JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 4441-4449

Microbiological and Chemical Evaluation of Decomposed Open Pollinated Tomato (*Lycopersicon esculentum*) Fruits in Storage

Rowland M.O. Kayode and Anthony J. Afolayan*

[1]Medicinal Plant and Economic Development Research Centre, Department of Botany, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa.

(Received: 20 July 2014; accepted: 10 September 2014)

Tomato spoilage is a major concept of annual revenue wastage in some countries in Africa. In the Northern part of Nigeria, tomato fruits glut has been major problem due to the activities of microorganisms during transportation and storage before sales to the final consumers. This has led to the spoilage of tones of this important fresh produce over decades. Spoilage was initiated in fresh tomato fruit by conditioning them to semi-humid environment and storage at 27±2°C for 14days during which the microbiological and chemical evaluation was examined. Seven organisms were isolated from the decomposed tomato fruits and characterized using genomic DNA extraction, polymerase chain amplification of rRNA and sequence determination. The bacterial isolates were identified on the basis of the 16S rDNA as *Bacillius laterosporus*, *Lactobacillus salivarius* and *Proteus vulgar*is while, yeasts and filamentous fungi were identified on the basis of 26S rDNA as *Saccharomyces cerevisiae, Saccharomycopsis fibuligera, Rhodotorula musilaginosa, Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum*. Three of the identified organisms namely; *Bacillus subtilis*, *Aspergillus niger* and *Saccharomyces cerevisiae* were isolated from the decomposed and control samples. The pH of the decomposing samples showed significant ($p<0.05$) increase with concomitant decrease in titratable acidity after storage for 8 days. There was significant ($p<0.05$) increase in the vitamin K of decomposed tomato (8.76 – 9.45 mg/100g) at the end of 14 days storage when compared control (1.09mg/100g). The carotenoid fractions of the decomposed samples decreased significantly ($p<0.05$) except malvidin which increased significantly (7.99 - 9.02mg/100g) compared with the values of fresh sample (4.96mg/100g) that was used as control.

Key words: Tomato, spoilage, microbes, vitamins, carotenoids.

Tomatoes are considered to be one of the most important fruit in the western diet, based on the amount consumed, mineral compositions and vitamins constituent mostly A and C. The fruits are high in carotenoids mainly beta-carotene; a vitamin A precursor and lycopene that constitute the bright red colour pigment.There are different varieties include *Solanum habrochaites, Solanum sitien, Lycopersicon chilense, Lycopersicon pennelli, Solanumlycopersicum var. cerasiforme* among others. Many hundreds of cultivar varieties with different type and size, either organic, hybrid or genetically modified exist. Most cultivars produce red fruits. However, a number of cultivars with yellow, orange, pink, purple, green, or white colours are also common scenes (Allen, 2008).

In Nigeria, annual total area of one million hectares is reported to be cultivated for tomato production and tomato makes up about 18% of average daily consumption. Nigeria is actually ranked the second largest producer of tomato in Africa and thirteen largest producers in the

* To whom all correspondence should be addressed.
Tel.: +234-08035850545;
E-mail: kayodermosnr@gmail.com

world.Despite being the second largest producer of tomatoes in Africa after Egypt, with the production capacity of 1.2 million metric tons annually, Nigeria is a net importer of tomatoes and tomato pastes spending over N11 billion on it annually, a situation which experts and farmers say is unnecessary and a waste of resources (Daily Trust, 2013). In the past decades in Nigeria, not less than 50% of tomato produced is lost due to attack by spoilage microorganisms during distribution to various parts of the country as a result of poor handling practices and transportation facilities, inadequate marketing structure in addition to poor storage and preservation methods.

Different tomato cultivars are grown in various regions of Africa and all over the world. "*Lycopersicon esculentum* is mass produced among farmers in Western parts of Africa especially in the Northern parts of Nigeria,while, *Trichosanthescucumerina* (Snake Tomato) is commonly grown in the South-Western region.Diversities of microbial genera including Pseudomonas, Shigella, Proteus, Geotrichum, Rhizopus, Mucor and yeasts have been isolated from decomposed tomato.Tomatoes that have been modified using genetic engineering have been developed but only commercially available in few countriessuch as South Africa where it is sometimes imported to few Nigerian supermarkets like Shoprite. A typical lesser known commercially available genetically modified tomato fruits was a variety named the '*FlavrSavr'*, which was engineered to have a longer shelf life (Redenbaugh*et al.*, 1992). Scientists have continued to develop tomatoes with new traits not found in natural crops in order to provide better nutrition, increased resistance to pests and environmental stress. The effort to reduce rate of spoilage in most African countries through conversion to various products such as tomato paste, tomato juice or other products had not been sufficient to stop this high rate of lost.

Therefore, this studyfocuseson the isolation and identification of microorganisms responsible for the spoilage and effects of the microbial activities on the inherent vitamin and carotenoid profiles of tomato (*Lycopersicon esculentum*) fruits in storage.

MATERIALS AND METHODS

Sources and identification of tomato sample

Tomato samples were purchased at four major raw fruits selling points in IlorinTownship, Nigeria. The selling points werelocated at different markets namely;Mandate (MD), Ipata(IP), Ganmo (GM)and Oja-oba(OB). The samples were packaged in a clean sterile polyethylene bags before transferred to the laboratory of the Department of Home Economics and Food Science,University of Ilorin.The tomato sample was identified as *Lycopersicon esculentum* (*Lycopersiconly copersicon*) at the Herbarium of the Department of Plant Biology,University of Ilorin before further analysis.

Initiation of tomato spoilage in anartificially humid environment

Fifteen healthy tomato fruitswere placed on three different porcelain discs inside glass desiccators containing moist cotton wool which created an artificially humid environmentto initiate spoilage. The tomato samples were store for 14 days in the desiccators during which spoilage parameters were monitored.

Isolation of microorganisms

Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were prepared according to the manufacturer's specifications before used for the isolation of bacteria, moulds and yeasts respectively. Samples were taken from the semi humid desiccators and aseptically mashed using sterilized pestle and mortar before used for microbiological analysis. Three sets of serial dilutions from 10^{-1} to 10^{-4} were prepared for the isolation of bacteria, while the dilutions 10^{-1} to 10^{-6} were prepared for the isolation of fungi (Oladoye *et al*., 2014).

Inoculation of samples on growth medium

Each of the diluents (0.1ml) was aseptically placed on nutrient agar, malt extract agar and potato dextrose agar plates in triplicate. Sterile glass spreader was used to spread the inoculums over the surface of the agar plates. After inoculation, the nutrient agar plates were incubated at 37±2°C for 24 hours, while the potato dextrose agar and malt extract agar plates were incubated at 27±2°C for 1-3 days. The growth of moulds and yeasts were assessed every 24 hours.

Evaluation of the microbial population in decomposing tomato fruit

Nutrient Agar, Potato Dextrose Agar and Malt Extract Agar (Oxoid, UK) were used for the enumeration of bacteria, moulds and yeasts respectively in the samples. At the end of 24 hours for bacteria and 48 hours for moulds and yeasts, culture plates with colonies having < 300 colonies for bacteria and between 10 and 130 discrete growth foci for moulds and yeasts were selected for counting. The figures obtained were thereafter expressed as colony forming unit (cfu) per gram (g) of the sample. Thereafter, the mixed cultures were sub-cultured repeatedly to obtain distinct axenic cultures.

Extraction of Deoxyribonucleic acid (DNA) from yeast

The cultures of moulds and yeasts DNAs were extracted according to the methods of Connerton(1994) and Oladoye*et al*. (2014). This was also done twice for each organism. Afterwards, 50µl of 2.5M NH4Ac and 300 µl of propan-2-ol were added to precipitate the DNA. This was followed by centrifugation at 12500 rpm for 5 minutes at room temperature (27^{o}C) before it was dried under vacuum and stored in the cold room at 4^{o}C.

Amplification of mould and yeast genomic DNA

Polymerase Chain Reaction (PCR) reactions were carried out according to James and Jack (2000) and Cocolin*et al*. (2002) as reported by Oladoye*et al*. (2014). The PCR products were electrophoresed in a 1% agarose gel for 3 hours before using primers to amplify the D1 region of the 26S rDNA.

Sequence of purified mould and yeast genomic DNA-PCR products

Each of the purified PCR products (20 µl aliquots) of amplified DNA of the organisms were transferred into PCR tubes labelled appropriate bar codes. The tubes were later sent to MWG Biotech in Germany for DNA sequencing using the amplification primers. Thereafter, the nucleotide sequences of all the isolates were matched with existing sequence databases using the Basic Local Alignment Search Tool (BLAST) to identify the moulds and yeasts isolates based on query coverage and maximum identity in the databases. Identification of the organisms was further confirmed by the use of the DISTANCE TREE matching.

Vitamins profiles of the decomposed tomato fruitsafter storage

The vitamin profiles of the sample was analysedaccording to the modified method of AOAC (2006). After removing the samples from the storage chamber at less than 4^{o}C, they were made to attain room temperature (27±2^{o}C) on the laboratory work bench before careful pressing and complete homogenizationusing a pestle and mortar to avoid ball formation. Then, 0.10gof eachsample was weighed into a 10ml beaker followed by extraction. Afterward, the extract was concentrated to the volume of1.0ml for chromatographic analysis.

Carotenoids profilesof the decomposedtomato fruitsafter storage

The carotenoids extraction was carried out according to the modified method of Shigeaki Takagi as described in: Determination of green leaf carotenoids. Ten grams of each sample was weighed and transferred to a glass stoppard flask and treated with ethanol until the sample was fully soaked and then shaken every hour for the first six hours and then filtered into a pre-cleaned borosilicate beaker. The filtrate (extract) was collected and evaporated to dryness using nitrogen stream. Then, 7.5 ml of acetone was added to 0.1g of each concentrated extract beforeleaving at room temperature (25±2^{o}C) for one hour in the dark. The homogenate was filtered through a 12mm Whatmanfilter paper. The extraction was repeated three times with the same volume of acetone; afterwards the extracts were combined and evaporated under reduced pressure. Theresidue was re-extracted by an equal proportion of the mixture of diethyl ether and petroleum ether. The re-extracted mixture was concentrated to 2ml in an agilent vial for gas chromatography analysis.

Statistical Analysis

Mean and standard error were calculated for all data using Microsoft Excel Software (Microsoft Corporation). While Least Significant Difference test was used to compare differencesbe tween treatments at 95% confidence level of individual variable (Chase andBown, 1997).

RESULTS

The pH which ranged between 4.79 and 4.87 before storage significantly ($p>0.05$) increased to the range between 5.13 and 6.05 on day 8 and

Table 1. pH and Titratable acidity of the tomato sample in storage

Market / Storage	Market Source of Tomato Sample							
	Ipata		Ganmo		Oja-oba		Mandate	
Day	pH	TA (g/100g)	pH	TA (g/100g)	pH	TA (g/100g)	pH	TA (g/100g)
1	4.80^{a}±0.05	0.16^{a}±2.04	4.87^{a}±0.32	0.15^{a}±1.42	4.79^{a}±0.12	0.18^{a}±3.54	4.86^{a}±0.18	0.15^{a}±2.72
3	4.85^{a}±0.17	0.12^{a}±2.89	4.82^{a}±0.05	0.10^{a}±2.25	4.82^{a}±0.15	0.14^{a}±2.05	4.89^{a}±0.21	0.09^{a}±2.89
5	4.87^{a}±0.00	0.09^{a}1.98	4.82^{a}±0.00	0.10^{a}±2.18	4.87^{a}0.05	0.09^{a}±2.43	4.95^{a}0.12	0.07^{b}±3.35
7	5.65^{a}±0.12	0.09^{a}±2.31	4.87^{a}±0.28	0.10^{a}±2.59	5.64^{a}±0.05	0.09^{a}±1.56	4.98^{a}±1.09	0.10^{a}±3.34
9	6.05^{b}±0.06	0.07^{b}±3.24	5.13^{b}±0.36	0.08^{b}±3.43	5.71^{b}±0.05	0.07^{b}±2.87	5.20^{a}±0.00	0.06^{b}±2.86
11	6.21^{b}±0.10	0.07^{b}±3.33	5.96^{b}±0.33	0.08^{a}±4.45	6.00^{b}±0.00	0.07^{b}±2.45	6.50^{b}±0.21	0.05^{b}±2.05
13	6.30^{b}±0.33	0.05^{b}±2.70	6.05^{b}±0.05	0.05^{b}±2.78	6.33^{b}±0.01	0.04^{b}±2.30	6.54^{b}±0.26	0.04^{b}±2.43
15	6.30^{b}±0.02	0.03^{b}±2.65	6.12^{b}±0.29	0.05^{b}±3.02	6.38^{b}±0.00	0.03^{b}±2.40	6.58^{b}±0.10	0.02^{b}±2.81

Means in the column not followed by the same superscripts are significantly ($p<0.05$) different; Values are means of three replicate determinations.

Table 2. Identified microbial isolates from the decomposed tomato samples

Isolate's Code	Maximum Identity	Identified Organism
NAIP 1	99	*Bacillus laterosporus*
NAGM 2	99	*Bacilluslaterosporus*
NAOB 3	100	*Bacilluslaterosporus*
NAMD 4	99	*Bacillus laterosporus*
NAIP 1	100	*Enterobacteraerogenes*
NAGM 2	100	*Enterobacteraerogenes*
NAOB 3	99	*Enterobacteraerogenes*
NAMD 4	100	*Enterobacteraerogenes*
NAOB	98	*Proteus vulgaris*
NAIP 1	99	*Lactobacillus salivarius*
NAGM 2	100	*Lactobacillus salivarius*
NAOB 3	98	*Lactobacillus salivarius*
NAMD 4	100	*Lactobacillus salivarius*
PDAGM	99	*Aspergillusflavus*
PDAIP 1	100	*Aspergillusniger*
PDAGM 2	100	*Aspergillusniger*
PDAOB 3	100	*Aspergillusniger*
PDAMD 4	100	*Aspergillusniger*
PDAIP 1	100	*Fusariumoxysporum*
PDAOB 2	100	*Fusariumoxysporum*
MEAIP 1	100	*Saccharomyces cerevisiae*
MEAGM 2	100	*Saccharomyces cerevisiae*
MEAOB 3	98	*Saccharomyces cerevisiae*
MEAMD 4	100	*Saccharomyces cerevisiae*
MEAIP 1	98	*Saccharomycopsisfibuligera*
MEAGM 2	96	*Saccharomycopsisfibuligera*
MEAIP	100	*Rhodotorulamusilaginosa*

Key: NA=nutrient agar, PDA=potato dextrose agar, MEA=malt extract agar, IP=Ipata market, GM=Ganmo market, OB=Oja-oba market, MD=Mandate market, 1 to 4=Isolates obtained from samples

Table 3. Occurrence of microbial isolates intomatoes purchased from the markets

Microbial isolate	Market Source of Tomato Sample				
	Control	Ipata	Ganmo	Oja-oba	Mandate
Enterobacteraerogenes	-	+	+	+	+
Lactobacillus salivarius	-	+	+	+	+
Bacillus laterosporus	+	+	+	+	+
Proteus vulgaris	-	-	-	+	-
Aspergillusflavus	-	-	+	-	-
Aspergillusniger	+	+	+	+	+
Fusariumoxysporum	-	+	-	+	-
Saccharomyces cerevisiae	+	+	+	+	+
Saccharomycopsisfibuligera	-	+	+	-	-
Rhodotorulamusilaginosa	-	+	-	-	-

Table 4. Vitamin profile of the decomposed tomato samples in storage

Vitamin (mg/100g)	Fresh Tomato (Control)	Decomposed Tomato Samples After Storage Period			
		Ipata	Ganmo	Oja-oba	Mandate
A	$2.04^{a}\pm0.54$	$1.23^{b}\pm1.12$	$1.25^{b}\pm0.97$	$1.24^{b}\pm0.76$	$1.30^{b}\pm1.00$
B1	$6.07^{a}\pm1.54$	$5.44^{b}\pm1.21$	$5.86^{ab}\pm0.76$	$5.57^{b}\pm1.00$	$5.02^{b}\pm0.97$
B2	$1.47^{a}\pm0.54$	$1.11^{b}\pm0.62$	$0.99^{b}\pm0.43$	$1.11^{b}\pm0.51$	$1.11^{b}\pm1.04$
B3	$3.19^{a}\pm1.13$	$2.97^{a}\pm1.27$	$2.85^{b}\pm1.12$	$2.92^{a}\pm2.05$	$2.97^{a}\pm1.07$
B5	$1.41^{a}\pm1.44$	$1.31^{ab}\pm1.32$	$1.39^{ab}\pm1.05$	$1.36^{ab}\pm1.65$	$1.27^{b}\pm1.58$
B6	$2.17^{a}\pm1.44$	$1.89^{b}\pm1.65$	$1.882^{b}\pm0.97$	$1.90^{a}\pm1.25$	$1.88^{b}\pm1.35$
B9	$6.18^{a}\pm1.47$	$4.70^{b}\pm1.32$	$4.70^{b}\pm1.33$	$4.69^{b}\pm1.09$	$4.07^{c}\pm1.22$
C	$20.55^{a}\pm0.87$	$18.44^{b}\pm0.03$	$18.45^{b}\pm1.08$	$17.87^{c}\pm0.00$	$18.01^{b}\pm1.43$
E	$4.31^{a}\pm0.00$	$4.041^{b}\pm2.02$	$4.10^{b}\pm1.59$	$4.06^{b}\pm1.21$	$4.13^{b}\pm0.97$
K	$1.10^{c}\pm1.76$	$9.45^{a}\pm0.98$	$8.76^{b}\pm1.33$	$9.31^{a}\pm1.62$	$8.99^{ab}\pm1.87$

Means in the role not followed by the same superscripts are significantly ($p<0.05$) different; Values are means of three replicate determinations.

Table 5. Carotenoid profile of the decomposed tomato samples in storage

Carotenoid Profiles (mg/100g)	Fresh Tomato (Control)	Decomposed Tomato Samples After Storage Period			
		Ipata	Ganmo	Oja-oba	Mandate
Malvidin	$4.96^{c}\pm1.02$	$8.62^{ab}\pm0.64$	$9.02^{a}\pm2.05$	$7.99^{b}\pm0.75$	$8.78^{ab}\pm0.89$
Beta-Crytoxanthin	$3.65^{a}\pm0.07$	$2.34^{b}\pm0.64$	$2.76^{c}\pm2.10$	$2.58^{bc}\pm1.55$	$2.67^{b}\pm1.70$
Lycopene	$6.23^{a}\pm2.05$	$5.64^{b}\pm1.61$	$5.49^{b}\pm1.23$	$5.67^{b}\pm0.00$	$5.39^{b}\pm1.05$
Carotene	$4.07^{a}\pm2.12$	$3.68^{b}\pm1.18$	$2.88^{c}\pm0.54$	$3.05^{c}\pm0.87$	$2.12^{c}\pm0.33$
Lutein	$1.47^{a}\pm0.94$	$1.08^{b}\pm1.07$	$1.55^{b}\pm1.44$	$1.18^{b}\pm1.33$	$1.86^{ab}\pm1.42$
Zeaxanthin	$4.24^{a}\pm0.00$	$3.37^{ab}\pm1.00$	$2.55^{b}\pm0.02$	$2.93^{b}\pm0.32$	$2.82^{b}\pm0.92$
Antherxanthin	$4.97^{a}\pm1.20$	$2.19^{b}\pm1.46$	$2.92^{b}\pm1.93$	$2.26^{b}\pm2.00$	$2.50^{c}\pm1.32$
Astaxanthin	$2.71^{a}\pm0.26$	$1.07^{b}\pm0.66$	$1.52^{b}\pm0.54$	$1.35^{b}\pm1.98$	$1.12^{b}\pm0.98$
Violaxanthin	$9.15^{a}\pm2.32$	$4.31^{b}\pm2.02$	$4.66^{b}\pm0.12$	$4.71^{b}\pm1.56$	$4.64_{b}\pm1.76$
Neoxanthin	$3.97^{a}\pm1.45$	$3.03^{b}\pm0.94$	$3.07^{b}\pm0.76$	$3.00^{b}\pm1.00$	$3.65^{b}\pm0.93$

Means in the role not followed by the same superscripts are significantly ($p<0.05$) different; Values are means of three replicate determinations.

steadily increased to between 6.12 and 6.58 on day 14 when the experiment was terminated. There was significant($p<0.05$) decrease in titratable acidity of the samples after storage for 8 days (Table 1). Figure 1 shows the result of bacterial count of the decomposed tomato samples during the storage period. The sample purchased from Ipata market (IPT)showed highest loads of viable bacterial count ($51x10^6 cfu^{-g}$).This value was significantly ($p<0.05$) different from the results of samples collected from the other markets.

Table 2 shows the microbial isolates identified from the decomposed tomato samples. Based on the query coverage and maximum identity in the databases, nine microbial species were identified. The isolated organisms consist of three bacterial species: *Bacillus laterosporus* (NAIP 1, NAGM 2, NAOB 3 and NAMD 4), *Proteus vulgaris* (NAOB), *Streptococcus lactis* (NAIP 1, NAGM 2, NAOB 3 and NAMD 4), three species of yeast: *Saccharomyces cerevisiae* (MAEIP 1, MEAGM 2, MEAOB 3, MEAMD 4), *Saccharomycopsis fibuligera* (MEAIP 1 and MEAGM 2) and *Rhodotorula musilaginosa* (MEAIP),and three filamentous fungi: *Aspergillus flavus*(PDAGM), *Aspergillusniger*(PDAIP 1, PDAGM 2, PDAOB 3 and PDAMD 4), and *Fusarium oxysporum*(PDAIP 1 and PDAOB 2) (Table 2).The isolates from the spoilt tomatoes were identified as *Bacillus megaterium*, *Listeria monocytogens*, *Bacillus laterosporus*, *Morganella morganii*, *Aspergillus niger*, *Aspergillus flavus*, *Absidia corymbifera* and *Fusarium oxysporum* (Ibrahim *et al*., 2011).

Table 3 indicated the frequency of occurrence ofthe various microbial isolates in the tomato samples. *Bacillus laterosporus*, *Aspergillus niger* and *Saccharomyces cerevisiae* were isolated from both decomposed and control tomato samples. While, *Enterobacter aerogenes* and *Lactobacillus salivarius* were isolated from thediverse decomposed samples(Table 3).

Table 4 shows the effect of deterioration on vitamin profiles of the open pollinatedtomatoes in storage. There was significant decreased inthe vitamins components of the decomposed samples except vitamin K (range from 8.76 – 9.45 mg/100g) which significantly increased ($p>0.05$) compared with fresh tomato (control) sample (1.09mg/100g). The presence of some microbial speciesin the decomposed samples isindicative of common contaminants of tomato fruits and some of the microbial strains may have been responsible for the significant ($p>0.05$) increase in vitamin K of the decomposed samples as observed in Table 4.Table 5 shows effect of spoilage on thecarotenoid profiles of the open pollinated tomatosamples in storage. At the end of storage period, the carotenoid fractions of the spoiled tomatoes samples significantly ($p<0.05$) decreased, except malvidin (7.799 – 9.02 mg/100g) that increased significantly ($p>0.05$) compared with the values (4.96mg/100g) of fresh tomato sample (control).

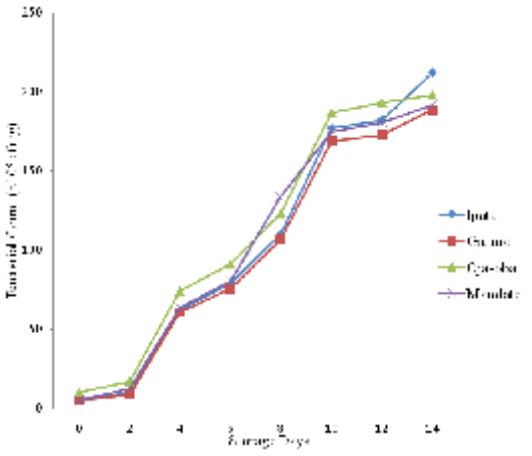

Fig. 1. Total bacterial count of decomposed tomato samples in storage

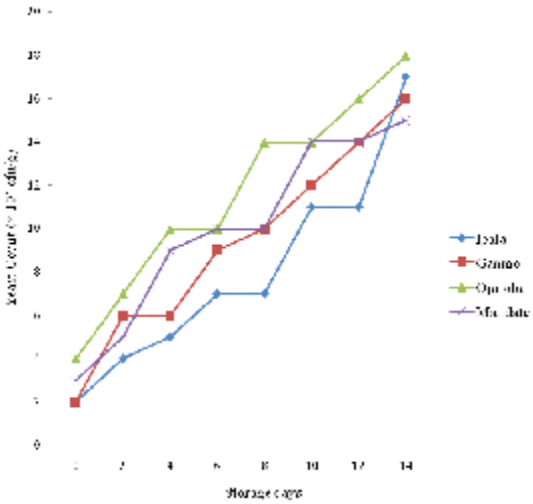

Fig. 2. Yeast count of decomposed tomato samples in storage

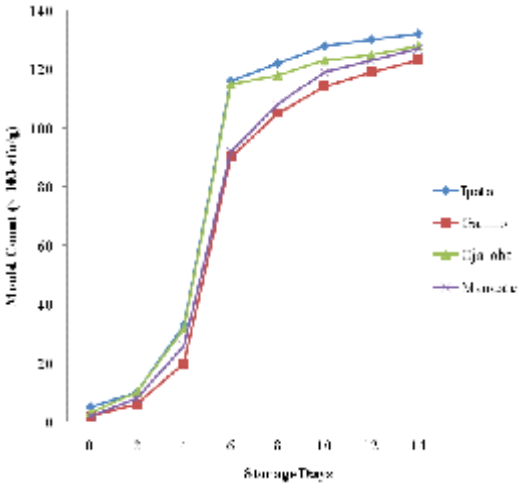

Fig. 3. Mould count of decomposed tomato samples in storage

DISCUSSION

The significant ($p<0.05$) decrease in titratable acidity of the samples after storage for 8 days as indicated in Table 1may be attributed to fruit senescence during storage (El-Ghaouth*et al*., 1991; Garcia *et al*., 1998a). A change in pH may also be associated with some factors such as effect of treatment on the biochemical condition of the fruit, slow rate of respiration and metabolic activity (Jitareerat*et al*., 2007). The artificially semi humid environment that was created in the glass container in which the samples were stored to initiate spoilage of the tomato may have effectively speed up fruit senescence and also created favourable environmental conditions that speed up the growth of microorganisms in the tomato during storage, which resulted in increased pH with concomitant decrease in acidity of the samples. The increase noted in pH may also be attributed to the breakup of acids with respiration during storage (Pesis*et al*., 1999). Increased activity of citric acid during ripening or reduction in acidity may be due to their conversion into simple sugars and their further utilization in the metabolic processes of the fruit (Abbasi*et al*., 2009). Acidity is an important property in assessing the quality and acceptability of fruits. As such,an extremely low or highacid content in fruits depicts bad quality and not recommended for good fruits (Jiang *et al*., 2004). The high loads of microbial count and diversities of microbial species isolated from the decomposing samples agreed with the reports of Brackett (1992) who established the presence of microorganisms in decomposed tomato fruits at different pH levels such as bacteria (pH 6.0-8.0), yeasts (pH 4.5-6.0 and filamentous fungi (pH 3.5-4.0). The presence of yeasts in the spoilt sample is in agreement with past findings. Garza*et al*.(1994) isolated three heat resistance strains of *Saccharomyces cerevisiae* from spoiled peach puree at pH 4 and 7.*Pichiaanomala*, *Hyphopichiaburtonii* and *Saccharomycopsisfibuligera* are spoilage yeasts that have been isolated from par-baked breads packaged under modified atmosphere; these yeasts are causative agents of chalk mould defects on foods (Deschuyffeleer*et al*.,2011).*Aspergillusniger*, *Aspergillusflavus* and *Rhizopusstolonifer* have been associated with the spoilage of bread (Okoko and Ogbomo, 2010), decomposed fruits and vegetables (Geetha*et al*. 2011). Ibrahim *et al*. (2011) isolated some bacterial and fungal species that are potential producers of volatile compounds from spoilt tomatoes; the authors identifiedthe organismsas *Bacillus megaterium*, *Listeria monocytogens*, *Bacillus laterosporus*, *Morganella morganii*,*Aspergillus niger*, *Aspergillus flavus*, *Absidia corymbifera and Fusarium oxysporum* (Ibrahim *et al*., 2011)

There significant increasein vitamin K of decomposed compared with control sample The presence of some microbial species in the decomposed samples is indicative of common contaminants of tomato fruits and some of the microbial strains may have been responsible for the significant ($p>0.05$) increase in vitamin K of the decomposed samples as observed in Table 4.Gorbach (1996) reported the capacity of some intestinal microflora synthesizing vitamin K as a cofactor in the production of important clotting factors such as prothrombin and formation of bones. The author reiterated the role played by the enzymes synthesized by some intestinal bacteria in the metabolism of numerous vitamins (Gorbach, 1996). The significant ($p>0.05$) increase in malvidin constituent of the spoilt tomatoes may be attributed to the quality and composition of inherent acids of the tomato fruits which may be precursors of this colour pigment. This observation is in agreement with the findings of Ribereau-Gayon*et al*. (2006) who reported some acids as precursors of volatile phenolic compounds

produced by microorganisms. Malvidin-3-monoglucoside is a major compound that constitutes the red pigment in many fruits such as grape and tomato. The compound is pH sensitive, unstable and changes from bright red at low pH because of the flavyliumcation to murky blue at high pH because of the quinoidal base which is uncharged (Ribereau-Gayon*et al*., 2006).

CONCLUSION

The artificially humid environment used for storage of the open pollinated tomatoes encouraged rapid spoilage at room temperature (27±2°C) with concomitant increase in the microbial loads, pH, and titratable acidity. It is evidenced from the results that different species of microorganisms group namely; bacteria, yeasts and filamentous fungi are common contaminants responsible for spoilage of open pollinated tomato samples. The markets from where the samples were purchased did not show clear disparity in pH, titratable acidity and microbial loads as evidenced by unique pattern of spoilage and effects on vitamins and carotenoids composition of the decomposed tomatoes. However, *Proteus vulgaris*, *Aspergillus flavus* and *Rhodotorula musilaginosa* had the least frequency of occurrence among the markets from where the samples were obtained. The identities of the organism (s) responsible for the significant ($p>0.05$) increase in vitamin K in the spoilt samples may be of interest to researchers and should be investigated; since the vitamin has earlier been reported useful as a cofactor in the production of prothrombin and formation of new bones.

REFERENCES

1. Allen, A. "A Passion for Tomatoes". *Smithsonian magazine*, 2008; 1-4.
2. Daily Trust.Local Tomatoes Wasting as N11.7 Billion Foreign is Imported. Nigeria daily newspaper 11th July, 2013.
3. Redenbaugh, K., Hiatt, W.R., Martineau, B., Kramer, K., Sheehy, S., Sanders, R.A., Houck, C. and Emlay, D. Safety assessment of genetically engineered fruits and vegetables: a case study of the FlavrSavr ä tomato. CRC Press, Inc., Boca Raton, FL. 1992.
4. Oladoye, C.O., Connerton I. F.,Kayode, R.M.O., and Omojasola, P.F.2014. Investigation on the activities of yeasts in the post-harvest spoilage of sweet potato. *Ethiopian J. Environ. Stud. Mangt*. 2014; **7**(4): 162-180.
5. Connerton, I. Preparation of DNA from *Saccharomyces cerevisiae*.An Experimental Protocol. 1994.
6. James, B., and Jack, H.R. PCR Primers that amplify fungi rRNA genes from environmental samples. *Applied and Environmental Microbiol.* 2000; **66**:4356-4360.
7. Cocolin, L., Aggio, D., Manzano, M., Cantoni, C., and Comi, G. 2002. An application of PCR-DGGE analysis to profile the yeast populations in raw milk.*International Dairy J*. 2002; **12**: 407-411.
8. AOAC. Official Methods of Analysis of Association of Official Analytical Chemists, 15TH ed., AOAC, Arlington, Virginia, USA. 2006.
9. Chase, W. and Bown, F. 1997. General Statistics. 3rd Ed John Willey and Sons. Inc. NY. 1997; 491-523.
10. El-Ghaouth, A., Arul, J., Ponnampalam, R., and Boulet, M. Chitosan coating effect on storability and quality of fresh strawberries. *J. Food Sci*. 1991; **56**: 1618-31.
11. Garcia, M.A., Martino, M.N., and Zaritzky, N.E. Plasticized starch-based coatings to improve strawberry quality and stability. *Jour. Agric. Food Chem*. 1998a; **46**: 3758-3767.
12. Jitareerat, P., Paumchai, S., and Kanlayanarat, S. Effect of chitosan on ripening enzymatic activity, and disease development in mango (*Mangiferaindica*) fruit. New Zealand *Jour. Crop Hort. Sci*. 2007; **35**: 211-218.
13. Pesis, E., Dvir, O., Feygenberg, O., Arie, R.B., and Ackerman, M. Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of litchi fruit. *Postharvest Biol. Technol*. 1999; **26**: 157-165.
14. Abbasi, N.A., Iqbal, Z., Maqbool, M., and Hafiz, I.A. Postharvest quality of mango (*MangiferaIndica*) fruit as affected by chitosan coating. *Pakistan J. Bot*., 2009; **41**(1): 343-357.
15. Jiang, Y., Li, J., and Jiang, W. Effect of Chitosan coating on shelf life of cold-stored Litchi fruit at ambient temperature LWT. 2004; 38:757-761.
16. Brackett, J. Shelf stability and safety of fresh produce as influenced by sanitation. 1992.
17. Garza. S., Teixidó, J.A., Sanchis, V., Viñas, I., and Condón, S.Heat resistance of *Saccharomyces cerevisiae* strains isolated from spoiled peach puree.*Internat. J. Food Microbiol*. 1994; **23**(2): 209-13.
18. Deschuyffeleer, N., Audenaert, K., Samapundo, S., Ameye, S., Eeckhout, M., and Devlieghere,

F. Identification and characterization of yeasts causing chalk mould defects on par-baked bread.*Food Microbiol*.2011; **28** (5): 1019-1027.

19. Okoko, F.J., and Ogbomo, O. Amylolytic Properties of Fungi Associated with Spoilage in Bread. *Contin. J. Microbiol*. 2010;4: 1 – 7.
20. Geetha, K.N., Jeyaprakash, K., and Nagaraja, Y.P. Isolation, screening of *Aspergillusflavus* and its production parameters for ±-amylase under solid state fermentation. *Journal of Applied and Natural Sci.* 2011; **3**(2): 268-273.
21. Ibrahim, A.D., Musa, K., Sani, A., Aliero, A.A., and Yusuf, B. S. Microorganisms associated with the production of volatile compounds in spoilt tomatoes. *Research in Biotechn.*2011; 2 (2): 82-89.
22. Gorbach, S.L. Microbiology of the Gastrointestinal Tract. In: Baron S, editor. Med.Microbiol. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston, 1996 ;Chapt. 95. *http://www.ncbi.nlm.nih.gov/books/NBK7670/*
23. Ribereau-Gayon*et al*. The Chemistry of Wine, Stabilization and Treatments. Wiley: West Sussex, Engl. 2006.Handbook of Enology: Vol. 2.