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Microbial Deterioration of Tomato Fruit (*Lycopersicon esculentum*) Sold in Three Popular Markets in Ilorin, Kwara State, Nigeria

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Abstract

The economic loss as a result of spoilage and proliferation of microorganisms on tomato fruits with the possible health risks were the justification for this study. One hundred and fifty tomato fruit samples in different stages of spoilage from three different markets in Ilorin, Kwara State, Nigeria were collected within five weeks and the effect of moisture content on each sample and resulting microflora examined. The pH of the samples ranged from 4.90 - 5.40, while the moisture content ranged from 89.10% - 90.70%. The bacteria counts ranged from 4.00×10^6 - 7.50×10^6 cfu/ml, while the fungal counts ranged from 1.60×10^6 - 3.50×10^6 cfu/ml. A total number of sixteen bacteria and eleven fungi including yeasts were associated with the samples. The bacterial isolates included *Aeromonas veronii*, *Bacillus* sp., *Neisseria* sp., *Corynebacterium renale*, *Pseudomonas fluorescens*, *Micrococcus varians*, *Moraxella* sp., *Bacillus polymyxa*, *Aeromonas hydrophila*, *Pseudomonas* sp., *Bacillus megaterium*, *Corynebacterium ulcerans*, *Vibrio* sp., *Proteus mirabilis*, *Corynebacterium xerosis*, and *Bacillus brevis* while the fungal isolates included *Alternaria* sp., *Botrytis* sp., *Candida albicans*, *Candida guilliermondii*, *Candida tropicalis*, *Chrysosporium tropicum*, *Curvularia* sp., *Doratomyces microsporus*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, and *Rhizopus stolonifer*. The high moisture content of the tomato samples provides an enabling environment for proliferation of the microbial load and hence its spoilage and potential to become health risk to human beings.

Keywords: Spoilage, proliferation, economic loss, pH and moisture

Introduction

Tomato *(Lycopersicon esculentum* Mill) is one of the world's most important vegetable crops with a current world wide fresh weight production of 80 million tons from a cropped area of about 3 million hectare (Fatih *et al.*, 2005).

It has its origin in Western South America and Central America. China is the largest producer followed by United States and Turkey (Denton, 2009). Economically, tomato tops the list in value among edible vegetables in Nigeria (Anyanwu *et al.*, 2003).

The fruit is one of the most important vegetable in most regions of the world and constitute an important source of food as well as

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cash in Nigeria (Alao, 2000; Ogbonna *et al.*, 2008).The fruit contributes to a healthy, well balanced diet. It is rich in vitamins, minerals, essential amino acids, sugars, and dietary fibers. Yellow varieties have high vitamin A content than red ones, but red tomato fruits contain lycopene, an anti-oxidant that may contribute to protection against carcinogenic substances (Seymour *et al.*, 2001; Ajayi and Olasehinde, 2009).

Although production figures are not available, production is seasonal resulting in a glut during the seasons and scarcity at off seasons. Tomato also does well with soil pH of between 5 and 7. Generally, harvested ripe fruits at room temperature (28±1°C) can store for 5 days. However, some tomato fruits referred to as iron tomatoes are so strong that they can store for a maximum of 10 days if kept in a favorable environment (Chuku *et al.*, 2008).

However, as beneficial as tomato fruit is to humans, large percentage of tomato fruits produced in Nigeria are being lost to post-harvest deteriorations caused by microorganisms (Ajayi and Olasehinde, 2009). Among the microbes infecting tomato fruits, fungal plant pathogen can cause extensive loss of the fruits (Salik *et al.*, 2008). Bacterial contamination is more dangerous, this is because the presence of highly dangerous toxin and bacteria spores is often not detected until after an outbreak of food poisoning and when laboratory examination and experiments uncovers the infective agent (Oladipo *et al.*, 2010).

The biological structure of tomato when disrupted (during harvesting, transportation and storage or by insect) can serve as a route of entry for opportunistic pathogen (Salik *et al.*, 2008). Due to their soft textures the fruits, are easily bruised through harvesting and other post-harvest handling operations such as packaging, transportation and storage (Sani and Alao, 2007). Tefera *et al.* (2007) states that due to poor storage conditions, resistance of fruits and vegetables to natural disease usually decline, leading to infection by pathogens.

For most microbial deteriorations to occur successfully, it usually requires cool weather temperatures with high relative humidity. The disease incidence increases with heavy rainfall (Iannotti, 2009).

The use of heat treatment at 38^0Cfor 24hour together with the application of *Pichia guilliermondii* is one of the most effective techniques in controlling post-harvest fungal spoilage in tomato fruit. The heat treatment inhibited hyphae growth and spore germination of *Rhizopus stolonifer,* while *P. guilliermondii* cells used on tomato after heat treatment multiplied rapidly in fruit wounds and had a strong capability of adhesion to the hyphae of *R. stolonifer* (Yan *et al.,* 2010).

The study was carried out to assess the opportunistic microorganisms involved in tomato fruit deterioration in the three major markets involved in retailing of these fruits in Ilorin, Kwara State, Nigeria and health-related hazards posed by these organisms to consumers.

Materials and Methods

Collection of Samples

One hundred and fifty tomato samples, some of which were showing signs of spoilage were bought from tomato fruit vendors at three different markets of Ipata, Oja-Oba, and Oja-Tuntun in Ilorin, Kwara State, Nigeria within five weeks. Ten samples were collected from each market site once a week in sterile polythene bags separately and taken to University of Ilorin, Ilorin laboratory for analysis.

Physicochemical Parameters Determination

pH

The pH values of all the tomato samples were obtained using a glass electrode pH meter, which was standardized prior to use each time with a neutral buffer. The liquid obtained from the mashed tomato sample was transferred into a sterile conical flask, after which the pH meter was inserted into the liquid. The readings on the pH meter were then recorded (Fawole and Oso, 2004).

Moisture Content

Moisture content of the samples was determined by weighing 1g of each tomato sample into a sterilized weighed crucible which was then dried at 105^{0}C for 24 hours. The crucible was then transferred into a dessicator and the sample allowed to cool. The crucible with the cooled dried sample was reweighed. The loss in weight and initial weight of the sample were noted and used to calculate the percentage moisture content using standard methods of APHA (2002) and Fawole and Oso (2004).

Total Bacterial and Fungal Counts

Total bacterial and fungal counts were determined using standard pour plate technique method. Nutrient agar and potato dextrose agar were used for isolating the bacteria and fungi respectively. One gram of sample was crushed in a mortar with pestle already sterilized before use and mixed properly with 9 ml sterile distilled water. A serial dilution up to 10^{-6} was prepared and 1 ml was used to inoculate the media. Nutrient agar plates were incubated at 37±2^{0}C and potato dextrose agar plates at room temperature. Plates were examined for growth after 24 hours. Colonies were counted after 24 hours for bacteria and 72 hours for fungi. Bacteria and fungi were identified using morphological and cultural characteristics as well as biochemical tests as described by APHA (2002) and Fawole and Oso (2004).

Statistical Analysis

The means of all the relevant data obtained were subjected to analysis of variance (ANOVA) (Norusis, 2006).

Results

pH of the tomato fruits in the three markets ranged from 4.90 – 5.40 indicating acidity of the fruits.

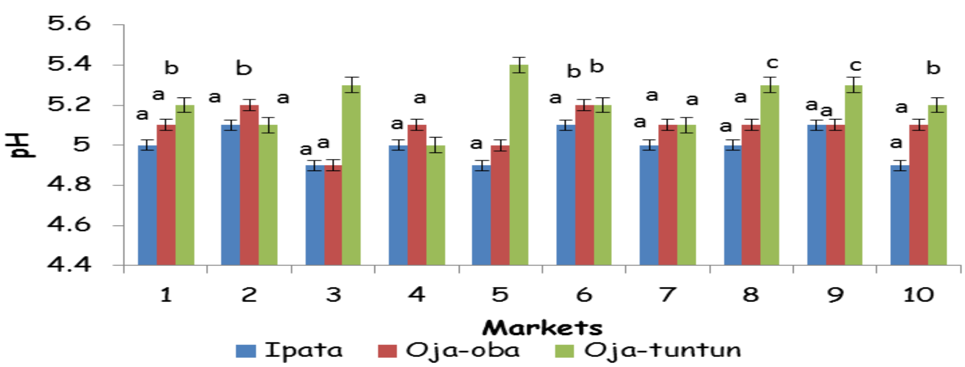
Mean of moisture content

The mean of moisture content of tomato fruits at the three markets ranged from 89.10 - 90.70 %

Microbial Isolates

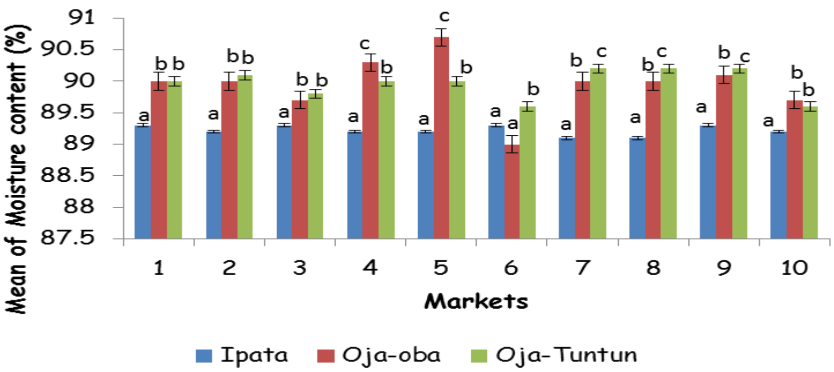
The mean of bacterial counts from the tomato samples ranged from 4.00 x 10^6 – 7.50 x 10^6 cfu/ml while that of fungal counts ranged from 1.60 x 10^6 – 3.50 x 10^6 cfu/ml. The lowest mean of bacterial counts was got at Oja-Oba market while the highest mean of bacterial counts was obtained from Oja-Tuntun market. The lowest mean of fungal counts of the samples was obtained from Ipata market and the highest mean of fungal counts was obtained from Oja-Oba market.

A total number of sixteen bacteria namely *Aeromonas veronii*, *Bacillus* sp., *Neisseria* sp., *Corynebacterium renale*, *Pseudomonas*



Mean followed by the same letter are not significantly different at α = 5% based on Duncan Multiple test

Figure 1: Mean of pH of tomato fruits in three markets in Ilorin, Nigeria.



Mean followed by the same letter are not significantly different at α = 5% based on Duncan Multiple test

Figure 2: Mean of moisture contents of tomato in three markets in Ilorin, Nigeria.

fluorescens, *Micrococcus varians*, *Moraxella* sp., *Bacillus polymyxa*, *Aeromonas hydrophila*, *Pseudomonas* sp., *Bacillus megaterium*, *Corynebacterium ulcerans*, *Vibrio* sp., *Proteus mirabilis*, *Corynebacterium xerosis*, and *Bacillus brevis* were isolated from the tomato samples while eleven fungi isolated included *Alternaria* sp., *Botrytis* sp., *Candida albicans*, *Candida guilliermondii*, *Candida tropicalis*, *Chrysosporium tropicum*, , *Curvularia* sp., *Doratomyces microsporus*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, and *Rhizopus stolonifer*.

Discussion

A total number of sixteen bacteria isolated from the tomato samples in the three markets of Ipata, Oja-Oba and Oja-Tuntun included *Aeromonas veronii*, *Bacillus* sp. *Neisseria* sp., *Corynebacterium renale*, *Pseudomonas fluorescens*, *Micrococcus varians*, *Moraxella* sp., *Bacillus polymyxa*, *Aeromonas hydrophila*, *Pseudomonas* sp., *Bacillus megaterium*, *Corynebacterium ulcerans*, *Vibrio* sp., *Proteus mirabilis*, *Corynebacterium xerosis*, and *Bacillus brevis* while eleven fungi were isolated from the same tomato samples, and these include *Alternaria* sp., *Botrytis* sp., *Candida albicans*, *Candida guilliermondii*, *Candida tropicalis*, *Chrysosporium tropicum*, *Curvularia* sp., *Doratomyces microsporus*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, and *Rhizopus stolonifer*.

The pH of deteriorated tomato fruits from the three markets ranged from 4.90-5.40 as shown in Figure 1 which is different from 4.2-4.3 obtained by Jay (2000), this slight increase in pH may be as a result of the neutralizing effect of the microbial metabolic products, produced by the initial contaminants of the tomato fruit. This statement is supported by ICMSF (2005) and Bartz *et al.* (2009).

Figure 2 indicated that ripe deteriorated tomato fruits had a high moisture content, which ranged from 89.10 - 90.70 % which tend to encourage microbial growth and multiplication in the fruits, as microorganisms require a little water film for growth. This result is similar to

Table 1: Mean of bacterial and fungal counts of the tomato samples in the markets

Markets	MTBC (cfu/ml)	MTFC (cfu/ml)
Ipata	4.40 $X10^6$	1.60 $X10^6$
Oja-Oba	4.00 $X10^6$	3.50 $X10^6$
Oja-Tuntun	7.50 $X10^6$	2.40 $X10^6$

Key: MTBC: Mean of total bacterial counts, MTFC: Mean of total fungal counts

Table 2: Distribution of bacterial isolates across the three markets

Bacterial Isolates	Ipata					Oja-Oba					Oja-Tuntun				
	WK1	WK2	WK3	WK4	WK5	WK1	WK2	WK3	WK4	WK5	WK1	WK2	WK3	WK4	WK5
Aeromonas veronii	+	+	–	–	–	–	–	–	–	+	+	–	–	–	–
Bacillus sp.	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Neisseria sp.	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–
C.renale	+	+	–	–	–	–	–	+	+	–	–	–	+	+	–
P. fluorescens	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+
M. varians	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Moraxella sp.	+	+	–	–	–	–	–	+	+	–	–	–	+	+	+
B. polymyxa	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Aeromonas hydrophila	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Pseudomonas sp.	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Bacillus megaterium	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. ulcerans	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–
Vibrio sp.	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
P. mirabilis		–	–	–	–	+	–	–	–	–	–	–	–	–	–
C. xerosis		–	–	+	+	–	–	–	–	–	–	–	–	–	–
B. brevis		–	–	–	+	+	–	–	–	–	–	–	–	–	–

Key: + present; - absent; WK week

Table 3: Occurrence of fungal isolates in the three markets

Fungal Isolates	Markets		
	Ipata	Oja-Oba	Oja-Tuntun
Alternaria sp	-	-	+
Botrytis sp.	-	-	+
Candida albicans	+	+	+
Candida guilliermondii	+	-	-
Candida tropicalis	+	-	-
Chrysosporium tropicum	-	+	-
Curvularia sp.	-	+	-
Doratomyces microsporus	-	+	-
Geotrichum candidum	-	-	+
Saccharomyces cerevisiae	-	+	+
Rhizopus stolonifer	-	-	+

Key: + present; - absent

that obtained by Bartz *et al.* (2009).

The isolate *Botrytis* sp. is an important plant pathogen involved in bio-deterioration of tomato which is also supported by the findings of Campbell and Stewart (1980), Mansfield (1980) and Cerkauskas, (2005).

Alternaria sp. with 4% occurrence isolated from fruit samples in Oja-tuntun market has been implicated in tomato early blight disease (Mark and Brooke, 2006), as well as tomato black mold rot. *Alternaria* sp. produces mycotoxins known as tenuzoic acid which is of great concerns worldwide due to its severe ill effects (Pitt, 2000; Deepak *et al.*, 2007).

Musa *et al.* (2002) reported that the highest incidence of fungi rot in the farm, and market samples of tomato fruit were caused respectively by *Saccharomyces cerevisiae* and *Rhizopus stolonifer*. *Geotrichum candidum* has been identified as the sour rot pathogen of tomato fruits, which produces a sour odur on lesions in the fruit (Bartz *et al.*, 2009).

Rhizopus stolonifer, an opportunistic plant pathogen was also isolated from fruit samples in Oja-Tuntun sample, with a 4% occurrence. Fajola (2006) reported that *Rhizopus stolonifer* is a soft rot pathogen of tomato in Nigeria. The mycelium of the fungus can infect adjacent fruits, through mechanical wounds and natural openings, creating a nest of mold growth on diseased fruits (Mahovic *et al.*, 2009).

Curvularia sp. and *Chrysosporium tropicum* were isolated from tomato fruit samples from Oja-Oba market. The fungi are dry rot tomato fruit pathogens, which are able to cause post-harvest deteriorations of fruits. This statement was supported by Fajola (2006) who stated that *Curvularia lunata* has been established as one of the dry rot pathogen of tomato fruit, accounting for 15% of the overall post-harvest loss of fruit in Nigeria. *Curvularia lunata* has also been verified as the causative agent of keratomycosis (eye invasion) (Campbell and Stewart, 1980).

Candida albicans and *C. guilliermondii* were isolated from the tomato samples, with *Candida albicans* having the highest percentage occurrence of 25% out of all the fungi isolated. *Candida albicans* was present in fruit samples from all the three markets, while *C. guilliermondii* occurred only in fruit samples from Ipata market. *C. guilliermondii* has been isolated from normal skin, sea water, feaces, animals, fig wasps, butter milk, leather, fish and beer (Kreger, 1984; Rippon, 1988). Its presence in tomato fruit may be as a result of poor hygienic conditions by the handler, through contamination with the handler's skin or feaces etc. *C. albicans* could be said to be widely distributed across the 3 markets. It occurred most in Oja-Tuntun market, and least in Ipata market. The source of *C. albicans* in tomato fruit may be as a result of contamination, through sneezing, coughing, talking and unhygienic conditions by the handler.

Doratomyces microsporus was isolated from fruit samples in Oja-Oba market with 8% occurrence level. *Doratomyces* sp. has been reported to cause rot in potatoes, oats, corns and some other vegetables (Webster and Weber, 2007).

Pseudomonas fluorescence, Pseudomonas sp*., Bacillus polymyxa, Bacillus brevis, Bacillus megaterium, and Bacillus* sp. were isolated from tomato samples, with each having 4% occurrences except *B. megaterium*, with 8% occurrence. According to Bartz *et al.* (2009), certain species of *Pseudomonas* and *Bacillus* can cause soft rot of tomato. Some *Bacillus* sp. can cause food poisoning, result into different kinds of intoxication including; nausea, vomiting, and abdominal cramps (Jamil, 2007). They can also cause anthracis (Moser *et al., 2006).* The genus *Bacillus* also has members that produce clinically useful antibiotics e.g. *Bacillus polymyxa,* the source of polymyxin (Marikawa, 2009).

Corynebacterium renale, C. ulcerans and C. xerosis were isolated from the tomato samples with occurrences of 13%, 8%, and 4% respectively. Tournas (2005) said that the most common spoilage bacteria of harvested vegetables are *Erwinia caratovora, Pseudomonas* spp., *Corynebacterium* spp., *Xanthomonas campestris* and lactic acid bacteria. *Corynebacterium renale* causes urinary tract infections in humans, *C. ulcerans* causes diphtheria-like syndrome (pharyngitis) in human, while *C. xerosis* causes endocarditis and disseminated infections in immunocompromised individual (Marikawa, 2009).

Oladipo *et al.* (2010) stated that bacteria strains such as *Bacillus* spp., *Aeromonas hydrophila, Proteus* sp., *Pseudomonas* sp. etc., spoil tomato fruit juices and that their presence may pose risks to consumer health and should not be taken for granted. Contamination of fruit juice by bacteria may occur when the organisms enter the processing plant or in the surface, the fruit having originated from water, dust, and decomposing fruits (Ryu and Beuchat, 1998; Oladipo *et al.,* 2010).The existence of human pathogenic bacteria in fresh fruits and vegetable products such as *Aeromonas hydrophila* has been reported (FAO, 2010). Prescott *et al.* (2008) reported that *Micrococcus* species are widely spread in soils, water and on the skin of mammals, which may be their natural habitat. Contamination of tomato fruit with *Micrococcus varians* may be from these sources.

Neisseria sp. was isolated from two of the market fruit samples (8%), while *Moraxella* sp. was isolated from fruit samples from the three markets with 12% occurrence level. Their presence on tomato fruit may be attributed to sneezing, coughing or poor sanitary conditions by the handler (AlaAlden, 2007; Prescott *et al.*, 2008). *Moraxella* spp. may cause tracheobronchitis and pneumonia in humans, and also cause disease in cattles, where it can be transmitted by flies (AlaAlden, 2007).

Comparing the mean total bacteria counts and the mean total fungal counts, a higher number with respect to bacteria than fungi can be found. This may be as a result of the ability of bacteria to produce growth inhibitory substances such as bacteriocins to inhibit the growth of fungi as well as other bacteria competitors (Jay, 2000).

Besides causing huge economic losses, some fungal species could produce toxic metabolites in the affected site of the fruit, constituting a potential health hazard for humans (Tournas, 2005). Additionally, vegetables have often served as vehicles for pathogenic bacteria, viruses and parasites and were implicated in many food- borne illness outbreaks. Therefore, in order to slow down vegetable spoilage and minimize the associated adverse health effect, great cautions should be taken to follow strict hygiene, good agricultural practices(GAPs) and good manufacturing practice (GMPs) during cultivation, harvesting, storage, transportation and marketing (Tournas, 2005).

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

Conclusion

Tomato fruit is the world's most popular home garden fruit and the second most consumed vegetables after potato in the world. Tomato fruit is highly used in stew and sauce making in Nigeria. As most Nigerians cannot do without taking stew daily in their meals and diets, the presence of potentially pathogenic organism in the fruits could lead to great economic and health hazard.

Effective control of bacteria and fungi plant pathogens, as well as contaminants in tomato fruit will bring about a great reduction in its deteriorations, improve the quality of tomato fruits delivered to consumer and also prevent the health hazards accompanied with the consumption of contaminated fruits and vegetables in Nigeria.

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