Post Harvest Microbial Deterioration of Tomato (*Lycopersicon esculentum*) Fruits

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ABSTRACT: This research looked into microbial, chemical and environmental causes of the rotting of this all important vegetable. A total of eight microorganisms (Fungi and Bacteria) were isolated from deteriorating tomato fruits. These isolates were used to carry out pathogenicity tests on wounded and unwounded healthy fruits and it was found out that fungi generally cause more deterioration than bacteria. Tests were carried out to ascertain the effect of temperature and milton on the rotting of the fruits. It was observed that deterioration increases with increase in temperature. Treatment with milton effectively reduced rotting. Ascorbic acid level in the fresh and deteriorating fruits was also experimented. The results showed a decrease in ascorbic level with increased deterioration.
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KEY WORDS: Post-harvest, Microbial, Deterioration, Tomato, Fruits.

INTRODUCTION

Tomato is an important vegetable grown for its edible fruit belonging to the genus *Lycopersicon*, being *Lycopersicon esculentum. Lycopersicon* species are native of South America, especially Peru, and the Galapagos Islands.

In Nigeria, the most important tomato producing areas are Bauchi, Kaduna, Kano and Oyo States (Aworth, 1985). Baskets of tomatoes are normally lined with grass leaves and guarded on top by woven cane used for inter-states trade. Observations showed that about 40% of the content of such baskets is lost to rotting and decay which is a severe financial loss to growers, middlemen and retailers. This level of spoilage suggests that the baskets are not suitable structures for transporting such a highly perishable crop like tomato (Aworth, 1985).

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable worldwide, in terms of the amount of vitamins and minerals it contributes to the diet. However, consumers are not satisfied with the quality of tomato (Sozzi and Fraschina, 1997).

Tomato is one of the most perishable vegetable. It is highly esteemed as a source of vitamins A and C, and some essential minerals. (Campbell, 1985).
Tomatoes are a good source of protein, but most of it is in the seeds. Tomato juice contain 19 amino acids principally glutamic acid. They are also a significant source of vitamin A. Red tomatoes have about 1000 IU (International Units) of vitamin A per 100g (McGraw-Hill, 1987).

The growth and subsequent disease development of the different micro-organisms on fruits is of varying degree and rate. One of the most common and obvious symptoms of deterioration results from the activity of fungi. Attack by most organisms follows physical injury or physiological breakdown of the commodity. In a few cases, pathogens can infect apparently healthy tissues and become the primary cause of deterioration (Kader, 1992).

Environmental factors such as soil type, temperature, frost and rainy weather can have an adverse effect on storage life and quality of fruits and vegetables (Bachmann & Earles, 2000). Post-harvest fruit quality depends on both textural durability during storage and nutritional and sensory attributes. The practice of harvesting many fruit prior to softening and full maturity, to improve resistance to damage during handling and shipping, has resulted in crops that typically fail to accumulate the full complement of nutrients. Thus, if softening could be uncoupled from other ripening-related processes, fruit could be harvested at a later stage, resulting in commodities with substantial resistance to over-softening and a dramatically enhanced flavor and nutritional and commercial value (Lytoychenko et al., 2009).

Tomato commercialization is limited by rotting caused by Alternaria alternata (Fr.:Fr.) Keissl., or by Botrytis cinerea Pers.:Fr. (Jones et al., 1993).

In developing countries, losses of fruits and vegetables during post- harvest fluctuate between 20 and 50% (Kader, 1992; Okezie, 1998). It has been estimated that 20-50% of tomato fruits harvested for human consumption are lost through microbial spoilage. Other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading (Aworth, 1985).

MATERIALS AND METHOD

ISOLOATION OF MICRORGANISMS FROM THE DETERIORATED TOMATO FRUITS

Two tomato varieties were considered:

(i) HUNKUYI LOCAL (with big, spherical fruits)
(ii) ROMA V.F. (with oblong or pear-shaped fruits)
Two samples of each variety, each sample containing 20 fruits, were collected from each of the two different markets namely:
(i) New Market Garki
(ii) New Market Gwagwalada

In all the samples, firm, ripe tomato fruits were used. The fruits were further exposed in open trays in the laboratory for two days to allow enough contact with aeromicroflora capable of inducing spoilage of the product. The fruits were then incubated in humidified desiccators for 10 days at room temperature (25°C ± 2) to facilitate the growth and sporulation of the micro organisms in, and on the fruits.

Humidity was created by placing 10 ml. of sterile distilled water in the lower chamber of the desiccators and by further moistening the filter paper on which the fruits were placed with additional 5 ml of sterile distilled water. The fruits and filter paper were held above the water in the lower chamber of the desiccators by wire gauze.

Small portions of the deteriorating parts of the fruits (showing softening, rotting and discoloration) were cut out and transferred aseptically onto each of the following:
(i) Nutrient Agar Plates
(ii) Potato Dextrose Agar Plates
(iii) Malt Extract Agar Plates

Plates were incubated at room temperature (25°C ±2°C) for fungi; and at 37^0C for suspected bacterial isolates. Subsequent sub culturing was continued until pure cultures were obtained and identified. For the bacterial isolates, Gram staining and biochemical tests were carried out. The frequencies of occurrence of the various isolates on the different samples were determined.

B. pH CHANGES ASSOCIATED WITH DETERIORATED TOMATO FRUITS

Fifteen ripe tomato fruits of each type (Hunkuyi local and Roma V.F) selected for uniformity in size and firmness were obtained from markets and left in open trays in the laboratory. Two fruits picked randomly at 3-day intervals were ground to a fine pest and the pH of the paste determined using the pH paper. The experiment was again repeated and the results were recorded.

C. EFFECT OF TEMPERATURE ON THE RATE OF DETERIORATION

Firm, ripe tomato fruits of both varieties were used. Each sample was held for 7-days at 15°C, 20°C, 25°C, 30^0 C and 35°C. At the end of 7 days, deterioration was scored using a modified "Assessment keys for plant diseases (James, 1971) as modified by Ladipo and Amosu (1975). The key was rated 0-5 as follows:

0 - Fruit not showing any deterioration
1 - Slight deterioration, up to 20% of the total surface area of fruit rotted
2 - 1-40% of total surface exhibiting rotting or showing deterioration signs
3 - Moderate degree of deterioration, 41-60% of total surface showing rotting or other forms of deterioration.
4 - High degree of deterioration, 61-80% of total Fruit rotted, softened or showing other forms of deterioration
5 - Fruit completely rotted and hence unmarketable

D. EFFECTIVENESS OF MILTON (SODIUM HYPOCHLORITE SOLUTION) AND TEMPERATURE IN THE PRESERVATION OF TOMATOES

Fifty fruits of each variety were soaked in hypochlorite solution (10 ml of milton (0.01%)) solution in 1 litre of distilled water (a dilution recommended for disinfecting fruits and vegetables) for 5 minutes and then transferred into a clean tray without rinsing. The fruits from each variety were grouped into 5 samples (of 10 fruits each) and placed in small polythene bags.

A sample each from the two groups (varieties) was maintained at temperatures of 15°C, 20°C, 25°C, 30°C and 35^0C for seven days. A control experiment was set up in a similar manner but without treatment with Milton. The extent of deterioration was recorded at the end of one week using the same key referred to above.

F. PATHOGENICITY TESTS

These tests were carried out to confirm the ability of various isolates to infect and cause deterioration of apparently healthy tomato fruits. Fresh and undamaged fruits of the two varieties were soaked in 0.01% sodium hypochlorite solution for 5-10 minutes, rinsed in 3 changes of sterile distilled water and blotted dry. Inoculations of fruits with the isolates were effected with and without wounding the fruits.

G. INOCULATION OF MECHANICALLY WOUNDED FRUITS

The method of Boston et al (1982) was employed.

A matchstick sterilized by soaking in NaOCl solution for 10 minutes was used to puncture the middle portion of the fruit. A 5 mm culture disc from the edge of an actively growing culture of the isolate was transferred into the wound, sealed immediately with a transparent adhesive tape before the fruits were placed in sterile polythene bags. The bags were tied with a rope and incubated at room temperature (25°C ± 2°C). Therefore each of the eight isolates listed in Table 1 was used to inoculate the test fruits in the same manner.

In the control treatment, fruits were similarly punctured but the wound was immediately sealed up with adhesive tape without being treated with the isolates. The extent of the rotting or lesion development from the spot of inoculation was used as a measure of deterioration. The experiment was repeated once using each variety of tomato.

H. DETERMINATION OF ASCORBIC ACID LEVELS OF HEALTHY AND DETERIORATING TOMATOES OF THE TWO VARIETIES.

The method of Plummer (1978) was used. 5ml of diluted fruit juice was pipetted into a boiling tube; 1 ml of chlorophorm and 1 ml of glacial acetic acid were added to the mixture. This was titrated with 2, 6- dichlorophenolindophenol solution (dye) until a faint, permanent, pink colour was obtained. The titre (T) was recorded. The titration was repeated with 5 ml of water for the blank (BL) and 5 ml of ascorbic acid standard solution (St) and the vitamin C (ascorbic acid) content of the test sample calculated thus:

Vit. C of test (mg/1OOml)=<u>T-Bl</u> x 2 x Dilution St-BI

RESULT

A total of eight micro-organisms were isolated from deteriorating portions of the tomato fruits as recorded in Table 1. *Aspergillus niger, Rhizopus nigricans, Rhizopus stolonifer, Candida* yeast, *penicillium spp., Mucor spp*. and two Gram negative coccal forms of bacteria.

Deterioration of tomato fruits increased with increase in, temperature. That is, the rate of deterioration is less at low temperature (l5°C), moderate at moderate temperatures range (20-25°C) and high at high temperatures (30 - 35°C). Surface disinfection with 0.01% NaOCl (Milton) germicide was, however, more effective at lower than at higher temperature levels and irrespective of the fruit variety.

Table III shows the severity or extent of rotting from pathogens isolated which were introduced into the wounded and unwounded fruits. Only little rotting was noticed in the unwounded fruits while severe rotting was noticed in wounded fruits inoculated with *Aspergillus niger, Rhizopus niger, Mucor spp. and Rhizopus stolonifer.* The rest showed a moderate rotting.

The method of Plummer (1978) used show a progressive reduction in ascorbic acid content of the deteriorating fruits with time (in days). The initial values of the fresh fruit juice and subsequent ones show a great disparity between the two varieties; with the Roma V. F having the ascorbic acid (Vitamin C) content about twice (double) that in Hunkuyi Local.

Analysis of the titre values obtained (Table IV) shows that the fruit variety and condition as well as the interaction between those two factors all influenced ascorbic acid variation in the fruit samples.

TABLE 1: Microorganisms isolated from deteriorating fruits :
Aspergillus niger
Rhizopus nigricans
Rhizopus stolonifer
Mucor spp.
Penicillium spp.
Candida yeast

2 Gram negative coccal forms of bacteria

TABLE 2: Mean fruit rot severity at five different temperatures

Temperature	Mean Rot	
(^{0}C)	**Variety 1: (Hunkuyi Local)**	**Variety 2 (Roma V.F)**
15	1	1
20	2	2
25	2	2
30	4	4
35	5	5

Key:
0-Fruit not showing any deterioration
1-Slight deterioration, up to 20% of the total surface area of fruit rotted, necrotic, discoloured or showing any other deterioration sign.
2.-21-40% of total surface exhibiting rotting or showing deterioration signs.
3-Moderate degree of deterioration, 41-60% of total surface showing rotting or other forms of deterioration.
4-High degree of deterioration, 61-80% of total fruit surface rotted, softened or showing other forms of deterioration
5-Fruit completely rotted and hence unmarketable.

TABLE 3A: Pathogenicity of fungal and bacterial isolates in artificially wounded and unwounded tomato fruits of Hunkuyi local variety

Microorganisms	Rot Development on:	
	Wounded Inoculated Fruits (a)	Unwounded but Inoculated fruits (b)
Aspergillus niger	++	+
Rhizopus nigricans	++	+
Mucor spp	++	+
Candida Yeast	+	-
Gram negative Bacteria (a)	+	-
Gram negative Bacteria (b)	+	-

++ Extensive rotting
\+ Moderate rotting
\- No rotting

TABLE 3B: Pathogenicity of fungal and bacterial isolates in artificially wounded and unwounded tomato fruits of Roma V. F. variety

Microorganisms	**Rot Development on:**	
	Wounded Inoculated Fruits (a)	**Unwounded but Inoculated fruits (b)**
Aspergillus niger	++	+
Penicillium spp	+	-
Rhizopus stolonifer	++	+

++ Extensive rotting
\+ Moderate rotting
\- No noticeable rotting

TABLE 4: Ascorbic Acid Level of Deteriorating Tomato Fruits of the Two Varieties

Days	**Mean Ascorbic Acid Value (in mg/ml)**	
	Hunkuyi Local	**Roma V.F**
1	15.97	30.49
3	15.42	28.64
6	13.37	27.44
9	11.37	24.94
12	9.22	23.79
15	6.98	22.66

DISCUSSION

Preservation of fruits and vegetables is of great importance because it makes provision for delayed use and eliminates wastage. Due to the perishable nature of tomato, post harvest lost is highly recorded. Therefore, production must go hand in hand with preservation and storage. In light of this, tomatoes have been processed into various products for the purpose of reduction in losses and enhancement of shell life.

The primary causative agents of microbial post-harvest spoilage of tomatoes are the bacteria, yeasts and moulds (Aworth, 1985). This report shows that wounded tomato fruits are most liable to microbial infection and hence deterioration. A cut on a broken tomato fruit may easily harbour pathogens that may spread and spoil all tomatoes in a lot (Villareal, 1980).

In this work, the most frequent and more damaging micro-organisms isolated were fungi viz: *Aspergillus niger, Rhizopus nigricans, Rhizopus sto1onifer and Mucor spp*. These have already been reported in other works. For example, Atherton and Rudich (1986) reported that the major causes of post-harvest losses of tomato are diseases; principally *Alternia, Rhizopus,* grey mould and bacteria. This study also shows that the rate of spoilage was higher in fruits of the Hunkuyi Local than in those of Roma V.F. variety. This is probably due to genetic difference between them.

More deterioration was noticed in wounded fruits inoculated with isolates than in the unwounded fruit. This confirmed the assertion that spoilage micro-organisms often gain entry into fruits through wounds (Kuku, 1980). The little deterioration noticed in unwounded fruits is seen to be caused by fungi. This confirms the assertion that fungi have an advantage over other microbial pathogens in that they grow at their tips (Garrett, 1970).

Tomato sellers leave these fruits in the open under the sun where they are heated up and subjected to rotting due to the heat. This study has proved that the temperature has an effect and in fact high temperatures encourage deterioration of tomato fruits. Same was reported by Stakman and Harrar (1957) when they stated that high temperature levels speed up the physiologic processes occurring in fruits leading to sub oxidation and accumulation of metabolic by products. Lutz and Hardenburg (1968) recommended a temperature range of 12.8^{o}C - 21.1^{o}C for the storage of mature green tomatoes and 7.2^{0}C -1O^{0}C for ripe ones.

From this study, the ascorbic acid content of healthy tomato fruits was found to be higher than deteriorated fruits. This is in line with what has been reported by Mudahar *et al.*, (1986)

When they found out from experiment that there is a significant loss of ascorbic acid during storage.

The supply of tomatoes the year round cannot be achieved through production alone, it must go hand in hand with preservation and storage of excess at harvest. However, it is also important to mention that the practices at cultivation and time of harvest could improve or mar the shelf life. Quality can not be improved after harvest, only maintained; therefore, it is important to harvest fruits and vegetables at the proper stage and size and at peak quality. Immature or over mature produce may not last as long in storage as that picked at proper maturity (Wilson et al., 1995).

Proper packaging and handling is able to improve the quality and storage life of fresh fruits and vegetables by protecting them against mechanical damage, excessive wilting, and invasion by decay organisms, softening and over ripening. Substantial reduction in losses of perishable fruits and vegetables in Nigeria can be achieved by improved packaging. Traditional packaging materials such as Jute-bags, bamboo and cane baskets offer very little protection to perishable horticulture produce and are not conducive for efficient handling or suitable for modern methods of transportation (Aworth, 1985).

Reduction in losses and enhancement of shelf life of tomato fruits can be achieved by careful method of harvesting, handling, packaging and mostly preservation and storage (Erile, 1983).

Milton (Sodium hypochlorite solution) effectively reduced the post harvest decay of tomato in this study and should be used.

Also, cold storage by refrigeration and controlled atmospheric environments should be seriously considered for tomatoes by farmers engaged in large scale production. An integrated approach in which all possible preservation techniques are combined, will give positive results.

RECOMMENDATIONS

We have recently identified a tomato line that we named the DFD (delayed fruit deterioration) mutant, which promises to provide a new perspective of the key molecular determinants of softening and other postharvest quality traits. The two major characteristics of DFD are substantially reduced fruit softening and complete resistance to postharvest disease, which collectively result in a remarkable shelf life (Saladie et al; 2005). A newly identified tomato mutant, Delayed Fruit Deterioration (DFD), provides an excellent opportunity to address this question since DFD fruit exhibit exceptional shelf life and are entirely resistant to postharvest microbial infection (Lytovchenko et al; 2009).

In light of this breakthrough, research activities should be sponsored by government in Nigeria to enable a transfer of such technology to our immense benefits.

I recommend that government agencies like National Agency for Food and Drug Administration and Control (NAFDAC), Standards Organization of Nigeria (SON) should embark on enlightenment of the public on the causes of tomato fruit decay and how it can be checked. They should make it mandatory for tomato sellers to display them in shades and also store in refrigerators where temperatures can be regulated. They should also be taught how to use disinfectants like Milton to wash the fruits and disinfect the containers in which these fruits are carried.

Other agencies like Agric Development Project (ADP) and Ministries of Agriculture through their extension workers should make available fungicides and teach the farmers to make pre-harvest and post harvest spray to check carrying these micro-organisms from the farm.

Expenses for all these will be justified if the over 40% loss of this important fruit is checked.

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