistency as well as the watery condition of the decayed tissues of plants. Mores so, the direct involvement of cellulolytic as well as pectic or pectinolytic enzymes produced by pathogens implicated to cause soft rot have also been described in 1990 and 1994 by Garber *et al*. and Walker *et al*. respectively, cited in Opara and Asuquo (2016).

However, under artificial epiphytotics, there have been a report of yield losses up to 98.8% described by Thinda and Payakab (1985). The survivability of the bacterium in soil or plant debris has been confirmed and this guarantees the infection of a new plant may continue whenever opportunity arises. The decay or rot process is aggravated when factors such as high temperature (about 30 ^{o}C) is coupled with high relative humidity, which results in a faster of multiplication rate of the soft rot pathogens according to the report of Agrios (2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

All materials such as glass wares were manufactured by Pyrex® in England. The media and reagents used for the research were obtained Vicdomstell limited, Lagos State, Nigeria, Pyrex- IG Scientific Company Benin and Equator Medics International limited, Lagos. The media used in this study were manufactured by Oxoid Limited, Basingstoke, Hamsphire, England. They include tryptone soya broth CM0129, Mueller Hinton agar, manitol salt agar, salmonella Shigella agar, Eosine methylene blue agar, *Pseudomonas* cetrimide agar and Bacillus cereus agar.

3.2 Collection of Samples

Samples were obtained from four markets, which are Oba, Osa, Uselu and New Benin markets. The samples were labelled as good (for fresh and healthy samples without any wound on its skin) and spoilt (for broken tomato samples with signs of spoilage). The samples were immediately transported to the laboratory for analysis and analysed within 24 hours after collection. The good samples were washed with sterile distilled water before they were serially diluted using a dilution factor of 10.

3.3 Preparation and Sterilization of Culture Media

All culture media such as Salmonella-shigella agar, Pseudomonas cetrimide agar, Oxacillin resistance screening agar base, and Mueller Hinton agar, which were all products of Oxoid, were prepared according to the manufacturer's instructions. Sterilization was at 121ºC at 15ppi for 15 min.

3.3.1 Salmonella-Shigella Agar (Ssa)

Salmonella-shigella agar (SSA) is a moderately selective and differential medium which was used for the isolation, cultivation and differentiation of *Salmonella* species and some strains of *Shigella*species. It was prepared by suspending 60g of the powdered commercially prepared SSA in 1 litre of distilled water and dissolved thoroughly. It was then aseptically dispensed into sterile petri dishes.

3.3.2 Pseudomonas Cetrimide Agar

Pseudomonas cetrimide agar is a selective and differential medium used to isolate and identify *Pseudomonas aeruginosa*. It is differential for *Pseudomonas aeruginosa* because the organism produces a number of water soluble iron chelators, including the yellow-green or yellow-brown fluorescent pyoverdin which combines with the blue water-soluble pyacinin to form the bright green colour characteristic of *Pseudomonas aeruginosa.* It was prepared by suspending 45.3g of the medium in 1 litre of distilled water and adding 10ml of glycerol. It was then dissolved thoroughly. The dissolved mixture was then sterilized by autoclaving at 121ºC for 15 min and was cooled to 45-50ºC before being dispensed aseptically into sterile petri dishes.

3.3.3 Mannitol Salt Agar

A selective medium for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited, with the exception of a few halophilic species. Presumptive coagulase-positive staphylococci produce colonies surrounded by bright yellow zones whilst non-pathogenic staphylococci produce colonies with reddish purple zones. Mannitol Salt Agar is recommendedfor the detection and enumeration of coagulase-positive staphylococci in milk, in food and other specimens. It is prepared by suspending 111 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

3.3.5 Mueller Hinton Agar (MHA)

Mueller Hinton agar is a non-selective, non-differential medium that is used majorly for antimicrobial susceptibility testing. It is the standard medium for the Bauer Kirby method. It was prepared by suspending 38g of the medium in 1 litre of distilled water and was dissolved properly to get a homogenous mixture. It was then autoclaved at 121ºC for 15 min and was cooled to 45ºC. The molten agar was aseptically dispensed into sterile petri dishes.

3.4 Enumeration of bacteria and fungi from Tomato

A sterile blade which was heated using a Bunsen flame was used to 10g from each the good tomato sample and was aseptically transferred to 90 ml of sterile saline water in a conical flask and then sealed with foil paper to prevent contamination. In the conical flask, the sample was vigorously shaken against the walls of the flask to cause disintegration in order to increase the surface area. 75ml of normal saline (which was prepared by dissolving 8.5g of NaCl in 1 litre of distilled water and then autoclaving at 121ºC for 15 min) was added to enhance the homogenization of the sample and to dissolve the sample. The resultant mixture was regarded as the stock suspension with a total volume of 100ml. Subsequently, 1 ml of suspension was taken from the stock and was added to a test tube containing 9 ml of normal saline and shaken properly to ensure homogeneity. Another 1 mlwas pipetted out of the first dilution test tube and was added to a second test tube containing 9 ml of normal saline water. This process was repeated until the fifth test tube from whence (first, and third tubes) 1 ml volume of inoculum was used for culturing using pour plate technique. From the appropriate dilution, 1000µl (1 ml) each was poured onto petri dish inoculated on the different media (Mueller hinton agar for bacteria and Czapek dox agar supplemented with streptomycin for fungi).

3.5 Isolation and Identification of Bacterial Isolates

The bacterial colonies of different morphology were sub-cultured on the various media to obtain pure cultures. All the plated petri dishes were incubated at 37ºC for 24h. The pure cultures were preserved for further biochemical analysis and antibiotic sensitivity testing.

3.5.1 Biochemical Characteristics

Bacterial isolates were biochemically characterized by performing tests which include Gram staining, Catalase, Oxidase, Indole, citrate and Citrate tests.

3.5.2 Gram staining

The technique is used to differentiate between gram positive and gram negative bacterial strains.

Procedure:

Dry bacterial colony was emulsified on a clean glass slide using a drop of normal saline and wire loop. A thin smear of the bacterial suspension was made on the glass slide and the smear was allowed to dry for some minutes. 2 drops of crystal violet was pipetted unto the smear and allowed to stay for 1min. This was rinsed with running water. 2 drops of iodine was added to the smear and allowed to stay for 3min. This was rinsed with running water. 3 drops of safranine was used to counter-stain for 1min, rinsed with running water, allowed to dry and observed under the microscope.

3.5.3 Indole test

This is a qualitative procedure for determining the ability of bacteria to produce indole by deamination of tryptophan.

Procedure:

Tryptone broth was inoculated with test isolates and incubated at 37˚C for 48hours. 1ml of Kovac's reagent was then added to each tube. The tubes were gently agitated for 15 minutes, allowed to stand. It was then observed and recorded.

3.5.4 Citrate test

This test is used to identify bacteria which utilize citrate. The test differentiates among enteric organisms by determining their ability to utilize citrate as their source of carbon and energy (Hemraj *et al*., 2013). This test is usually used in characterizing Gram-negative pathogens and environmental isolates. As citrate is utilized from the medium by the bacteria, the pH of the medium rises (from green to blue on the agar slant). The alkaline carbonates and bicarbonates produced as by-products of citrate metabolism raise the pH of the medium to above 7.6, causing the bromothymol to change from the original green colour to blue. No colour change indicates a negative reaction as the medium will retain the deep forest green colour of the un-inoculated agar (Cheesebrough, 1987).

Procedure:

Test organisms from pure cultures were inoculated into Simmons Citrate agar and incubated at 37 ^{0}C for 24 hours. It was then observed and recorded.

3.5.5 Catalase test

This test is used to differentiate organisms which can synthesize catalase rapidly and hence can degrade Hydrogen peroxide (H_2O_2) which is poisonous to microorganisms.

Procedure:

A few drops of 5% hydrogen peroxide were placed on glass slide using a sterile dropping pipette and a sterile inoculating loop was used to immerse a loopful of pure bacterial cells into the hydrogen peroxide solution. Formation of gas bubbles is indicative of the production of catalase enzyme which means a positive result while the absence of bubbles indicated a negative result (Kumari *et al*., 2013).

3.5.6 Oxidase test

This test is a key test to differentiate between the families of Pseudomonadaceae and Enterobacteriaceae, and is useful for speciation and identification of many other bacteria, those that have to use oxygen as the final electron acceptor in aerobic respiration. It is used to determine the production of the enzyme 'oxidase' by an organism which also indicates the presence of Cytochrome C (Hemraj *et al*., 2013).

Procedure:

A filter paper was saturated with a few drops of 10% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride (Kovac's oxidase reagent) and was left to air dry. An inoculating loop was used to pick a portion of the test culture and smeared unto the filter paper. A development of deep purple colour within 5-10 seconds indicates a positive result (Hemraj *et al*., 2013).

3.6 Determination of Moisture Content

10 g of chopped tomato was into a preweighed petri-dish dried in an oven at 105^{o}C for four hours and then allowed to cool. The petri dish was then weighed. This process was repeated many times until the weight of the petri-dish with its content remained constant. Triplicate determinations were made for each cultivar (Gharezi *et al.,* 2012).

3.7 Determination of Total Soluble Solids

10 g of homogenized tomato sample was placed into a 50 cm3 centrifuge vial and span at 300 rpm for 10 min. Then, 2 cm3 of the supernatant was measured into pre-weighed glass petri-dish and the weight taken before drying in an oven at 60 ºC for 17 hr. Samples were weighed after oven drying and the results expressed in percentages. All determinations were carried out in triplicate (Quartey *et al.,* 2012).

3.8 Crude Fiber Determination

100 g of the chopped sample was weighed into a beaker and 50 cm^3 of H_2SO_4 (1.25%) was added. The mixture was then boiled for 1 hour, filtered and the residue boiled with distilled water to dilute the excess acid. 50 cm3 of NaOH (1.25%) was added and the mixture was boiled for another 1 hour. It was then filtered, washed with distilled water until free from alkali. The residue was then rinsed with acetone and dried in oven at 110oC for 2 h. The dried residue was ashed in a muffle furnace at 600oC for 3 hours, cooled in a desiccator and weighed. The crude fiber content was calculated by difference (Adebooye *et al.,* 2006).

3.9 Crude Protein Determination

0.2 g of each homogenized sample was weighed into the digestion tube followed with the addition of 5 g of Kjeldahl catalyst mixture and 15 cm^3 of concentrated sulphuric acid. The tube was swirled gently until the mixture has thoroughly mixed. The mixture was heated continuously for 2 hr until the solution became clear and 15 cm3 of 40% NaOH was added. The mixture was allowed to cool and then transferred into 100 cm3 volumetric flask and diluted mark with 1000 Ldistilled water. Another 10 cm^3 of 2% boric acid was measured into 100 cm3 Erlenmeyer flask and few drops of Methyl red indicator were added. Furthermore, 10 cm3 of digested aliquot was transferred into a distillation apparatus and then distilled into the boric/indicator for 15 min. The distillate was then titrated with 0.025M HCl to a pink end point (AOAC, 1990).

3.10 Determination of Titratable Acidity

10 cm3 of the filtered tomato juice was added to 50 cm3 of distilled water and titrated with 0.1M NaOH using phenolphthalein indicator (Gharezi *et al.,* 2012).

3.11 Determination of Ash Content

2.0 g of the chopped, dried tomato sample was placed in a porcelain crucible and ashed in a muffle furnace at 600oC for 3 hr. The crucible was allowed to cool and the weight of the ash taken (Owusu *et al.,* 2012)

3.12 Statistical Analysis

Statistical analysis was performed using SPSS version 21. Results were expressed as means standard error. Analysis of Variance (ANOVA) test (5% Confidence Interval) was used to determine significant differences between the results. Correlation analysis was examined by using the protocol for unpaired t-test.

CHAPTER FOUR

RESULTS

The results of the bacteriological as well as the proximate analysis of good and spoilt tomatoes sold in various markets in Benin metropolis is shown in this chapter. The total heterotrophic bacterial and total fungal count for spoilt tomato samples are shown in table 1. THE Bacterial Count Ranged From 7.13 ±0.11 X 10^4cfu/G In Uselu market to 4.25 ±0.96 X 10^4cfu/g in Osa market. There was however, a reduction in the fungal count compared to bacterial count. The mean fungal count ranged between 0.04 ±0.01 x 10^4CFu/g to 0.55 ±0.12 X 10^4cfu/g.

Table 1. Total heterotrophic bacterial and fungal count of spoilt or broken tomatoes samples

Sample location	Mean ± S.E x 10^4 cfu/g	
	THBC	TFC
Osa	8.33 ±0.18	0.55 ±0.12
Uselu	7.13 ±0.11	0.06 ±0.01

Legend: values are Mean ± S.E of triplicate determination

The number of heterotrophic bacteria and total fungal count was drastically reduced in the good tomato samples analyzed in the study. There was no heterotrophic bacterial or fungal count in certain good samples analyzed in this study (but I must be quick to point that before the sample analysis, the surface of tomato was rinsed with sterile distilled water). The few samples that had count was less than 100 colonies (table 2).

Table 2. Total heterotrophic bacterial and fungal count of fresh/good tomato samples

Sample location	Mean ± S.E x 10^1 cfu/g	
	THBC	TFC
Osa	4.00 ±1.70	0.10 ±0.00
Uselu	28.50 ±7.71	0.00 ±0.00

Legend: values are Mean ± S.E of triplicate determination

The cultural, morphological and biochemical characteristics of the isolated bacteria are shown in table 3. From their respective growth on selective and differential agar, it was revealed that *Pseudomonas aeruginosa, E. coli, Bacillus subtilis* and *Salmonella enteritidis* were the identified bacterial isolates from tomato samples in the study.

Table 3. Cultural, morphological and biochemical characteristics of bacterial isolated from spoilt tomato samples

Parameter	1	2	3	4	5
Cultural					
Elevation	Low convex	Convex	Low convex	Low convex	Smooth
Margin	Smooth	Smooth	Entire	Entire	Low convex
Colour	Cream	Cream	Cream	Cream	Smooth
Shape	Circular	Circular	Circular	Circular	Cream
Morphological					Circular
Gram stain	Negative	Negative	Negative	Negative	Negative
Cell type	Rod	Rod	Rod	Rod	Rod
Arrangement	Single	Single	Single	Single	Single
Spore staining	Positive	ND	ND	ND	ND
Biochemical					
Catalase	Positive	Positive	Positive	Positive	Positive
Indole	Positive	Negative	Negative	Negative	Positive
Citrate	Positive	Positive	positive	positive	Positive
Urease	ND	Positive	Positive	Positive	ND
Oxidase	ND	Negative	Positive	Positive	ND
Gr. Diff. Agar	BCA	SSA	PCA	PCA	BCA
Identity	*Bacillus subtilis*	*Salmonella*	*Pseudomonas fluorescens*	*Alcaligenes faecalis*	*Bacillus cereus*

Legend: MSA= Mannitol salt agar, PCA= *Pseudomonas* cetrimide agar; EMB = Eosin methylene blue agar, SSA = salmonella Shigella agar

ND=Not determined

Gr. Diff. Agar = growth on differential agar

Concerning the good or healthy tomato samples, only two bacteria where found which happens to be *Salmonella* and *Staphylococcus epidermidis*. The reason for the fewer bacterial isolates could be due to the fact that the samples were washed as well as ought not to contain microorganisms. The fewer bacteria present in this samples were also reflected in the count of the good sample compared to the bad sample.

Table 4. Cultural, morphological and biochemical characteristics of bacterial isolated from good tomato samples

	2	4
Cultural		
Elevation	Convex	Low convex
Margin	Smooth	Smooth
Colour	Cream	Cream
Shape	Circular	Circular
Morphological		
Gram stain	Negative	Negative
Cell type	Rod	Rod
Arrangement	Single	Single
Spore staining	ND	Positive
Biochemical		
Catalase	Positive	Positive
Indole	Negative	Positive
Citrate	Positive	Positive
Urease	Positive	Negative
Oxidase	Negative	Negative
Gr. Diff. Agar	SSA	BCA
Identity	*Salmonella enteritidis*	*Bacillus subtilis*

The proximate composition of diseased or spoilt samples of *Lycopersicum esculentum* (tomato) is shown in the table below. There was high moisture content of 42.49 % in samples obtained from Uselu market compared to 37.87 % for samples in Osa market. However, there were higher lipid, crude fibre and carbohydrate contents of 2.00 %, 11.31 % and 43.03 % respectively for samples obtained from Osa market compared to 0.50 %, 10.99 % and 39.12 % for same proximate parameters but obtained from samples in Uselu market.

Table 5. Proximate composition of diseased or spoilt samples of *Lycopersicum esculentum* (tomato)

Parameters (%)	Uselu market	Osa market
Moisture	42.29	37.87
Lipids	0.50	2.00
Crude fibre	10.99	11.31
Crude protein	5.95	4.38
Ash	1.55	1.41
Carbohydrates	39.12	43.03

The proximate composition of good or healthy samples of *Lycopersicum esculentum* (tomato) is shown in the table 6 below. There was high moisture content of 45.87 % in samples obtained from Uselu market compared to 47.85 % for samples in Osa market. However, there were higher lipid and crude protein content of 4.00 % and 8.58 % for samples obtained from Uselu market compared to 2.50 % and 7.35 % for same proximate parameters but obtained from samples in Osa market.

Table 6. Proximate composition of good/healthy samples of *Lycopersicum esculentum* (tomato)

Parameters (%)	Uselu market	Osa market
Moisture	45.87	47.85
Lipids	4.00	2.50
Crude fibre	6.06	9.73
Crude protein	8.58	7.35
Ash	2.25	2.30
Carbohydrates	33.24	30.27

The comparative evaluation of the proximate composition of healthy and diseased tomato samples is shown in table 7 below. Overall, one can conclude that healthy samples of *Lycopersicum esculentum* has higher values of moisture content (46.99±1.11) compared to diseased samples (41.14±1.46). More so, there were also higher values for other proximate parameters such as lipids (2.37±0.71), crude fibre (11.49±2.89), crude protein (7.61±0.75) and ash (2.01±0.29) compared to diseased samples with respective values of 1.12±0.37 (lipids), 11.10±0.21 (crude fibre), 5.29±0.33 (crude protein) and 1.29±0.11 (ash). Although, there was no significant difference ($p>0.05$) in certain of the proximate parameters such as lipids, crude fibre, ash and carbohydrates. There was observable difference in the moisture and crude protein content of healthy and diseased fruits of *Lycopersicum esculentum*

Table 7. Comparative evaluation of the proximate composition of healthy and diseased tomato samples

Parameters (%)	Healthy	Diseased	*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

CHAPTER FIVE

5.1 DISCUSSION

Fresh vegetable fruits including tomato have natural protective cover (epidermal layer) that effectively guide against most pathogenic microbes and plant spoilage. This protection however could be hindered and the fruits may be contaminated during field cultivation, harvesting, post-harvest handling and distribution (Beuchat, 2002). The identified bacteria were *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Salmonella enterica.* It is reported that the isolation of soil bacteria *Bacillus subtilis* isolated from tomato fruits, was an evidence of opportunistic contamination due to poor handling processes of the tomato fruit (Wogu and Ofuase, 2014). The occurrence of *E. coli and S. aureus* in tomato fruits contamination has been reported to be associated with faecal matters through inadequate human handling processes (Ghosh, 2009). This is consistent with the studies of Oyemaechi *et al.* (2014), who suggested that the presence of *S. aureus* in the decayed tomato fruit possibly due to contamination with organic manure and/ or faecal matter. It is noteworthy that the observations in this study negate some other researchers' reports outside Nigeria. For instance, Garg *et al.* (2013), isolated lactic acid bacteria, *Vibrio furnissii, Serratia marcenscens* and *Aeromonas hydrophila* in India.

Concerning the proximate composition of diseased and healthy tomato samples, Overall, one can conclude that healthy samples of *Lycopersicum esculentum* has higher values of moisture content (46.99±1.11) compared to diseased samples (41.14±1.46). More so, there were also higher values for other proximate parameters such as lipids (2.37±0.71), crude fibre (11.49±2.89), crude protein (7.61±0.75) and ash (2.01±0.29) compared to diseased samples with respective values of 1.12±0.37 (lipids), 11.10±0.21 (crude fibre), 5.29±0.33 (crude protein) and 1.29±0.11 (ash). Although, there was no significant difference (*p>0.05*) in certain of the proximate parameters such as lipids, crude fibre, ash and carbohydrates. There was observable difference in the moisture and crude protein content of healthy and diseased fruits of *Lycopersicum esculentum*. The moisture content of the good tomato is in conformity with the finding of Romain (2001) and Harry (1994). Too much of moisture in any food sample can make the sample viable for Microorganism growth. This accounts for most of the biochemical and physiological reactions in the plant (Guisseppe and Baratta, 2000). The difference of processing mechanism involved in the processes of preservation might have a different effect on the fat content. Also, geographical differences may also be a contributing factor for the difference. In agreement with the present findings, Nguyen and Schwartz (1998) reported that the availability of lipid from tomato products is increased when these foods are processed at high temperature or packaged with oil. They also reported that mechanical treatment (homogenization) and heating enhance the release of lycopene from the tomato matrix and may explain the improved bioavailability seen with consumption of processed tomato products (cooked tomato, tomato paste). Furthermore, canned tomato and ketchup contain more lipid than fresh tomato because cooking at high temperature breaks down cell walls, releasing and concentrating carotenoids There was a significant difference in lipid content amongst the tomato sample under review .This is also in agreement with the findings of (Rao and Agarwal 1998) who reported that significant differences exist between different tomato products in terms of lipid release and its gastrointestinal absorption of the commonly consumed tomato products.

5.2 CONCLUSION

The presence of disease or spoilage of tomato may have impacted or influence the choice of consumers and thus bring a loss to vendors and farmers in the long run. In this light, there is no difference in the nutritional or proximate parameters between healthy and good looking fruits of *Lycopersicum esculentum* and diseased or broken samples of the same crop.

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APPENDIX A

CULTURE MEDIA

SALMONELLA, SHIGELLA AGAR (SSA)

Lab-lemco powder	5.0g/L
Peptone	5.0g/L
Lactose	10.0g/L
Bile salts	8.5g/l
Sodium citreate	10.0g/L
Sodium thiosulphate	8.5g/l
Ferric citrate	1 .0g/L
Brillant gree	0.00033g/L
Neutral red	0.025g/L
Agar	15.0g/L

BILE AESCULIN AGAR

Peptone	14.0g/L
Bile salts	15.0g/L
Ferric citrate	0.5g/L
Aesculin	1.0g/L
Agar	15.0g/L

OXACILLIN RESISTANCE SCREENING AGAR BASE (ORSAB)

Peptone	11.6g/L
Yeast extract	9.0g/L
Mannitol	10.0g/L
Sodium chloride	55.0g/L
Lithium chloride	5.0g/L
Aniline blue	0.2g/L
Agar	12.5g/L

MUELLER-HINTON AGAR

Dehydrated infusion from beef	300.0g/L
Casein	17.5g/L
Starch	1.5g/L
Agar	17.0g/L

MAC-CONKEY AGAR

Formula gm/litre

Peptone 20.0

Lactose 10.0

Bile salts 5.0

Sodium chloride 5.0

Neutral red 0.075

Agar 12.0

pH 7.4 ± 0.2

APPENDIX B

GRAM STAINING AND BIOCHEMICAL REAGENTS

STAIN AND REAGENT

Gram stain

The Gram stain was prepared using two stains (crystal violet and safranin or carbol fuchsin), Gram's iodine, and a decolorizing agent (ethyl alcohol).

A. Gram crystal violet

Solution A

Crystal violet - 2.0 g

Dissolved in ethanol (95%) - 20.0 ml

Solution B

Ammonium oxalate - 0.8 g

Distilled water - 80.0 ml

Gram iodine

Iodine (crystalline) - 1.0 g

Potassium - 2.0 g

Distilled water - 300.0 ml

3.0g of medium was dissolved in 300.0 ml of distilled water.

It is very important to note that; crystalline iodine, potassium and distilled water were combined to produce iodine solution and that Gram's iodine solution was stored in a dark bottle and protected from light so that it does not degrade.

Decolorizer

95 % ethyl alcohol was used.

Gram safranin

Safranin-O (certified) - 0.25 g

Ethyl alcohol (95 %) - 100.0 ml

Working solution:

Safranin stock solution – 10.0ml

Distilled water – 90.0 ml

Biochemical reagents

Indole medium

Peptone – 20.0 g

Sodium chloride – 5.0 g

Distilled water – 1000 ml

pH – 7.4

25.0 g of indole medium was dissolved in 1000 ml of distilled water and autoclaved for 15 min at 121 ºC and dispensed aseptically into sterile test tubes.

Oxidase reagent (Kovac's oxidase)

Amul-alcohol – 15.0 ml

p-dimethyl-aminobenaldehyde – 0.5 ml

Concentrated HCl – 50ml

Small quantity of Kovac's reagent was prepared by dissolving the aldehyde into alcohol and adding the acid slowly and then kept inside the refrigerator.

Catalase test

3% Hydrogen peroxide