Original Research Article

Isolation and identification of microbial deteriogens of fresh tomatoes stored at ambient temperature

Abstract

This study investigated ~~the~~ microorganisms associated with the deterioration of fresh tomato, *Lycopersicum esculentum,* stored at ambient temperature. A total of 60 tomatoes obtained from three different markets in Owerri metropolis were sampled and a total of 134 bacteria isolates and 140 fungal isolates were encountered. Bacteria isolated and identified were *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp and Salmonella spp.* The most prevalent bacteria isolate was *Bacillus subtilis* with 52.24% while *Salmonella spp* was the least prevalent isolate with 1.49%. The fungal isolates were *Fusarium spp, Aspergillus niger, Rhizopus stolonifer, Saccharomyces spp.* Whereas *Aspergillus niger* was the most prevalent with 60.71% and was found in tomato sample from all the market, *Rhizopus stolonifer* had the least prevalence of 0.71%. The presence of toxin producing fungi, *Aspergillus niger and Fusarium sp* which are capable of causing food poisoning as well as some bacterial isolates, raises concern over public health risks that may be associated with the consumption of unwholesome tomato.

Introduction

Tomato (*Lycopersicum esculentum)* is a berry plant in the *solanaceae* family. It is a short lived perennial plant grown as an annual plant, typically growing about 3-5m approximately in height. Tomatoes are brightly red coloured berry which are eaten fresh in salads or processed and can be stewed, fried, baked and also used to produce soup, or used as juice. Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well balanced diet which is rich in vitamins such as vitamin, B, C, and E .carbohydrates such as fructose and glucose; and trace elements like iron, copper, zinc, and dietary fiber(Faostat,2010).

Tomatoes grow best in light, free draining, and fertile loam soil with pH of 5 – 7. However tomatoes can be grown in a variety of soils (Naika e*t al*., 2005) Regarding fertilizer requirements, tomatoes require an abundance of the three major elements namely, nitrogen, phosphorus, and potassium (Ohio,2009). The deep red colouration of ripened tomato is due to the presence of LYCOPENE, a form of B-carotenoid pigment and a powerful antioxidant that help to protect against prostate cancer, cardiovascular disease and diabetes, thus there is a great demand for tomatoes by consumer as a result of their healthy, tasty, convenient, and fresh appeal

(Passam *et al,* 2007). Tomato, however, have serious challenges to their existence. These include changes in climatic condition, pest, bacterial and fungal attack and over the years there has been an increase in the need to identify and isolate the microorganism associated with their deterioration.

deterioration refers to any change in ~~the condition of~~ food in which the food becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture(Berdegue *et al,* 2005) . Numerous microbial defects (Signs and Symptoms) of tomatoes are characterized by the type of micro-organism responsible for the deterioration, in the process of infection in the case of fungal invasion follows the development of fungal penetrating structure. The colonization process involves the ability of the micro-organism to establish itself within the produce. Susceptibility of tomato to microbial colonization is due to its differential chemical composition such as high level of sugar, low pH (4.9-6.5) and its high water activity (p>0.99) which favors the growth of micro-organism in tomato is recognized as a source of potential health hazard to man and animals, this is due to their production of toxins which are capable of causing disease like respiratory infection, meningitis, gastroenteritis, diarrhoea in man following ingestion (Beuchat *et al,* 2006).

Tomatoes ~~were~~ chosen for this study because they are referred to as ready-to-eat food since they are minimally processed and many people take tomatoes raw directly or via meals of salad usually served cold. Microbial spoilage and contaminating pathogens on this product poses a serious problem in food safety . The ~~centre~~ of ~~disease control~~ and ~~prevention~~ (CDC) estimates that there are 76 million cases of food borne illness every year. Outbreaks with identified etiology are predominantly of microbial origins (Bihn *et al*, 2006), so that studying about the microbial ecosystem of fresh raw and spoilt tomato is necessary.

Materials and methods

Materials used for these analyses were standard microbiological materials and were ~~gotten~~ from the microbiology laboratory except the tomato samples that were purchased from different markets.

Samples collection

Fresh tomatoes were obtained from the major markets in owerri metropolis, which include Ekeonunwa, Relief, and student market. A total of 80 tomatoes, 20 tomatoes from each market were sampled. The ripened tomato fruits selected were fresh, undamaged, firm and healthy. The sample were taken to the laboratory, washed and drained of water. The fruit samples were kept from dust and insects at room temperature for up to 14 days to undergo a natural process of deterioration before being used for study.

Processing of fresh spoilt tomatoes stored at ambient temperature

The tomatoes fruit samples were ground using a sterile mortar and pestle. A homogenate of each sample was made by blending one gram in 9ml of sterile water and shaking them together. Serial dilution of

up to 10^5 of the homogenate was made in sterile test tubes.

Enumeration of microbial load

A ten-fold serial dilution of each of the samples was carried out. Spread plate technique was employed by inoculating 0.1ml aliquot aseptically from the 10^{-3} and $10^{-}4$ dilutions onto nutrient and MacConkey agar plates for enumeration of bacteria and sabouraud dextrose agar for fungi count. The agar plates were incubated at 37^{o}C for 24-48hours for bacterial count and at 25^{o}C - 28^{0}C for 5-8 days for fungal count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial and fungal counts were recorded as colony forming unit per ml (cfu/ml).

Purification (subculture) of bacterial isolates

Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The subculture plates were incubated at 37°C for 24 hours to 48 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C which were stored at 4^{0}C and used subsequently for microscopic characterization and biochemical analyses.

Purification of fungal isolates

Colonies from the primary plates were aseptically picked with a sterile inoculation needle and transferred onto a freshly prepared sterile SDA plate with a streaking method and incubated for 5- 7days at 25^{0}C-28^{0}C. Discrete colonies were aseptically transferred and stocked on slant and incubated for another 5 days at25^{0}C-28^{0}C. Pure colonies were stored in the refrigerator at 10^{0}C-15^{0}Cuntil needed for characterization and identification.

Characterization and identification of bacterial isolates

All bacterial isolates were characterized and identified based on their cultural, morphological, microscopic examination and biochemical characteristics following the methods prescribed by (Cheesbrough, 2005). Biochemical test conducted include the following: Gram stain, Catalase test, Oxidase test, Motility test, Methyl red test, Citrate test and Urease test.

Identification of fungi isolate

The complete identification of fungi isolate was done by comparing the result of their morphological and biochemical characteristics with those of known taxa.

Results and Discussion

The results on the isolation and identification of microbial deteriogens of fresh tomato stored at ambient temperature are described as follows: The total heterotrophic count, total coliform count, total fungi counts are shown in table1.The result shows that tomato fruit samples from Relief market recorded the highest bacterial and fungal count while the samples from

Ekeonuwa market recorded the lowest bacterial and fungal count. Table 2 shows the characterization and identification of the isolates following biochemical procedures. The bacteria isolates identified from spoilt fresh tomatoes stored at ambient temperature were: *Bacillus subtilitis, Escherichia coli, Pseudomonas aeruginosa, salmonella spp, Klebsiella spp.* The result on the identification of fungi are shown in table 3, the fungal isolates identified from spoilt fresh tomatoes stored at ambient temperature were *Fusariumspp, Aspergillusniger, Rhizopusstolonifer, Saccharomyces spp.* The percentage occurrence of bacteria isolates from fruit samples obtained from different markets are presented in table 4 which indicates that From all the tomato fruit samples obtained from the 3 market, 134 bacteria was isolated of which *Bacillus subtilitis* was the most prevalent with 52.24%, *Escherichia coli(14.93%), Pseudomonas aeruginosa (23.88%), salmonella spp (1.49%), klebsiellaspp (6.72%).*The isolation of soil bacteria *Bacillus substilis,* from the fruit samples, suggests evidence of opportunistic contamination from human activity. The percentage occurrence of fungi isolates as presented in table 5 are as follows: in the characterization of fungi, a total of 140 organism were isolated of which 85*(60.71%) were Aspergillus niger, followed by Fusarium spp 44(31.43%), Saccharomyces spp 10(7.14%), Rhizopus stolonifer was not significant* ($P>0.05$). Similar findings were reported by (Gosh,2009) who also asserted that *Aspergillus niger, and Fusarium spp.* were the major microorganisms that are responsible for the spoilage of tomato fruits. The implications of microbial contamination and growth on tomato produce causes spoilage, decreased sensory appeal and decreased shelf life leading to loss and wastage of product that have significant economic consequences. The microbiological safety of these products has also become a significant issue, as the incidence of food borne disease outbreaks associated with their consumption.

As a consumer we need to recognize that food safety is important for fresh fruits and vegetable. Also individuals of the population especially those in developing countries who usually use spoilt and slightly decaying tomatoes as a result of their cheaper prices should be educated that these spoilage are often not due to mechanical damages but microbial colonization and physiological decays, they should be made to know that these organisms produces toxins and spores which are relatively heat resistant and can cause severe food poisoning resulting in fatal outcome.

TABLE1: MICROBIAL LOAD OF SPOILT FRESH TOMATOES STORED AT AMBIENT TEMPERATURE (cfu/ml)

	Sample 1	Sample 2	Sample 3
Total bacterial count	$3.6x10^4$	$5.0x\ 10^4$	$4.8x10^4$
Total coliform count	$1.6x10^4$	$2.5x10^4$	$2.0x10^4$
Total fungi count	$4.0x10^4$	$5.3x10^4$	$4.6x10^4$

KEY

Sample 1= Result from tomatoes gotten from ekonunwa

Sample 2= Result from tomatoes gotten from relief

Sample 3 = Result from tomatoes gotten from Student market

	Size	Shape	Elevation	Margin	Surface	Opacity	Colour	texture	Suspecting Organism
Sample 1(A)	Small	Round	Slighty raised	Entires	Smooth	Shiny	Grayish white	Mucoid	Escherichia coli
Sample 1(b)	Large	Round	Umbonate	Undulate	Smooth	Transluscent	White	Mucoid	Klebsiellaspp
Sample1(c)	Medium	Oval	Unbonate	Wavy	Smooth	Transparent	Diffusible green	Mucoid	Pseudomonas spp
Sample 1(D)	Large	Irregular	Umbonate	undulate	Rough	Opaque	White	Dry	Bacillus spp
Sample2(A)	Medium	Oval	Unbonate	Wavy	Smooth	Transparent	Diffusible green	Mucoid	Pseudomonas spp
Sample 2(B)	Small	Round	Slighty raised	Entires	Smooth	Shiny	Grayish white	Mucoid	Escherichia coli

	Size	Shape	Elevation	Margin	Surface	Opacity	Colour	texture	Suspecting Organism
Sample2(C)	Large	irregular	Umbonate	undulate	Rough	Opaque	White	Dry	Bacillus spp
Sample 2(D)	Large	Round	Umbonate	undulate	Smooth	Transluscent	White	Mucoid	Klebsiellaspp
Sample 3(A)	Small	Round	Slighty raised	Entires	Smooth	Shiny	Grayish white	Mucoid	Escherichia coli
Sample 3(B)	Large	Round	Umbonate	undulate	Smooth	Transluscent	White	Mucoid	Klebsiellaspp
Sample 3(C)	Medium	Oval	Unbonate	Wavy	Smooth	Transparent	Diffusible green	Mucoid	Pseudomonas spp
Sample 3(D)	Large	irregular	Umbonate	undulate	Rough	Opaque	White	Dry	Bacillus spp

COLONIAL MORPHOLOGY OF BACTERIA ISOLATES

TABLE 2 Identification of bacteria isolates

A, B, C, D, E represents each colony on the culture plate from each sample

TABLE 2.1 BIOCHEMICAL CHARACTERISTICS OF BACTERIAL ISOLATES

	Gram staining	catalase	Coagulase	Methyl red	Vp test (Voges proskauer)	motility	Citrate test	Indole test	Oxidase test	Probable organism
Sample 1(A)	-ve single rods	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	*E.coli*
Sample 1(b)	-ve single rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Klebsiellaspp*
Sample1(c)	-ve single rods	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	*Pseudomonas spp*
Sample 1(D)	+ve single rods	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	*Bacillus spp*
Sample2(A)	-ve single rods	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	*Pseudomonas spp*
Sample 2(B)	-ve single rods	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	*E.coli*
Sample2(C)	+ve single	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	*Bacillus spp*

	rods									
Sample 2(D)	-ve single rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Klebsiellaspp*
Sample 3(A)	-ve single rods	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	*E.coli*
Sample 3(B)	-ve single rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Klebsiellaspp*
Sample 3(C)	-ve single rods	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	*Pseudomonas spp*
Sample 3(D)	+ve single rods	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	*Bacillus spp*
Sample 3(E)	-ve single rods	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	*Salmonella spp*

KEY:

+ = Positive

- = Negative

TABLE 3: IDENTIFICATION OF FUNGAL ISOLATES

	Macroscopy	Microscopy	Fungal isolates
Sample 1(A)	Greenish, filamentous with profuse proliferation of black velvety spores	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesicle-like chains	*Aspergillusniger*
Sample2(B)	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.	*Fusarium sp.*
Sample2(A)	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.	*Fusarium sp.*
Sample2(B)	Greenish, filamentous with profuse proliferation of black velvety spores	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesicle-like chains	*Aspergillusniger*

	Macroscopy	Microscopy	Fungal isolates
Sample3(A)	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.	*Fusarium sp.*
Sample3(B)	Greenish, filamentous with profuse proliferation of black velvety spores	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesicle-like chains	*Aspergillusniger*
Sample3(C)	Cottony white, filamentous,coenocytic,stolons, rhizoids.	Ovoid sporangiospores, tall sporangiospores in groups	*Rhizopusstolonifer*
Sample3(D)	Colonies of Saccharomyces sp. grow rapidly. They are flat, smooth, moist glistening or dull, and cream to tannish cream in color	Multilateral budding is typical Pseudohyphae, if present are rudimentary. Hyphae are absent. Saccharomyces sp. produces ascospores.	*Sacharomycescerevisiae*

TABLE 4: THE PERCENTAGE OCCURRENCE OF BACTERIA ISOLATES IN SPOILT FRESH TOMATOES STORED AT AMBIENT TEMPERATURE

Bacteria isolates	Sample 1(no of colonies)	Sample 2(no of colonies)	Sample 3(no of colonies)	Frequency	Percentage(%)
Bacillus spp	20	25	25	70	52.24
Pseudomonas spp	8	10	14	32	23.88
Salmonella spp	Nil	Nil	2	2	1.49
Escherichia coli	5	10	5	20	14.93
Klebsiellaspp	3	5	1	9	6.72
Total	36	50	48	134	100

KEY

Sample 1= Result from tomatoes gotten from ekonunwa market

Sample 2= Result from tomatoes gotten from relief market

Sample 3 = Result from tomatoes gotten from Student market

TABLE 5: THE PERCENTAGE OCCURRENCE OF FUNGI ISOLATES IN SPOILT FRESH TOMATOES STORED AT AMBIENT TEMPERATURE

Fungal isolates	Sample1	Sample 2	Sample 3	Percentage (%)
Fusarium spp	20	16	8	31.43
Aspergillus niger	20	37	28	60.71
Rhizopus stolonifer	Nil	1	Nil	0.71
Saccharomyces spp	Nil	Nil	10	7.14
Total	40	54	46	100

Sample 1= Result from tomatoes gotten from ekonunwa market

Sample 2= Result from tomatoes gotten from relief market

Sample 3 = Result from tomatoes gotten from Student market

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