MICROBIAL ASSESSMENT AND PROXIMATE COMPOSTION OF PEPPER (*Capsicum annum*) AND TOMATO (*Solanum lycopersicum)* SOLD IN THE MARKET

ABSTRACT

Tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) are among the world's most important vegetable crops. A total of 20 samples made up of fresh tomatoes, spoilt tomatoes, fresh pepper and spoilt pepper were analysed in this study. Standard microbiological practices were carried out on the samples. Total Heterotrophic Bacterial (THB) Count for Spoilt tomatoes ranged from $1.98x10^7$cfu/g to $2.39x10^7$cfu/g. Fresh tomatoes samples had a THB counts ranging from $1.43x10^6$cfu/g to $2.14x10^6$cfu/g. Spoilt pepper total heterotrophic bacteria counts ranged from $1.46x10^7$cfu/g to $2.01x10^7$cfu/g. Fresh pepper had a THB count of $1.22x10^6$cfu/g to $1.66x10^6$cfu/g. Spoilt tomatoes samples had a higher fungal count that ranged from $3.5x10^5$cfu/g to $5.25x10^5$cfu/g while the fresh tomatoes samples had lower fungal counts ranging from $1.95x10^4$cfu/g to $3.45x10^4$cfu/g. The spoilt pepper had fungal counts ranging from $1.45x10^5$cfu/g to $2.65x10^5$cfu/g which was higher than that of fresh pepper with a low count of $1.6x10^4$cfu/g to $2.75x10^4$cfu/g. The bacterial isolates identified during this study are *Staphylococcus* sp., *Escherichia coli, Bacillus* sp., *klebsiella* sp., *Pseudomonas* sp., *Shigella* sp., *Protues* sp., *Enterobacter* sp., *Citrobacter* sp., *Lactobacillus* sp. *Micrococcus* sp. *Listeria* sp. *Streptococcus* sp. and *Serratia* sp. Spoilt tomatoes had the highest coliform count $1.02x10^5$cfu/g to $9.0x10^5$cfu/g while fresh tomatoes had a lower count of $4.65x10^5$cfu/g to $6.75x10^5$cfu/g. Spoilt pepper recorded coliform counts ranging from $3.8x10^5$cfu/g to $9.4x10^5$cfu/g. Most of the fungi isolated from both the tomatoes and pepper samples were molds and yeast which include *Aspergillus niger, Aspergillus flavus, Candida* sp., *Saccharomyces* sp., *Penicillium* sp. *Mucor* sp. and *Fusarium* sp. Adequate cooking is recommended before consumption.

Keywords: *Solanum lycopersicum,* bacterial isolates, molds, coliform, Total Heterotrophic Bacterial

Introduction

All foods are great sources for nutrient that can contribute to growth. Food materials contain organic substances in plenty and also sufficient amount of water. They may be either neutral or slightly acidic in nature (Singh, 2013). They are subjected to natural contamination by many

different kinds of microorganisms, including pathogens. Food spoilage refers to various changes in which the food becomes less palatable or even toxic to consumers these changes may be accompanied by alterations in taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of microorganisms responsible for their deterioration (Akinmusire, 2011). These fruits and vegetables are usually kept on tables and in baskets for prospective customers in the open markets until it is bought , thereby making it easy for further microbial infections beside those associate with these whole fruit and vegetables surface and those from adjacent infected fruits (Baiyewu et al., 2007).

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 to microbial deterioration is largely due to differential chemical composition such as pH and moisture contents are associated with greater predisposition to microbial spoilage. 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 (naturally occurring toxic chemical often of aromatic structure) which are capable of producing aflatoxin in man, following ingestion or inhalation.

These 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, like Nigeria, post-harvest deterioration are often more severe due

to inadequate storage and transportation facilities. Microbial fruits infection may occur during the growth season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007). Studies by Li-Cohen and Bruhn (2002) has shown that fungi can survive and/or grow on fresh produce and that the nutrient content (carbohydrate, protein and fat) of fresh produce support pathogens.

Fruits are affected by a wide array of microorganisms causing its decay. Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post-harvest handling or during storage and distribution (loading and offloading) (Barth *et al*, 2009). Those types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipments, on handling equipment, in the packaging house, in the storage facility, and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in the yield loss due to deterioration at all subsequent steps in the food (Barth *et al*, 2009).

Tomato and pepper fruits are very rich in mineral, vitamins, and carbohydrate (Udoh*et al*., 2005) In view of these, these fruits are often attacked by microorganisms especially after harvest, thus a fast and high rate of spoilage is often observed in storage (Barth *et al*., 2009)

Tomato and pepper fruits are very rich in mineral, vitamins, and carbohydrate and hence as may serve as good breeding sites for microbial proliferation. Growth of microorganisms on these

fruits contributes to their spoilage and deterioration and render them unhealthy for consumption as they may harbour microorganisms of potential public health importance.

This study sets out to determine the microbial profile of fresh and spoilt pepper (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) sold in the market in Port Harcourt, Nigeria.Microbial contamination sources of Tomato and pepper fruits include raw materials and contact with processing equipment. The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of these fruit and vegetables. Sapers' *et al*., (2001) reported that, compared with the good surface sanitization practices, no decontamination treatment or an ineffective antimicrobial treatment on whole tomatoes and pepper resulted in premature microbiological spoilage of tomato and pepper.. Products can also be contaminated by spoilage microorganisms through contact by people or equipment during processing possibly by air during processing and packaging steps, especially in market places

MATERIALS AND METHODS

Twenty samples each of both fresh and spoilt pepper and tomato (were sourced randomly from different vendors in Choba market within Obio-Akpor Local Government Area, of Rivers State and was transported in a sterilized bag to the Microbiology laboratory for analysis.

Commercially available nutrient media were used for isolation, identification and characterization of microorganisms. The media used include: Nutrient agar, Peptone water, *Salmonella Shigella* agar, MacConkey agar, Mannitol salt agar, Potato dextrose agar.

All the media, diluents and glass wares used (Petri plates, bijou bottles, test tubes, pipette) were sterilized by autoclaving at 121^{o}C for 15 minutes at 15 pounds per square inch (psi), unless stated otherwise and the work benches were disinfected with disinfectant, the wire loop was sterilized by passing through red hot flame from a Bunsen burner before use.

ISOLATION OF MICROORGANISMS

10g of the sample from different locations were added into 90 ml of peptone water, swirled and allowed to stay for few minutes after which a ten-fold serial dilution was done by pipetting 1 ml from the stock solution into the next test tube (10^{-2}), the process was done repeatedly up to (10^{-5}).From the prepared diluents, 0.1 ml of each last two prepared dilutions were transferred into sterile Petri plates containing the different media used and was spread gently using sterile glass rod. The plates were incubated at 37^{o}C for 18-24 hours for the bacteriological media used for bacteria growth and Potato dextrose agar for fungi isolation. The microbial count for each sample was obtained from the previously incubated Petri plates and was expressed as a colony forming unit (cfu/g).

Identification and characterization of isolates

Single colonies of bacteria growth were randomly selected from different media plates based on their morphology and were sub-cultured and incubated at 37^{o}C for 24 hours to obtain pure colonies.

Examination of bacteria

Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents and protocols according to (Cheesebrough, 2000) were used to identify discrete colonies from the bacteriological media of sub-cultured isolates.

Examination of fungi

The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope.

Proximate analysis was done as described by Garuba *etal*.,2018

RESULT/DISCUSSION

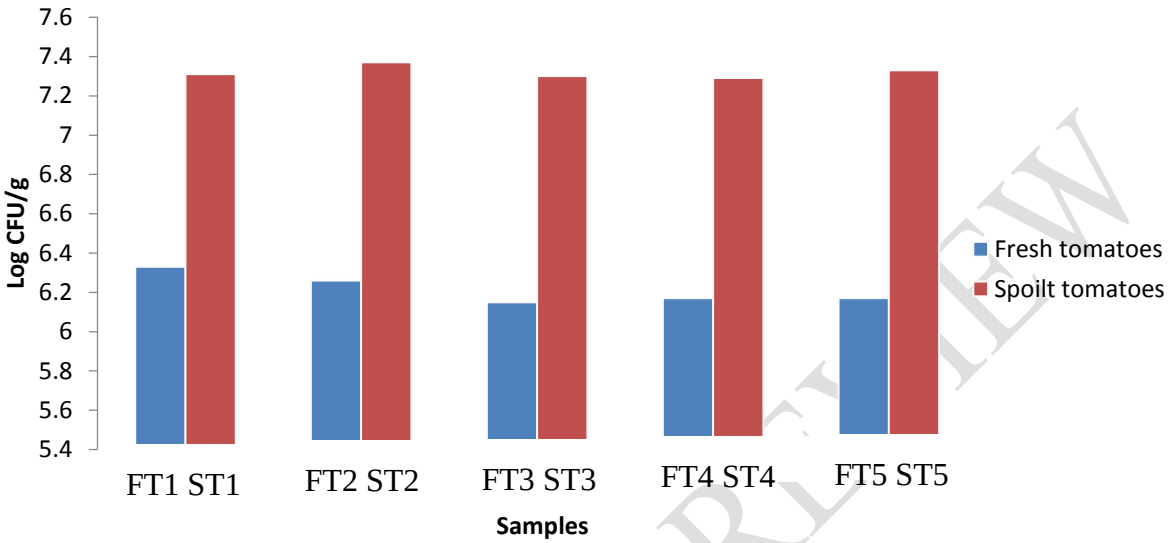

Figure 1`: heterotrophic bacteria count from fresh and spoilt tomatoes samples

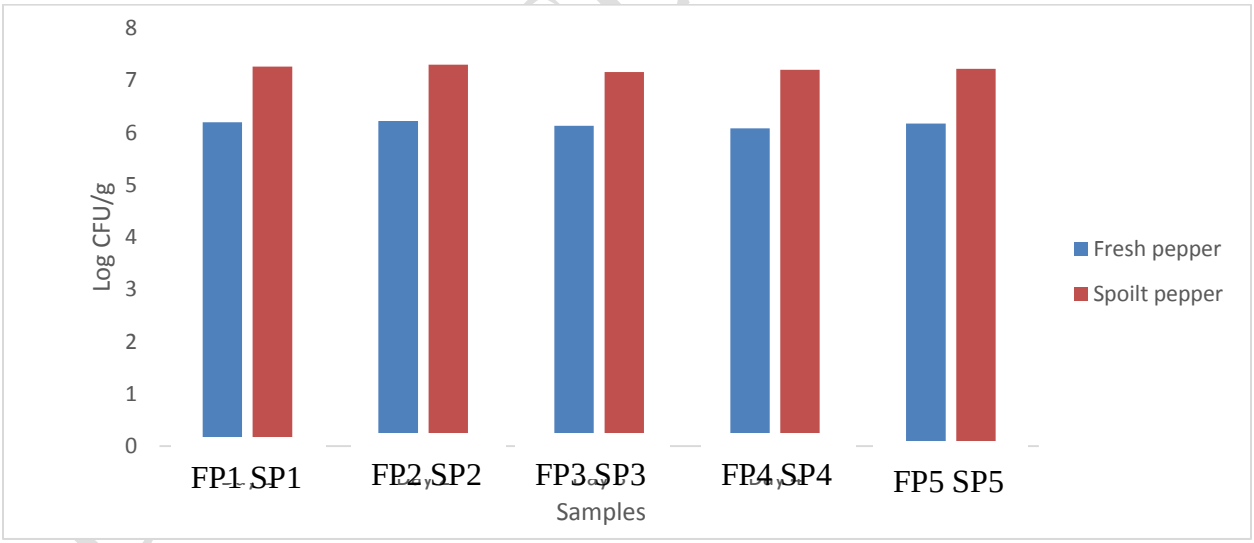

Figure 2: The heterotrophic bacteria count from fresh and spoilt pepper samples

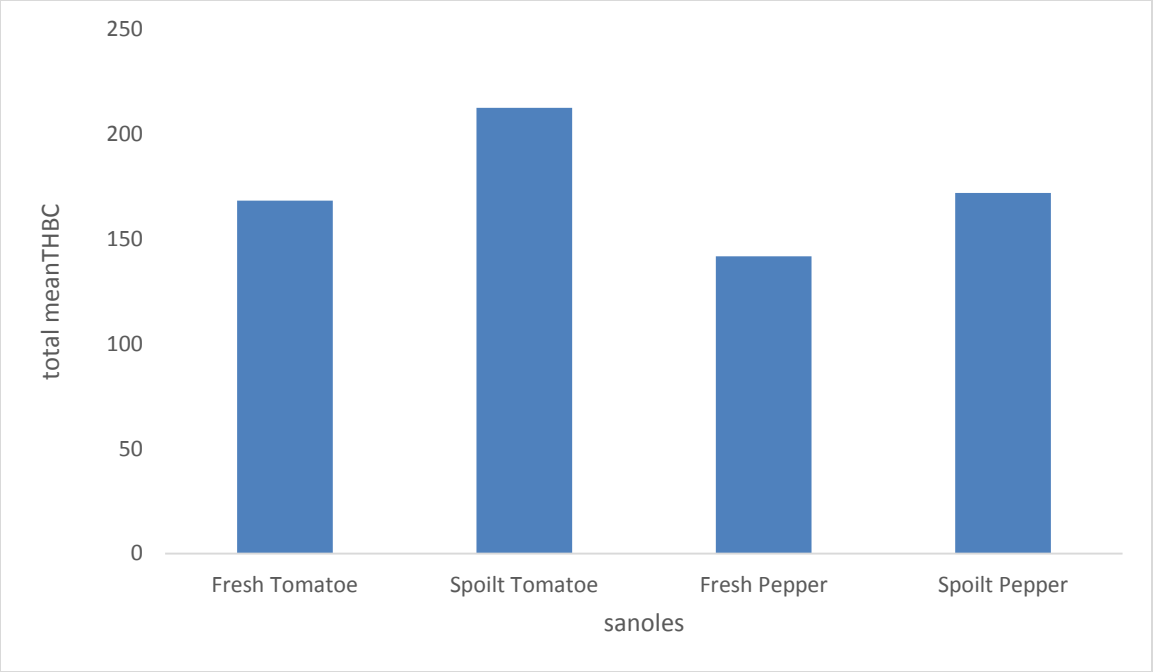

FIG 3 Total Mean of THBC in Fresh Tomatoes /pepper, and Spoilt Tomatoes and Pepper

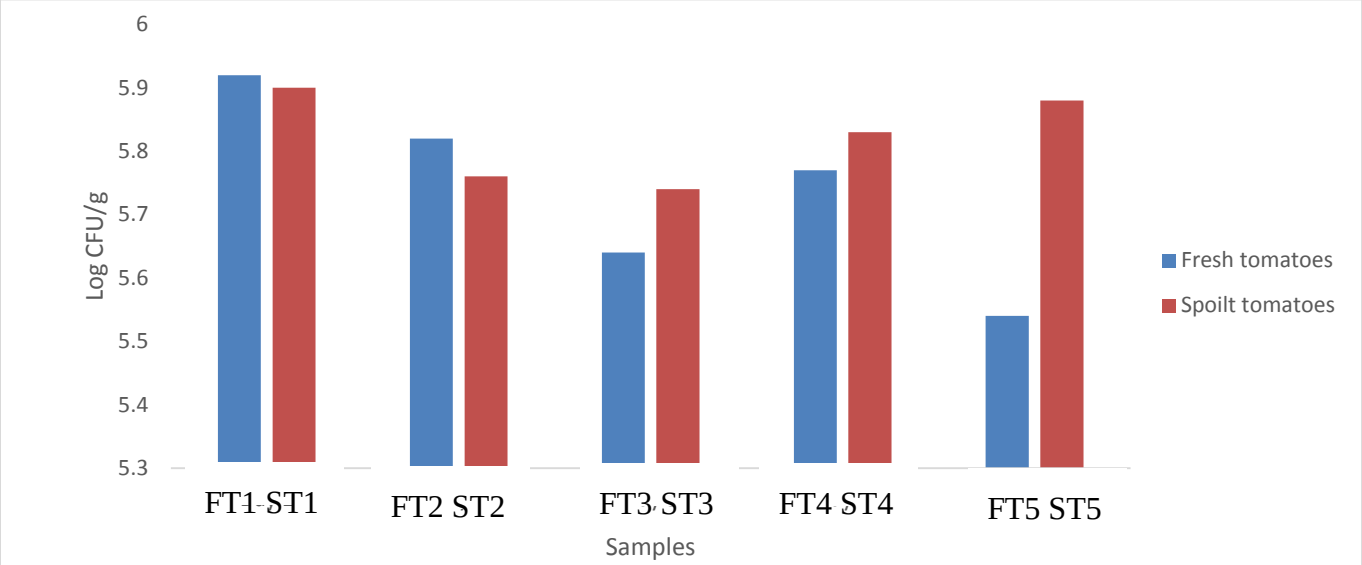

Fig 4. *Staphylococcus* count from fresh and Spoilt tomatoes samples

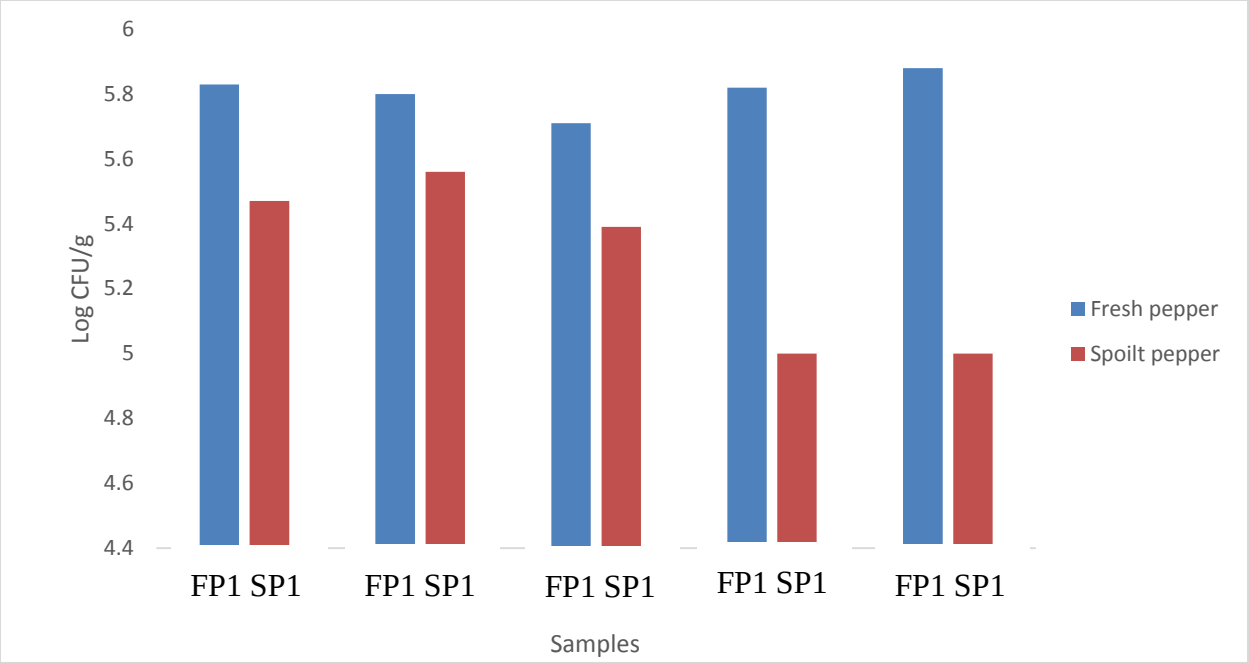

Figure 5: The *Staphylococcus* count from fresh and Spoilt pepper samples

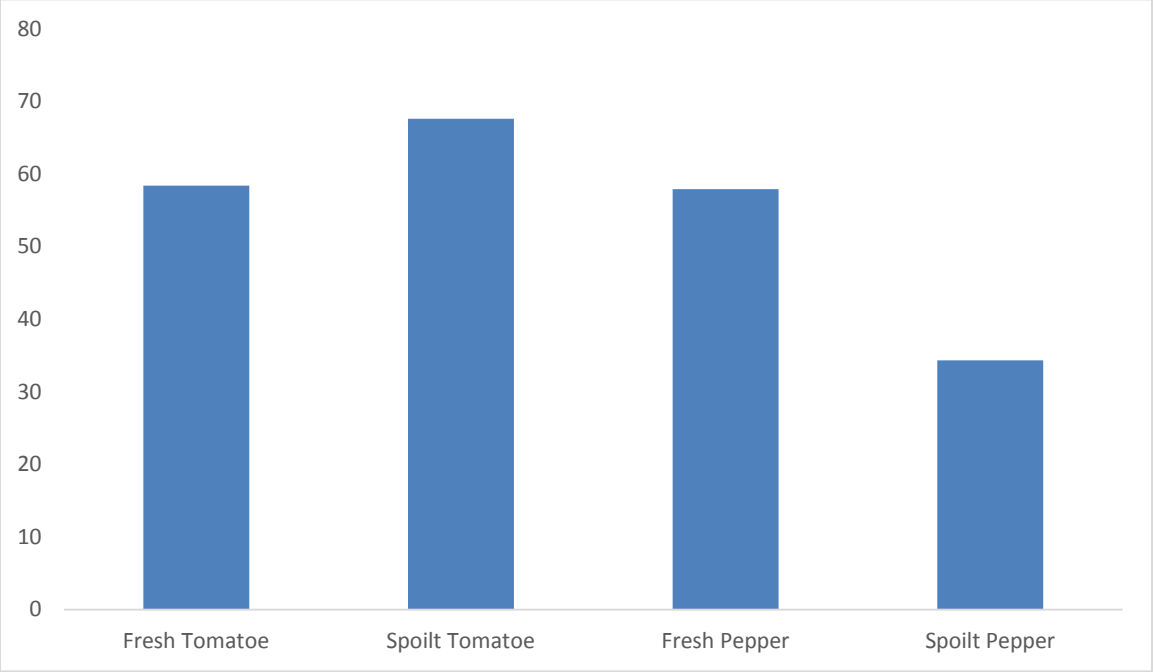

Fig. 6 Total Mean *Staphylococcus* Count in Fresh Tomatoes /pepper, and Spoilt Tomatoes and Pepper

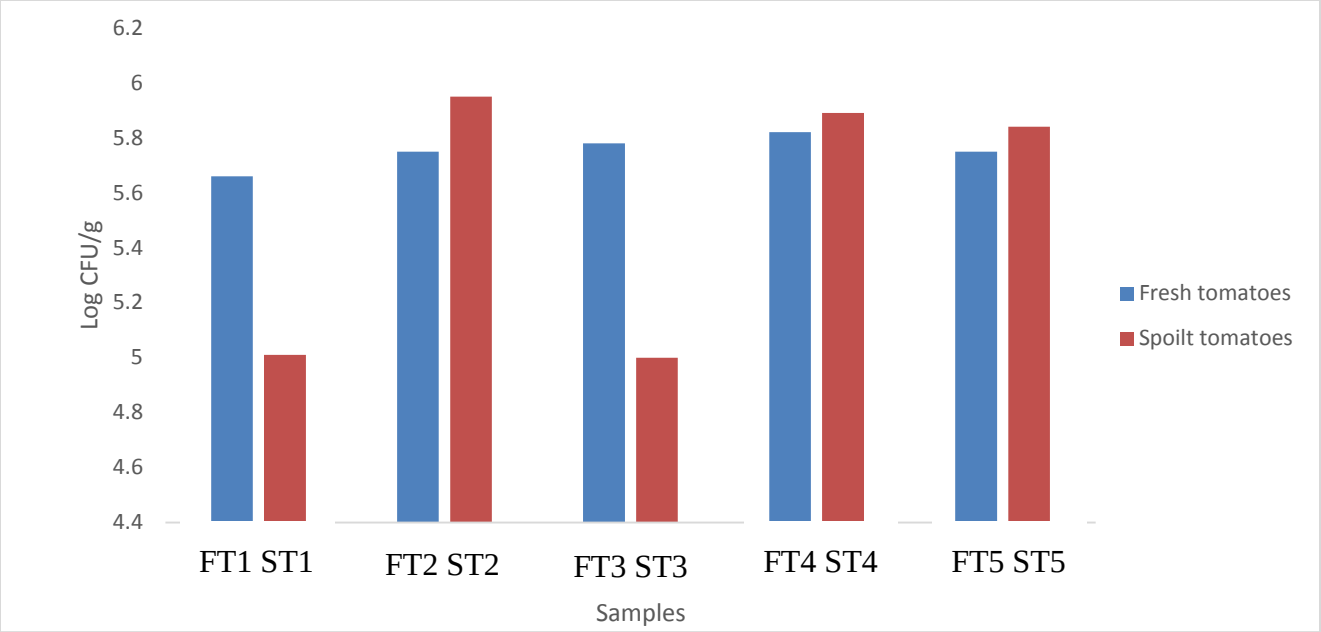

Figure 7; Total coliform count from fresh and spoilt tomatoes samples

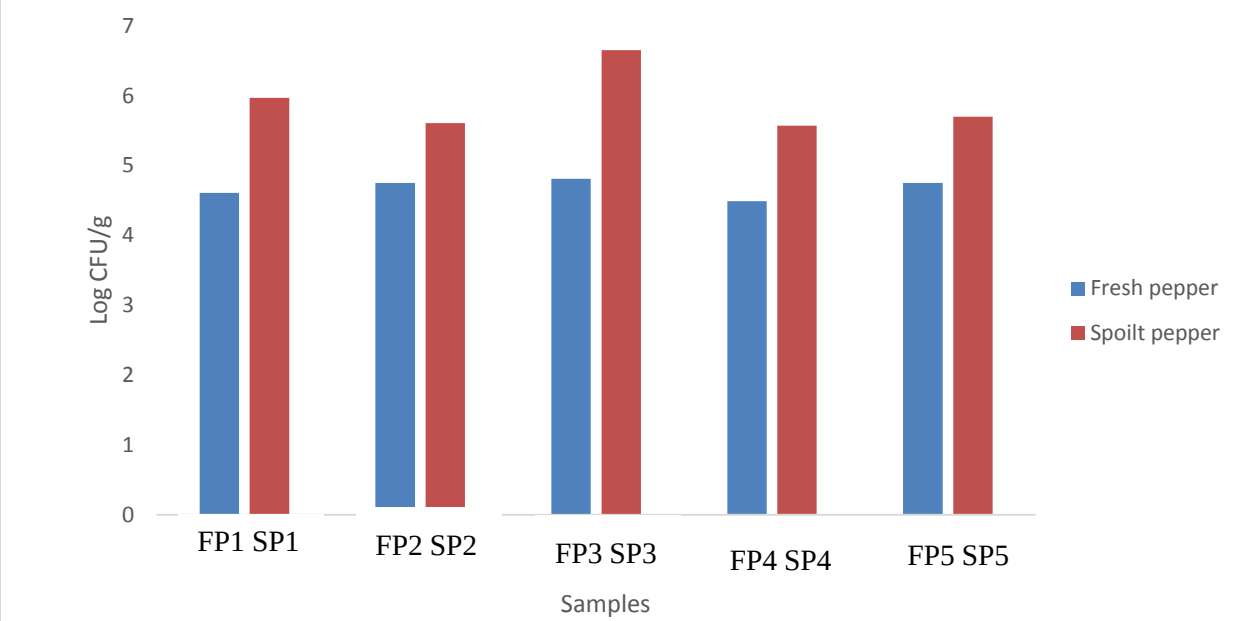

Figure 8: coliform count from fresh and spoilt pepper samples.

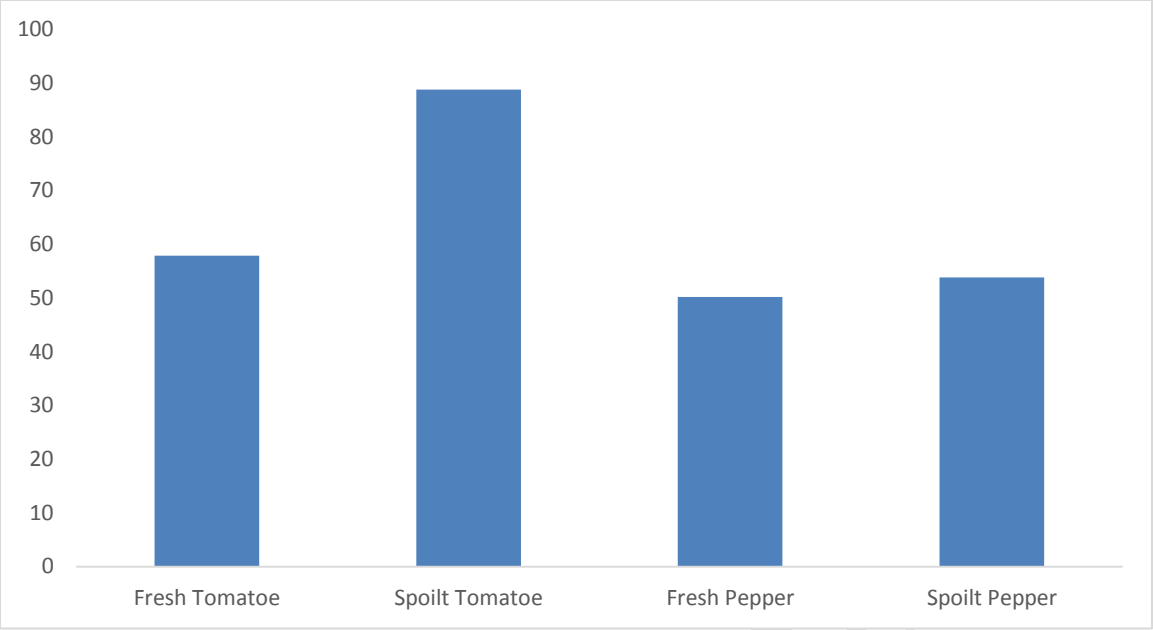

Fig. 9: Total mean of coliform count in fresh tomatoes/pepper and spoilt tomatoes and pepper

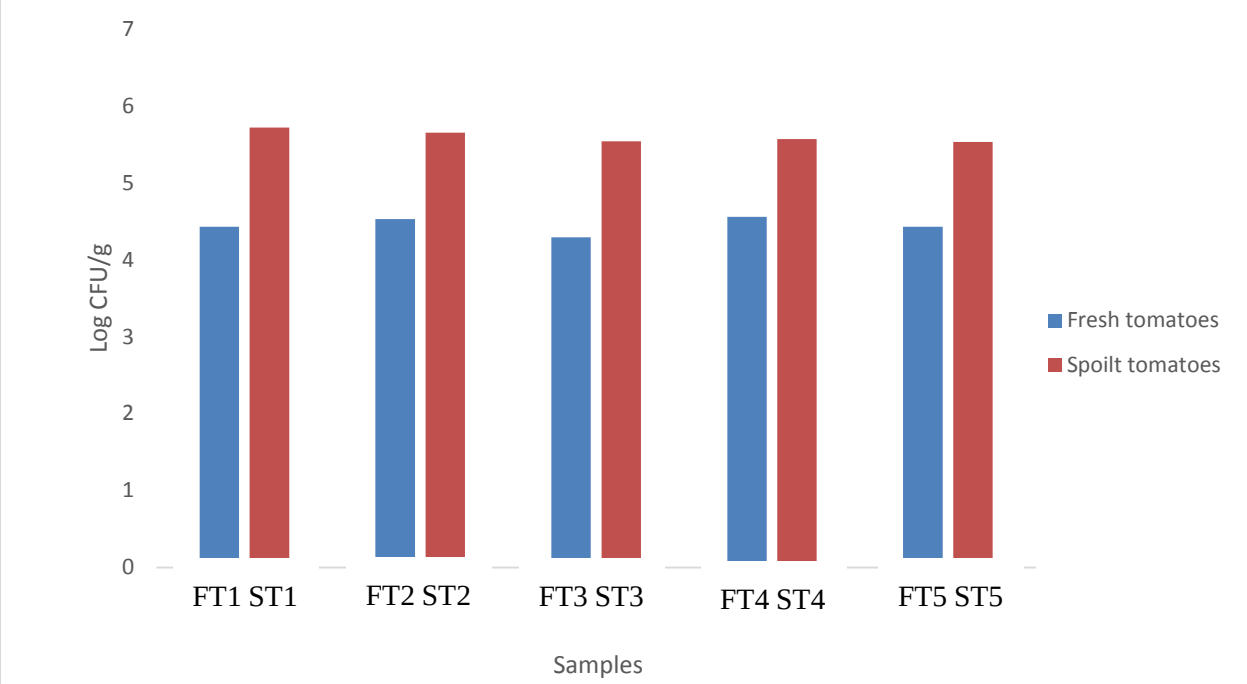

Figure 10 Total heterotrophic fungal count from fresh and spoilt tomatoes samples

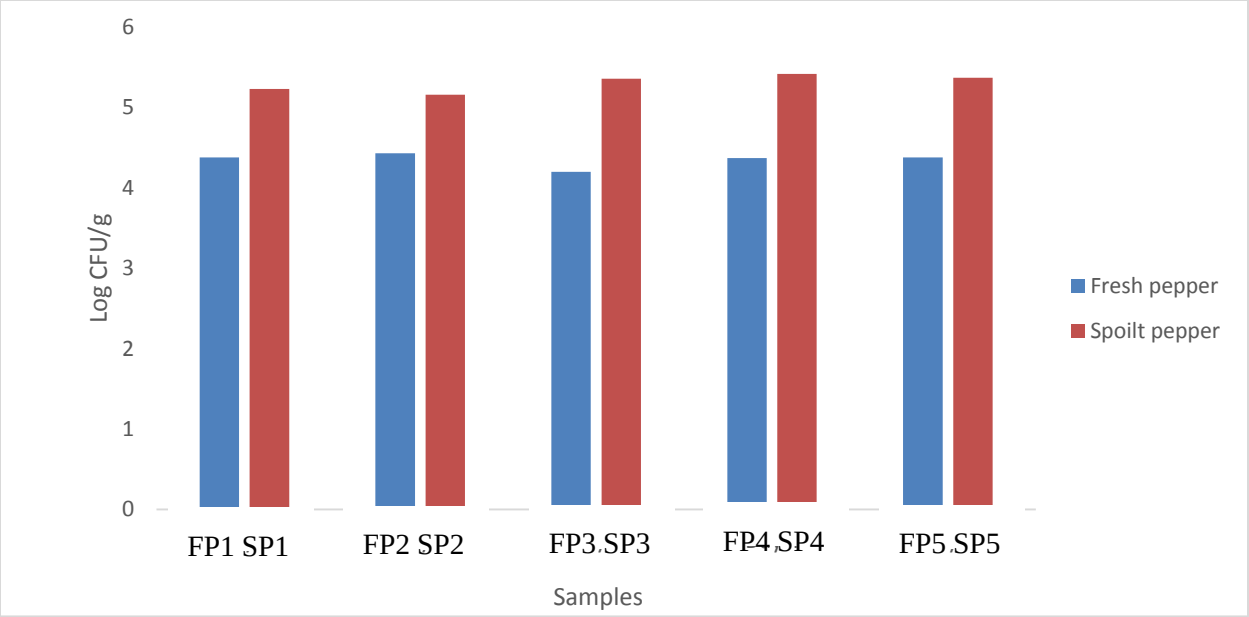

Figure 11: Total heterotrophic fungal count from spoilt pepper samples

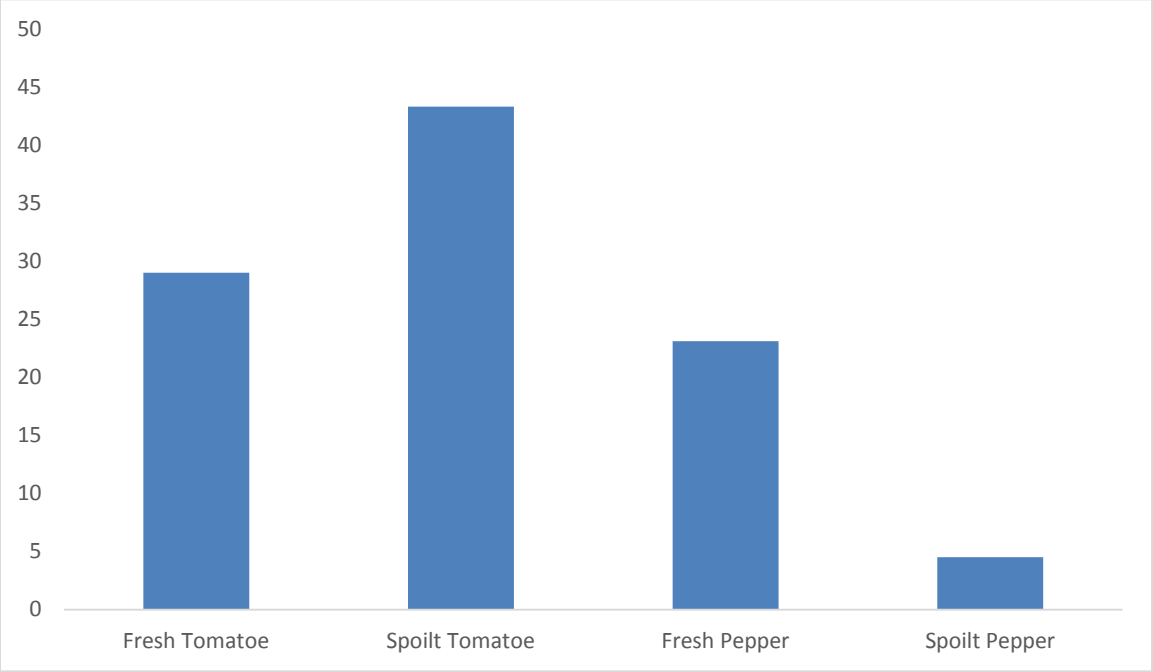

Fig. 12: Total Mean of Fungal count in fresh tomatoes/pepper and spoilt Tomatoes and Pepper

MICROORGANISMS ISOLATED FROM PEPPER AND TOMATOES SAMPLES

A total of one hundred and fourteen (114) bacterial isolates were obtained from both the tomatoes and pepper samples; 23 from fresh pepper samples and 28 from the spoilt pepper samples, 26 from fresh tomatoes samples and 37 from spoilt tomatoes samples. The bacterial isolates identified during this study include *Staphylococcus* sp., *Escherichia coli*, *Bacillus* sp., *klebsiella* sp., *Pseudomonas* sp., *Shigella* sp., *Proteus* sp., *Enterobacter* sp., *Citrobacter* sp.,

The percentage frequency of occurrence of bacteria isolated from the fresh tomatoes samples are in decreasing order *Klebsiella* sp. (19.23%), *Lactobacillus* sp. (15.38%), *Bacillus* sp. (15.38%), *Micrococcus* sp. (11.5%), *Citrobacter* sp. (11.5%) and *Staphylococcus* sp. (11.5%) and *Enterobacter* sp. (7.69%). For the spoilt tomatoes samples, *Lactobacillus* sp. (16.2%), *Klebsiella* sp. (13.51%) and *Bacillus* sp. (13.51%), *Enterobacter* sp. (8.10%), *Proteus* sp. (8.10%), *Listeria* sp. (8.10%), *Shigella* sp. (5.40%), *Pseudomonas* sp. (5.40%) and *Escherichia coli* (5.40%). Bacteria isolated from the fresh pepper had a percentage frequency of occurrence with *Enterobacter* sp., *Streptococcus* sp. and *Micrococcus* sp. had a least occurrence of (13.04%), *Staphylococcus* sp. and *Klebsiella* sp. (17.39%), and *Bacillus* sp. (26.08%). Spoilt pepper samples had a frequency of occurrence for *Serratia* sp. (7.14%), *Enterobacter* sp. and *Escherichia coli* (10.71%), *Proteus* sp. and *Pseudomonas* sp. (14.28%), *Klebsiella* sp. (21.14%). These are represented in the figures below

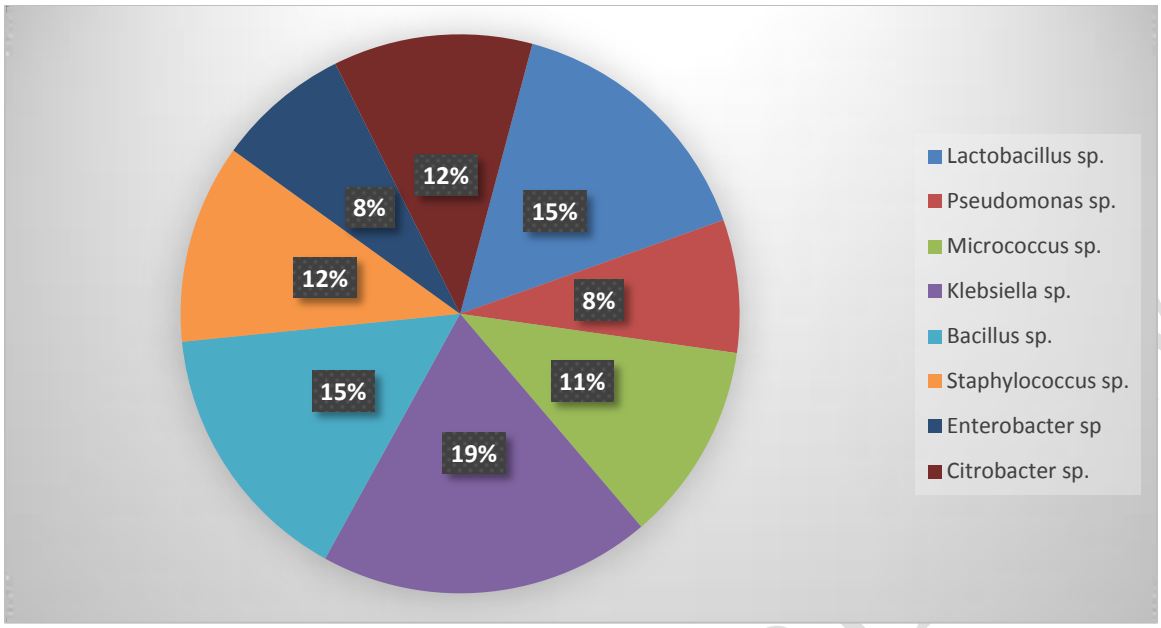

Fig 13: Percentage frequency of bacterial isolates obtained from fresh tomatoes samples

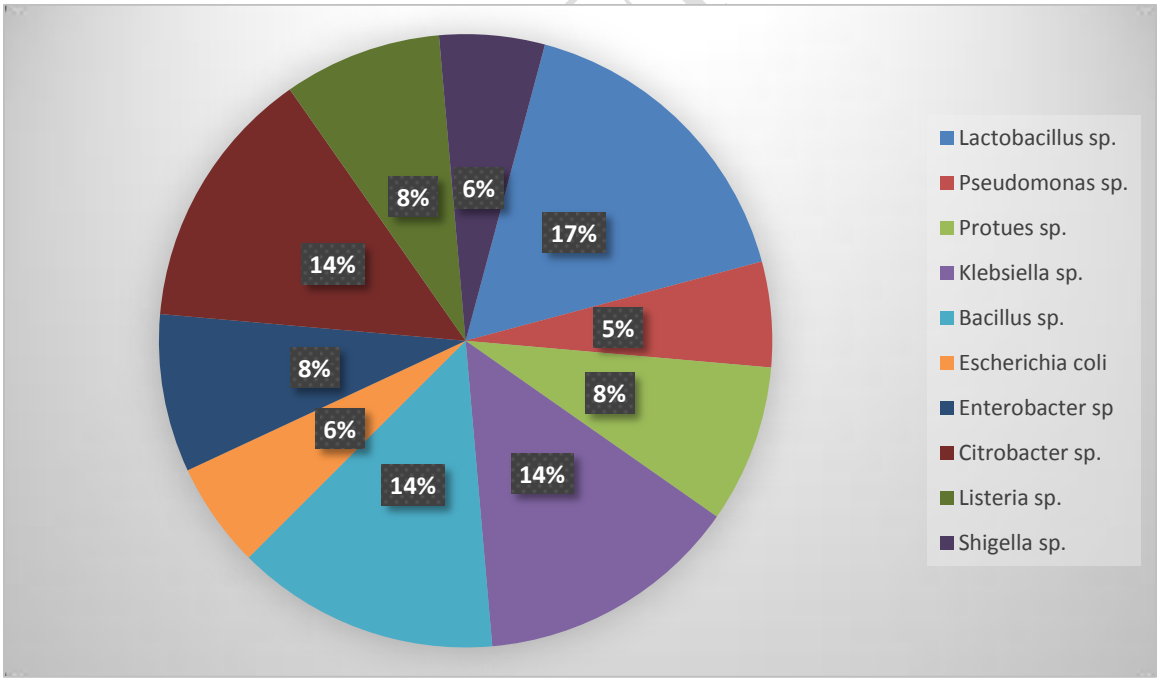

Fig 14: Percentage frequency of bacterial isolates obtained from spoilt tomatoes sample

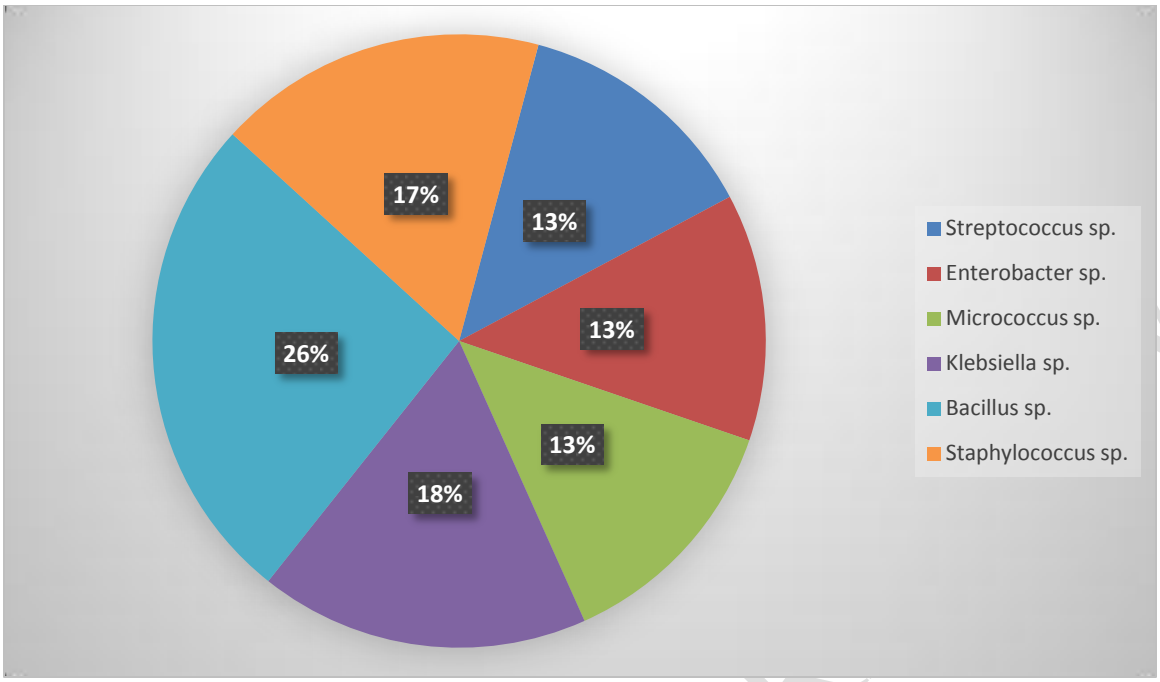

Fig 15: Percentage frequency of bacterial isolates obtained from fresh pepper samples

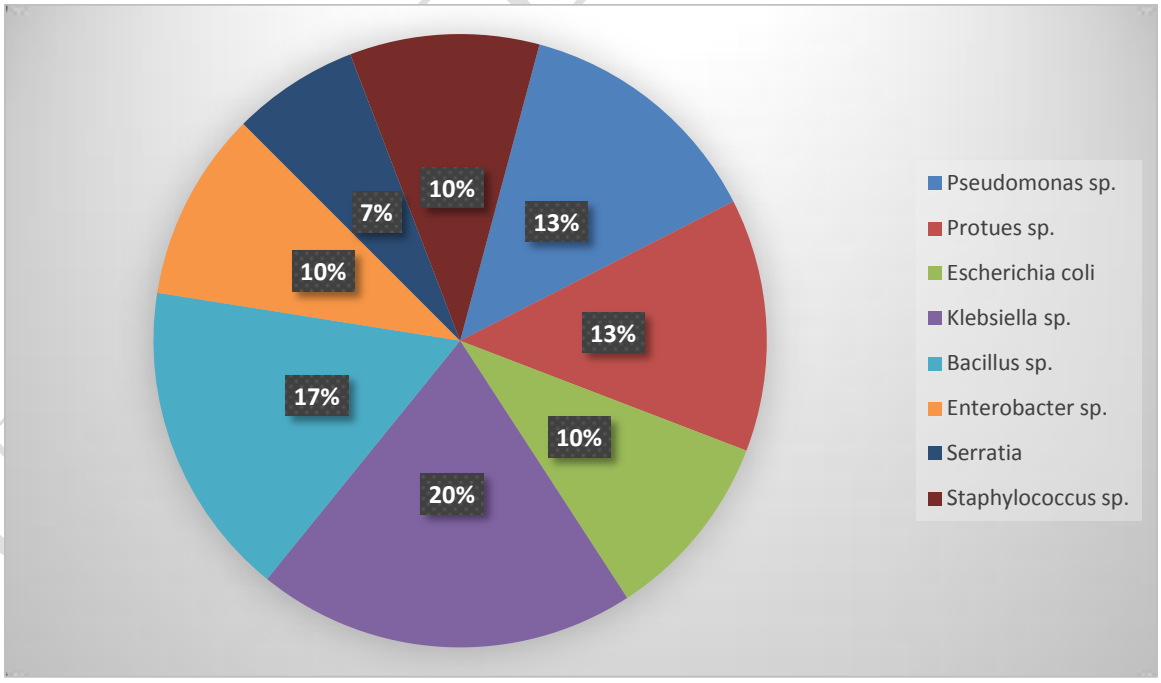

Fig 16: Percentage frequency of bacterial isolates obtained from spoilt pepper samples

Table 1: Proximate Analysis on Tomatoes and Pepper Samples

S/no	Sample code	Moisture	Ash	CHO	Protein	Lipid	Fibre
1	Fresh tomatoes	91.50	0.98	5.00	3.25	0.067	<0.01
2	Fresh pepper	84.67	0.63	7.87	2.79	1.72	2.42
3	spoilt tomatoes	91.09	0.35	5.94	2.45	0.17	<0.01
4	spoilt pepper	87.39	0.81	3.90	3.82	1.86	2.52

Results obtained from this research shows that tomatoes and pepper harbor an array of microorganisms. The total heterotrophic counts for fresh tomatoes samples ranging from $1.43x10^6$ cfu/g to $2.14x10^6$ cfu/g is lower than that of spoilt tomatoes with counts ranging from $1.98x10^7$ cfu/g to $2.39x10^7$ cfu/g. This is higher than results reported by Ibrahim *et al*., (2011). According to Gosh (2009), the high-water content of tomatoes makes them readily susceptible to microbial spoilage. The invasion of microorganisms and their quick multiplication can cause spoilage. This is a possible reason for the increased counts in the spoilt tomatoes than in the fresh one. Also, advisory guidelines for microbiological quality have suggested that satisfactory food products should contain no more than 10^5 cfu/g of starter organisms (Ibrahim *et al*., 2011), hence, considering counts obtained for both fresh and spoilt tomatoes samples in this study, the allowable limit was exceeded.

The spoilt tomatoes had a higher *Staphylococcus* count $5.5x10^5$ cfu/g to $7.95x10^5$ cfu/g while fresh tomatoes had counts of $3.5x10^5$ cfu/g to $8.5x10^5$ cfu/g . The *Staphylococcus* limit set by the Food and Drug Agency (FDA) for foods is $<10^5$. Results obtained from this study fell within the acceptable *Staphylococcus* limits for foods, the coliform count of fresh tomatoes

ranged from $4.56x10^5$ cfu/g to $6.75x10^5$ cfu/g, while spoilt tomatoes had a count of $1.02x10^5$ cfu/g to $9.0x10^5$ cfu/g. The work of Shenge *et al*., (2015) tried to establish possible pathways for coliform contamination of tomatoes. Although, no one single pathway was conclusive enough, they suggested that lack of hygienic practices in handling the product, source of irrigation water, cross contamination during transportation and other unknown factors were responsible for contaminating tomatoes.

The fungal counts of fresh tomatoes ranged from $1.95x10^4$ cfu/g to $3.65x10^4$ cfu/g and spoilt tomatoes had a count of $3.5x10^5$ cfu/g to $5.25x10^5$ cfu/g. Obunukwu et al., (2018) reported fungal counts for spoilt fresh tomatoes stored at ambient temperature similar to fungal counts obtained from fresh tomatoes in this study. In another study, Mwekaven *et al*., (2019) reported counts similar to those obtained from spoilt tomatoes in this study.

Bacterial species isolated from fresh and spoilt tomatoes samples include *Lactobacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus* sp., *Enterobacter* sp., *Citrobacter* sp. *Escherichia coli* was isolated only from the spoilt tomatoes samples. The organism isolated in this study is consistent with the works of Ibrahim *et al*., (2011), Obunukwu et al., (2018), and Mwekaven *et al*., (2019) who isolated similar bacterial species from fresh and spoilt tomatoes respectively. Six (6) bacterial isolates- *Lactobacillus fermenti*, *Pseudomonas stutzeri*, *Listeria monocytogenes*, *Leuconostoc* sp., *Rothia* sp., were found in tomato. (Ajayi, 2013) isolated *Leuconostoc* sp. and *Lactobacillus* sp. as tomatoes natural flora which could participate in spoilage of such fruit. Presence of *Micrococcus* sp. in foods causes dental decay and *Bacillus subtilis* causes flat sour of fruits and denaturing of body (Nester *et al*., 1995). Most of the bacterial isolates are opportunistic infection agents and could lead to food borne bacterial diseases (Mwekaven *et al*., 2019).

Eight (8) fungal isolates (filamentous fungi) were identified in both fresh and spoilt tomatoes samples. These fungal isolates are *Aspergillus niger*, *Penicillium* sp., *Saccharomyces* sp., *Mucor* sp., *Candida* sp., *Aspergillus flavus*, *Aspergillus fumigates* and *Rhizopus stolonifer*. The isolation of *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor* species from rotten tomato confirmed the studies of (Chuku *et al*., 2008). According to Ghosh (2009), fungi were the source of spoilage in most of tomatoes samples rather than bacteria. Also, Akinmusire (2011) had reported that *A. flavus* and *A. fumigatus* caused tomato spoilage. These fungal isolates are also potential sources of disease condition. Some like species of *Aspergillus* are known to produce mycotoxins which can be fetal when consumed (Ibrahim *et al*., 2011).

Fresh pepper had a total heterotrophic bacterial count ranging from $1.22x10^6$ cfu/g to $1.66x10^6$ cfu/g . This was lower than that of the spoilt pepper with counts ranging from $1.46x10^7$ cfu/g to $2.01x10^7$ cfu/g. Table shows that the *Staphylococcus* counts of fresh pepper ranged from $5.2x10^5$ cfu/g to $7.7x10^5$ cfu/g while that of spoilt pepper ranged from $2.5x10^5$ cfu/g to $4.0x10^5$ cfu/g. The coliform counts are shown in table. Spoilt pepper had a count ranging from $3.8x10^5$ cfu/g to $9.4x10^5$ cfu/g and fresh pepper had a count of $3.1x10^4$ cfu/g to $6.5x10^4$ cfu/g, the bacterial isolates obtained from fresh and spoilt pepper samples, respectively. These include *Enterobacter* sp., *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Micrococcus* sp. while *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Klebsiella* sp., *Bacillus* sp., *Enterobacter* sp., and *Serratia* sp.

While the fungal isolates obtained from the pepper samples which include *Aspergillus niger*, *Penicillium* sp., *Rhizopus* sp., *Aspergillus fumigates*, *Rhizopus stolonifer*, *Mucor* sp., *Saccharomyces* sp. These agree partly with the findings of (Li-Cohen and Bruhn, 2002) who discovered that species of fungi associated with the spoilage of some edible fruits including

tomatoes include species of *Aspergillus*, *fusarium*, *Penicillium* and *Rhizopus*. The most frequent of the isolated molds from tomato and pepper belongs to *Aspergillus* sp. and *Penicillium* sp. and these confirms their prevalence in fruits and foods exposed to tropical humid climate, thus consisting potential health risks to consumers. In a similar study carried out on fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria, *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Penicillium digitatum* and *Geotrichum candidum* were identified (Onuorah and Orji, 2015). (Abel-Mallek *et al*., 1995) also reported on the common occurrence of *Aspergillus niger* in healthy tomato fruits collected from markets in Assiut, Egypt. Several studies have also reported that *Aspergillus* sp. are associated with spoilage of tomatoes, apricot, orange, lemon, peach, apple, kiwi, mango etc. (Rashad *et al*., 2011). Onuorah and Orji, (2015) showed that *Aspergillus* had the highest decay diameter among other fungi associated with tomatoes spoilage. Studies have shown that *Aspergillus* produce aflatoxins. Aflatoxins are associated with some diseases in live stocks and humans throughout the world. *Aspergillus flavus* is the main producer of the well-known carcinogenic aflatoxins and its presence in food is of huge concern in terms of food safety, and they are toxic at low concentrations (Rodriques *et al*., 2007). The dominance of *Aspergillus* in rotten tomatoes could pose a serious health risk especially when the tomatoes are not well cooked. Healthy tomatoes fruit should be preferred as they seldom contain microbes (Ugwu *et al*., 2014)

Penicillium sp. were found next to *Aspergillus* in abundance. *Fusarium* are among the most important genera of mycotoxigenic fungi (Zain, 2011). The mycotoxins are of greatest agro-economic importance. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species (Zain, 2011).

Fruits and vegetables are very important and have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other dietary factors cannot be over emphasized. Its spoilage often results to wastage of economic resources as well as food poisoning, especially, when consumed. From the results obtained in this study, it was discovered that few organisms encountered are food borne pathogens. It is also revealed that some spoilage microorganism (mostly fungi) gained access into these fruits during the processes of cultivating, harvesting, grading and packing and environmental contaminant which have in one time or the other been involved in food poisoning. The prevalence frequency of occurrence of fungi was higher than that of bacteria in both fruits. The high amounts of fungi and bacteria demand that appropriate control measures against infection should be employed. Adequate microbiological knowledge and hygienic handling practices of these produce would help minimize wastes due to deterioration. It is therefore, important that both the farmers who harvest and package the fruits into bags for transportation, the marketers, and consumers take necessary precautions to prevent contamination and eating of contaminated fruits. This will however, enhance reduction in the risk of microbial toxins that are deleterious to human health.

The result obtained from Proximate analysis indicates that the fresh tomatoes sample had higher Moisture contents, Ash, Lipid and Protein. The carbohydrate content for fresh tomatoes was less than those of spoilt tomatoes sample. Both fresh and spoilt tomatoes sample showed they have equal fiber content. The spoilt pepper sample had higher Moisture content, Ash, Protein, Lipid and Fiber content. The fresh pepper sample however showed a higher Carbohydrate content. The result however, contrasts with the works of Ikuomola *et al*., (2015), Garuba *et al*., (2018), Ismail *et al*., (2016) who had varying values for Moisture, Ash, Carbohydrates, Protein, Lipid and Fibre.

Conclusion

It is concluded that most of the fungi isolated from both the tomatoes and pepper samples were molds and yeast which include *Aspergillus niger, Aspergillus flavus*, *Candida* sp., *Saccharomyces* sp., *Penicillium* sp. *Mucor* sp. and *Fusarium* sp. Adequate cooking is recommended before consumption.

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