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Microorganisms associated with the production of volatile compounds in spoilt tomatoes

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The microorganisms associated with the production of volatile compounds in spoilt tomatoes has been isolated and identified. The mean heterotrophic bacterial count recorded range between 8.7 x 10^6 to 9.1 x 10^6 cfu/g. The highest value was obtained from Bado and the least from Dundaye market. The organisms isolated and identified include three species of bacteria and four fungal species. These include *Bacillus, Listeria, Morganella, Aspergillus, Absidia,* and *Fusarium.* GC-MS analysis revealed the presence of eight compounds dominated by 9, 12 – octadecadienoic acid (31.08%), 2, 3 – Butandiol (28.79%), and n-Hexadecanoic acid (19.85%). This study suggests that spoilt tomatoes could be exploited for the biogeneration of some volatile compounds that could provide baseline knowledge for curbing post harvest lost.

Keywords: *Aspergillus, Bacillus, Fusarium*, spoilt tomatoes, volatile compounds.

Tomato (*Lycopersicum esculentum* Mill.) is grown in many parts of Nigeria both as wet and dry season crops. Although most tomato production is at a small scale in backyard gardens, there are a few commercial fields (Wokoma, 2008). In Northern Nigeria where dry season tomato is grown under furrow irrigation, foliar diseases are less. Consequently, tomato crops grown in Northern Nigeria have higher yields and better quality fruits. Wilts caused by *Fusarium oxysporum*; *Lycopersici, Sclerotium rolfsii* and *Pseudomonas solanacearum* are the important diseases of tomato in the Savanna and forest zones of Nigeria (Erinle, 1986). Among the wilt diseases, *Fusarium* wilt is most prevalent, particularly among the local varieties, which are very susceptible. Wilts of tomato are also prevalent and damaging to tomato in other countries (El-Abyad *et al*., 1993; El-Shanshoury *et al*., 1996; Quasem, 1996; Sharma and Norwak, 1998; De Cal *et al*., 1999).

According to Ghosh (2009) fungi were the source of spoilage in most of tomatoes samples accessed rather than bacteria. Among the fungi, it was found that *Aspergillus niger* and *Fusarium* were found in most of the spoiled samples with a few samples containing *Penicillium sp*.

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Tomato flavour is a combination of taste and aroma sensations. The four (4) taste, sweet, sour, salty and bitter that are perceived by certain regions of the tongue, while volatiles are perceived by olfactory nerve endings of the nose (Acree, 1993). The pleasant sweet sour taste of tomatoes is mainly due to the sugar and organic acid contents. Over 400 volatiles determined in tomatoes and 30 have proved to be the most important compounds contributing to the aroma of tomatoes (Yilmaz, 2001). The characteristic tomato flavour is produced by the complex interaction of the volatiles and non volatile components (Petro-turza, 1987; Bultery, 1993). This research aimed at identifying microorganisms associated with spoilage of tomatoes and to identify the volatile compounds in spoilt tomatoes.

MATERIALS AND METHODS

Sample Collection

Ten spoilt and two of healthy tomatoes sample were collected from Kasuwan Daji, Dundaye, Bado, Mabera and sokoto central market all in Sokoto metropolis. Two samples were collected from each of the locations into clean polythene bags and were then brought to the laboratory for the analysis.

Identification of Bacteria and Fungi

The bacteria isolate were identified following series of biochemical test as described by Holt *et al.* (1994). Fungal colonies were studied by using Lactophenol Cotton Blue Mount (LPCB) as described by Oyeleke and Manga (2008).

Proximate Composition

Samples were analyzed in triplicate for proximate composition in accordance with the Official Methods of the Association of Official Analytical Chemists (AOAC, 1995). Ash was determined by incinerating two grams (2g) each of spoilt and healthy tomatoes at 550^{O}C in lenton furnaces (England) over night. Fiber was determined by drying two gram (2g) each of spoilt and healthy tomatoes over night at 105^{0}C in the oven (Gallenhamp Oven BS) and incinerated at 550^{0}C for 90 minutes in lenton furnaces (England). Moisture Content was determined by drying two gram (2g) each of spoilt and healthy tomatoes over night at 105^{0}C in the oven (Gallenhamp Oven BS). Crude lipid was determined by weighing a known weight of the dried sample into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted for 15 hours. It was drained into an empty flask, earlier weighed and designated W_1. It was placed in an oven to allow the N-hexane to evaporate in the oven (Gallenhamp Oven BS). Protein (% N * 6.25) was determined by the Micro-kjeldahl Method. Soluble carbohydrate is not determined directly but obtained as a difference between crude protein and the sum of ash, protein, crude lipid and crude fiber.

Mineral Content

Analyses of minerals of spoilt and healthy tomatoes were done in triplicate according to methods described by Walinga *et al.* (1989); Black *et al.* (1965). The investigated minerals include potassium, sodium, calcium, and magnesium. Potassium, sodium was determined using flame photometer (Corning 400 Essex, England), determination of calcium and magnesium was done by ethylene diamine tetra acetic acid (EDTA) Titration Method.

Extraction of volatile Metabolites

Volatile compounds were extracted using general purpose solvent Parliment (1997) as described by Ibrahim et al. (2011). Extraction of volatile compounds was done by direct solvent extraction method. Two gram of spoilt mango fruits and healthy ripe mango fruits was weighed into a bottle and saturated

with 20ml of diethyl ether. It was allowed to stand at room temperature for 24 hours, filtered using Whatman No. 1 filter Paper and the filtrate was collected in a sterile bottle, closed tightly before the GC-MS analysis.

Gas chromatography mass spectrometry (GC-MS) analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250 ^{0}C for 3min by using an inlet of 0.75mm i.d to minimize peak broadening. Chromatographic separations were performed by using DB-WAX analytical column 30 m 0.25 mm, 0.25mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/Min. The oven temperature was programmed at 60 ^{o}C for 5min, followed by an increase (held for 5 min), and finally at 10^{0}C/min to 280 ^{o}C (held for 10min). The temperature of the FID was set to 250 ^{o}C. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200 ^{o}C, ionization voltage of 70 eV and mass scan range of m/z 23- 450 at 2.76 scans/s.

Identification and quantification of volatile Metabolites

The chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). According to the method of (Wanakhachornkrai and Lertsiri, 2003) approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results are presented as the peak area normalized (%).

RESULTS

The results of the heterotrophic bacteria count from spoilt tomatoes were assessed and it ranges between 8.7 x 10^6 to 9.1 x 10^6 cfu/g for the samples. The highest and least count was recorded for samples from bado and dundaye market (Table 1).

Table 1: Heterotrophic bacteria counts obtained from spoilt tomatoes fruits

Collection locality	Mean Plate count (cfu/g)
Kasuwan Daji	8.9 x 10^6
Dundaye market	8.7 x 10^6
Central market	8.9 x 10^6
Mabera market	8.8 x 10^6
Bado market	9.1 x 10^6

The values are mean of two replicates.

Microorganisms isolated and identified from the spoilt tomatoes sample in Sokoto metropolis are presented in Table 2. The results revealed the presence of *Bacillus megaterium, Listeria monocytogens, Bacillus laterosporus, Morganella morganii, Aspergillus niger, Aspergillus flavus, Absidia corymbifera* and *Fusarium oxysporum*. These revealed that both bacteria and fungi are responsible for the spoilage of tomatoes. Table 3 shows that *A. niger* and *A. flavus* had the highest occurrence and the least was *Absidia corymbifera*.

The proximate analysis of tomatoes samples (healthy and spoilt), showed no difference in moisture, ash and fiber content but there are difference in lipid, nitrogen, protein and carbohydrates. The result of proximate composition is presented in Table 4. The mineral content of both healthy and spoilt tomatoes sample is presented in Table 5. The spoilt samples have high content of nitrogen, potassium and magnesium. The calcium content was unchanged in the healthy and spoilt tomatoes.

The result of GC-MS analysis of spoilt tomatoes samples evaluated is presented in Table 6. The result revealed the presence of 8 – volatiles compounds dominated by 9, 12 – octadecadienoic acid (31.08%), 2, 3 – Butandiol (28.79%), and n-Hexadecanoic acid (19.85%).

Table 2: Colonial and morphological characteristics of fungal species

Colonial Characteristics on SDA	Morphological Characteristics	Probable Identity
Colony floccose, light grayish, growing rapidly, covering the whole petridish within one week.	Smooth walled, with an occasional septum, often terminating in a large sporangium. Rhizoids borne on stolon, in frequently branched.	*Absidia corymbifera*
Colonies on SDA attaining a diameter of 3-5cm within 7 days, consisting of a dense felt of dark green conideiophores intermixed with aerial hyphae brearing condiophores	Conidial heads typically radiate, later splitting into several loose columns, conidiophores hyaline, coarsely roughened.	*Aspergillus flavus*
While to yellow basal felt with a dense layer of dark brown to black condiophores.	Septate branching hyphae. Conidiophores non septate. Conidia round, smooth and in clain.	*Apspergillus niger*
Pale (whitish to cream), brownish, pink, reddish, violet, aerial mycelium sparse.	Micro-conidia septate, borne on laterial short branched conidiophores shape & size ovoid – ellipsoidal to cylindrical.	*Fusarium oxysporum*

Table 3: Frequency of occurrence of fungal organisms

Plate number	***Aspergillus niger***	***A. flavus***	***Absidia corymbefera***	***Fusarium oxysporum***
1	+	+	-	+
2	+	+	-	+
3	+	+	+	+
4	+	+	+	-
5	+	+	-	+
6	+	+	+	+
7	+	+	-	+
8	+	+	-	+
9	+	+	-	+
10	+	+	+	+
Occurrence	**10**	**10**	**4**	**9**

Table 4: Proximate Composition of tomatoes sample (healthy and spoilt)

Sample	Moisture	Ash	Lipid	Fiber	Nitrogen	Protein	CHO
Healthy	90	5	4	Trace	0.095	0.592	90.408
Spoilt	90	5	4.5	Trace	0.14	0.875	89.625

Table 5: Mineral Composition of tomatoes sample (fresh and spoilt)

Sample	Na	K	Ca	Mg
Healthy	1.8	2.2	0.06	0.06
Spoilt	3.4	2.6	0.06	0.11

Table 6: Volatiles Composition of spoilt tomatoes

RT^{-1} (min)	Compounds	Area normalized (%)
3.974	3, 3 – Butanediol {R – (R, R)}	28.79
23.922	Butylated hydroxytoluene	3.52
28.067	n – Hexadecanoic acid	19.85
29.240	9,12 -Octadecadienoic acid (z,z)	31.08
30.582	9 – Octadecenamide (z)	6.57
31.133	(z) 6 (z) 9 – pentadecadien – 1 – ol	3.07
32.285	Linoleic acid Chloride	1.24
33.593	4, 22 – cholestadien – 3 – one	5.88

DISCUSSION

Microbial loads and kinds from the various spoilt tomato fruits sold in Sokoto Metropolis had been identified and the results revealed the presence of both bacteria and fungi. In the characterization of Bacteria, all the samples obtained had very high counts. The bacteria isolated are; *Bacillus, laterosporous, Bacillus megaterium, Morganella morganii and Listeria monocytogens,* with *Listeria monocytogens* being pathogenic contradicts the work of Goldoni *et al*. (1992) who isolated *Bacillus coagulans, B. stearothermophilus,* and *lactic acid bacteria.* The fungal identified are *Aspergillus niger, A. flavus, Absidia corymbefera, and Fusarium oxysporum.* Similar observation was made by Ghosh (2009). *Bacillus spp, Aspergillus spp* and *Fusarium spp* are the major microorganisms responsible for the spoilage of tomatoes. This is not surprising because of the water content of the tomato fruits which support the growth of fungi with *Bacillus spp* probably emerging at a later stage of spoilage. Advisory guidelines for microbiological quality have suggested that satisfactory food products should contain not more than 10^5 cfu/g of starter organisms, less than 1 coliform/g as evident from this research the

highest bacterial load was found to be 8.9 x 10^6. With the guidelines, it makes the consumption of spoilt tomatoes fruits unwholesome for human or animals.

Aspergillus species and *Fusarium* species being the major cause of spoilage in tomato are a source of potent mycotoxins which exhibits a wide spectrum of diseases which can even be fatal. *A. niger* is a source of ochratoxin while *Fusarium* produces *Trichothecenes*. Both these toxins have detrimental effect in humans which can even be fatal. *Ochratoxin* is considered to be a potent carcinogen and suspected of playing a role in the etiology of esophageal cancer and Balkan endemic nephrotoxicity. Hence spoiled tomatoes should not be consumed under any circumstances and should not be fed to cattles as well (Ghosh, 2009). It is therefore important that, both the tomato producers and the marketers take the necessary precautions in preventing contamination of the fruits to reduce possible contamination and hence reduce the risk of mycotoxins, enterotoxins and other metabolites that are deleterious to human health.

In the proximate analysis, of the two sample (healthy and spoilt) the two results reveals that, there is no difference in moisture (90%), Ash (7%), fiber (Trace) but there is difference in lipid (4%), (4.5%); protein (0.875%), (0.592%); carbohydrate (CHO) (90.408%), (89.625%) for healthy and spoilt samples respectively. Lipid, nitrogen, and protein are higher in spoilt sample. The variation could be attributed to microbial biomass in the spoilt samples. Carbohydrate (CHO) content is higher in fresh samples; the variation could be attributed to the facts that these organisms might have selectively utilized some carbohydrate content in the tomato to produce some volatile compounds which may be responsible for the flavor in spoilt tomatoes.

The difference in mineral content could probably due to the fact that these minerals are important components of the biomolecules of the spoilage organisms.

The GC-MS analysis revealed the presence of eight (8) volatile compounds 2, 3 – Butanediol, Butylated Hydroxytoluence, n-Hexadecanoic acid, 9-octadecenamide, (z) (6) (z) 9 – pentadecadien-1-ol, linolic acid chloride and 4, 22 – cholestadien – 3 – one; 2, 3 – Buanediol is a colorless and odorless liquid chemical with a very high boiling point and low freezing point it is largely used as a monomer for polymer synthesis. The commercial applications of this diol are not limited to the manufacture of bactadience or to its use as an antifreeze agent (Perego *et al.,* 2003). It is known as 2, 3 – butylenes glycol, a valuable chemical feedstock because of its application as solvent, a liquid fuel, and as a precursor of many sysnthetic polymers and resins. 2, 3 – butandiol compares favorably with ethanol and methanol for use as a liquid fuel and fuel additives. Dehydration yields 1, 3 – butanediene which is the starting material for synthetic rubber and is also an important monomer in the polymer industry. (Saha and Bothast, 1999). Interest in microbial production of 2, 3 – butanediol has been increasing recently due to the large number of industrial applications of this product (Perego *et al.,* 2003). Microbially produced 2, 3 – butandiol can be converted into 1, 3 – butandiene, a feedstock chemical currently supplied by the petrochemical industry. 1, 3 – butadiene can, in turn be utilized in the manufacture of plastics, pharmaceuticals and synthetic rubber (Mallonee and speckman, 1988). The microorganism that has so far been used in the production of 2, 3 butanediol is *Klebsiella pneumoniae.*

Another volatile compound detected is 4, 22 – Cholestadien – 3 – one, 9, 12 – octadecanoic acid and 9 – octadecenamide are colourless and have been reported as being

important contributors to fresh tomato aroma. Linoleic acid chloride, (z) 6 (z) 9 – pentadecadien-1-ol and Butylated Hydroxytoluene are responsible for medicinal and chalky odor perceived in the spoilt tomatoes. And n-Hexadecanoic acid is a good solvent to many substrates.

Conclusion

From the result obtained in this research, it can be concluded that the microorganisms isolated from the spoilt tomatoes sold in Sokoto metropolis are *Bacillus laterosporus, Bacillus megaterium, Morganella morganii, Listeria monocytogens, Aspergillus niger, A. flavus, Absidia corymbefera* and *Fusarium oxysporum.* High bacteria count were encountered, these organisms causes problems by producing toxins such as; *mycotoxins* and *Aspergillosis* and makes spoilt tomatoes not fit for human consumption but can serve as a substrate for the biogeneration of important volatiles compounds.

It is therefore a great concern for further research on biogeneration of volatile compounds from spoilt tomato, which is regarded as waste can be converted to wealth therefore meeting the adage "from waste to wealth" or "waste are no longer wasted". Therefore, waste tomatoes are a substrate for biogeneration of volatiles. It is also concluded that the carbohydrate content of tomatoes appear to be the major component utilized by spoilage organisms to produce off-flavor compounds. Therefore, studies need to be carried out to ascertain the exact volatile produced by each of the isolated organisms.

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