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Research Article

Microbial Flora of Post-Harvested Tomatoes and the Effect of Sodium Hypochlorite on the Microbial Load

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ABSTRACT

As beneficial as Tomato (*Lycopersicon esculentum*) is to humans, large percentage of tomato fruits purchased in Nigeria are being lost to post harvest deterioration caused by microorganisms. The study was aimed to determine the microbial flora of post-harvested tomato and effect of sodium hypochlorite on the microbial load. Post harvested tomatoes were analysed for microbiological qualities and the effect of sodium hypochlorite on microbial load was also assessed. The microbiological quantities analysed were total aerobic count, coliform count and fungal count. A set of Intact and cracked tomatoes were treated with Sodium hypochlorite (1 w/v %) and another set was used as control (analyzed untreated). The total aerobic count, coliform count and fungal count of $47x10^4$ cfu/ml, $40x10^4$ cfu/ml, and $28x10^4$ cfu/ml were obtained from the untreated samples respectively, while none was recorded for the treated samples. Five genera of bacteria namely; *Bacillus, Pseudomonas, Escherichia, Staphylococcus, Enterobacter* and four genera of fungi namely; *Aspergillus, Pencillium, Candida* and Mucor were isolated from the tomatoes analysed. The absence of microorganisms in the treated tomatoes shows the effectiveness of sodium hypochlorite as a disinfectant in controlling/reducing the microbial load in food, which could subsequently lead to rapid spoilage.

Key words: Tomatoes, Microbial Flora, Sodium hypochlorite.

INTRODUCTION

The versatility of some fruits and vegetables like tomatoes due to their role as important parts of a healthy diet has made them subject to contamination by microbes (FDA, 2001). Tomatoes, when cooked serves as a vegetable and when eaten raw serves as a fruit (Bartz, 1999) It is one of the most common raw agricultural products, though perishable, but of great importance as a food supplement. It is one of the most important vegetable in most regions of the world and constitute an important source of food as well as cash (Ogbonna, 2008). The microbial flora of post- harvested tomatoes are those group of organisms that can live on or within the body of the above fresh produce (Andrews, 1992). However, microbial flora encompasses broad genera of bacteria and fungi that can survive or grow on or within the body of post harvested tomatoes (Osuji, 2002). The fact that tomatoes are grown on land which has been fertilized with organic manure has made it a subject of contamination by plant pathogens and other opportunists that can arise from applied manure (Yakav *et al*., 2017). Recent food borne illnesses associated with consumption of fresh produce (i.e., fresh fruits and vegetables) have raised concerns about microbial safety hazards associated with agricultural and manufacturing practices for the production of fresh produce (CDC, 2000) Contamination of the fresh produce (tomatoes) with human source pathogens and flora is considered significant, thus the need for prior sanitization. Sodium hypochlorite is a chemical compound with the formular, NaOCl, comprising of a sodium cation and hypochlorite anion; a household chemical widely used as a disinfectant or a bleaching agent (Peter, 2006). Sodium hypochlorite in solution exhibits broad spectrum anti-microbial activity and is widely used in healthcare facilities in a variety of settings. It is usually diluted in water depending on its intended use. (Rutala and Weber, 2017). Therefore, this research is aimed at evaluating the microbial flora of post-harvested tomatoes and assessing the effectiveness of sodium hypochlorite application on the microbial load.

MATERIALS AND METHODS

Sample Source

Tomato samples used for this work was sourced from three major local markets in Owerri, Imo state, Nigeria.

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Table 1: Mean Count of Isolates from Tomatoes

Tomatoes	Total aerobic count (cfu/ml)	Coliform count cfu/ml	Fungal count cfu/ml
Untreated	$47x10^4$	$40x10^4$	$28x10^4$
Treated	Nil	Nil	Nil

Table 2: Bacterial Isolates from Intact and Cracked Tomatoes

Isolates	Intact	Cracked
Staphylococcus sp.	+	+
Pseudomonas sp	+	-
Escherichia sp.	-	+
Bacillus sp.	-	+
Enterobacter sp.	-	+

Table 3: Fungal Isolates from Intact and Cracked Tomatoes

Isolates	Intact	Cracked
Aspergillus niger	-	+
Mucor sp.	+	+
Cladosporium sp	-	+
Penicillium sp	+	-

Table 4: Percentage occurrence of Bacterial Isolates

Bacterial Isolates	Number of occurrences	Percentage occurence (%)
Staphylococcus sp.	7	31.8
Pseudomonas sp.	1	4.5
Escherichia sp.	5	22.7
Bacillus sp.	4	18.1
Enterobacter sp.	2	9.1

Table 5: Percentage occurrence of fungal Isolates

Bacterial Isolates	Number of occurrences	Percentage occurence (%)
Aspergillus niger	6	31.57
Mucor sp.	1	52.63
Cladosporium sp.	2	10.52
Penicillium sp.	10	5.2

Table 6: Mean Bacterial count of Isolates

Bacterial Isolates	cfu/ml x 10^4
Staphylococcus sp.	33
Pseudomonas sp.	11
Escherichia sp.	25
Bacillus sp.	19
Enterobacter sp.	12

Table 7: Mean Fungal count of Isolates

Fungal Isolates	cfu/ml x 10^4
Aspergillus niger	12
Penicillium sp.	5
Mucor sp.	8
Cladosporium sp.	3

Mode and method of sample collection

A total of 45 samples were collected with the use of sterile hand gloves into a clean ziplock bag by which it was transferred to the laboratory for processing.

Analysis of untreated samples

Ten grams of the tomatoes were weighed and washed in 100ml of sterile distilled water using a sterile hand glove. Tenfold serial dilution was made with the "wash water" to 10^{-3} dilution. 0.1ml of the desired dilution was plated on Nutrient agar for aerobic count, MacConkey agar for coliform count and Sabouraud dextrose agar for fungal count. The sterile distilled water used for washing the tomatoes was also inoculated on NA to serve as control. The plates were incubated at 37^0C for 24-48 hours except for Sabouraud dextrose agar which was at 30^0C for 2-5 days.

Analysis of treated samples

Ten grams of the tomatoes were weighed and soaked in 1% w/v of sodium hypochlorite for about 30 minutes and then washed in 100ml of sterile distilled water. Tenfold serial dilution was made with the "wash water" and plated out as in 2.3.

Identification of Isolates

The isolates were identified using standard methods (Cheesbrough, 2002; Ogbulie *et al*., 1998; Abbey, 1995; Allen et al., 1979).

RESULTS AND DISCUSSION

The mean count of the isolates as shown in Table 4.1 recorded the total aerobic count, coliform count and fungal count as $47x10^4$ cfu/ml, $40x10^4$ cfu/ml and $28x10^4$ cfu/ml respectively. For the treated samples, no growth was recorded. Five genera of bacteria and four genera of fungi were isolated from the tomato samples. The bacteria genera were *Enterobacter, Bacillus, Pseudomonas, Staphylococcus* and *Escherichia* while the fungal genera were *Aspergillus, Pencillium, Cladosporium* and *Mucor.*

Thus, putting the ubiquity of microorganisms into consideration, a line cannot and should not be drawn that only these genera are associated with tomatoes, some other factors which could be attributed to chance might have hindered the possibility of isolation of other existing floras. Also, the fact that nature selects for its own organisms should not be left out. Generally, two of the bacterial isolates belong to the family Enterobacteriacae (*Escherichia, Pseudomonas*). Members of this group inhabit the soil, water and decaying matter. They are isolated as both normal flora and pathogens. Pseudomonads ranks among the most important plant pathogens because the species are resistant to soaps, dyes, quartenary ammonium disinfectants, drugs, drying and temperature extremes.

On the other hand, the genus *Bacillus* include a large assembly of most saprobic bacteria widely distributed in the earth's habitat. The priming habitat of many species of Bacillus is the soil and spores are continuously dispersed by means of dust onto the bodies of plants and animals. The genus *Staphylococcus* are inhabitant of the skin and the mucous membranes. The most common species are *Staphylococcus aureus* with the resistance factor ranking highest among all non-spore forming pathogens.

Among the fungal isolates, the genus *Aspergillus* is the most pervasive of all fungi with wide distribution in air, dust, vegetation, food and compost heaps. *Mucor* are extremely abundant saprobic fungi found in soil, water, organic debris and food. These genera are most often involved in Mucormycoses. The pathology of this disease depends on the portal of entry of the fungus which can be gastrointestinal, nasal etc and invades mostly the lungs.

These organisms could be said to have gained entrance into the analysed post-harvestd tomatoes through practice and manner of cultivation of growers, post harvesting practices, 'wash water' used before storage and conditions

Table 8: Biochemical and other Characteristics of Bacterial Isolates

Gram Staining	Motility test	Oxidase test	Catalase test	Indole test	Methyl red test	Voges proskaeur	citrate test	Sugar Fermentation Test					Possible isolate
								Glu	Mal	Suc	Ara	Fru	
Gram-ve rods	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Enterobacter sp
Gram+ve rods	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	Bacillius sp.
Gram-ve rod	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	Escherichia sp.
Gram –ve rod	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Pseudomonas sp.
Gram +ve cocci	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	ve	+ve	Staphylococcus sp.

Table 9: Morhological Characteristics of Fungal Isolates

Isolates	Colonial Morphology	Microscopic examination
Aspergillus niger	A salt pepper effect with surface covered with black	Hyphae were distinctively septate long and usually not seen. Spores are spherical and black
Penicillium sp.	Yellow radial rugose are often formed	Hyphae were hyaline and septate conidiophores give rise to branch-ing. Phialides forming a brush.
Mucor sp.	Rapid growth spreads and fill the plate, gray white and fluffy mycelium	Spores were borne within their fruiting bodies enclosed in a sac-like structure called Sporangia
Cladosporium	Colonial types varying from deep Brown to black, smooth rugose and Leathery	Hyphae were distinctively septate Conidiophores are freely branched having the appearance of a brush

of storage. This is because most of the isolates are saprobes, opportunists and organisms abundant in air, dust and soil and there should be no doubt pertaining to their entrances or attachment unto post-harvested tomatoes analysed.

There was a positive effect of Sodium hypochlorite (1%) used as a treatment/surface sterilizer reflected above (3.1) on the microbial load recorded.

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