



Nature of Tomatoes Microflora under Storage

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Author's contribution

The only author performed the whole research work. Author AOA wrote the first draft of the paper. Author AOA read and approved the final manuscript.

Research Article

Received 30th August 2012
Accepted 17th November 2012
Published 20th December 2012

ABSTRACT

Aims: The aim of this study is to determine microbial load of tomatoes including microbial species it constitute under storage with particular reference to raw and canned tomatoes.

Study Design: Random sampling of Tomatoes, from selected sources in Ondo State, Nigeria.

Place and Duration of Study: Sample: Akungba-Akoko and some communities in Ondo State. Analysis at Microbiology Laboratory, Adekunle Ajasin University, Akungba-Akoko. August, 2011 to July, 2012.

Methodology: Pour plate techniques were used to enumerate microbial load of the samples. Discrete isolated colonies from these sources were sub-cultured using streak plate method to get purified cultures for the study. The bacterial isolates were Gram stained while fungal species were examined using lactophenol cotton blue stain. Standard microbiological methods were subsequently used to identify the tomatoes microflora.

Result: The bacterial load observed during the study ranged from 1×10^5 in CT3 to 38×10^5 in CT2 which are the canned sample sources. Similar range of 10 to 21 cfu/mL was recorded for the raw tomatoes. Microorganisms generally encountered include eight bacteria species such as, *Leuconostoc* Species, *Pediococcus* species, *Staphylococcus* species, *Planococcus* species, *Micrococcus* Species, *Bacillus* Species, *Streptococcus* species and *Clostridium* species. Similarly, three fungal species were encountered during the study. These include *Saccharomyces* species, *Aspergillus* species and *Neurospora* species.

Conclusion: The microbial load and isolates from raw (local) tomatoes sources are at close range to those isolated from the canned tomatoes sources which still shows the

natural protective lactic acid components of this food source. Exposure of canned tomato to air gives room for deterioration by microorganisms as shown in this study. So, both raw and canned tomato should be well protected from contaminants to limit proliferation of spoilage organisms coupled with improvement of canning Technology. Similarly, the nature of microorganism encountered can be used to determine some food preservation systems in the study area.

Keywords: Growth conditions; microflora; microorganisms; preservation; tomatoes; nutrients.

1. INTRODUCTION

Tomato scientifically known as *Solanum lycopersicum* is a herbaceous plant usually sprawling on ground in the Solanaceae family. They are close family to tobacco, chili peppers and egg plant. It is a perennial often grown outdoors in temperate climate as an annual, typically reaching to 1-3m (3-10feet) in height, weak woody stem that often vines over other plants [1]. Tomato is widely used as condiment or as food dietary supplement in various part of the world and also valuable in the food industry [2]. Canene-Adams et al. [3] in their study stated that tomatoes are the fourth most commonly consumed fresh vegetable and the most frequently consumed canned vegetable in the American diet and showed the connection between increased tomato consumption and reduced risk for both cardiovascular disease and prostate cancer thus makes it valuable food source all over the world.

The word tomato comes from a word in the Nahuatt language tomatt. The specific name, *Lycopersicum* means "wolf peach" (compare the related specie, *Solanum lycopersicum* whose specific name means "wolf fruit". Common name "wolf apple" and they are a major food of wild candies in South America [3]. Various species of microorganism can alter adversely the nutritional quality of tomatoes. Microorganisms that cause spoilage like moulds, yeast and bacteria are destroyed by heat processing. Both raw and processed tomato and tomato products were directly associated with plasma lycopene concentrate with association being strongest for processed tomato and products thus, it is desirable as dietary choices for vulnerable population groups such as the elderly [4,5,6].

In areas and situations where, in season, perfectly ripe tomatoes are not available, canned tomatoes are of alternative to prepare dishes such as sauce or pizza. The top uses of canned tomato are in Italian, chill, soup, pizza, stew, casseroles and Mexican dishes. Industrially prepared canned tomato may be prepared in a number of ways. However safety measures need to be taken because imperfectly canned tomato can cause botulism poisoning [7]. Temperature higher than 100°C is required to destroy the spores of *Clostridium botulinum* in low acids foods which includes; vegetable, red meat, poultry, fish and wider game. To reach temperature higher than the boiling point of water (100°C), a pressure canner can be used. The temperature of at least 115.6°C is needed for the destruction of *Clostridium botulinum* spores. Therefore canning of low acid food is done in the pressure canner at 10 or 11 pounds of pressure (115.6°C) or at 15 pounds of pressure (121°C). Adjustment in canner pressure made according to elevation made above sea level. You don't have to worry about botulism in home canned tomatoes because tomatoes are too acidic for bacteria growth [7,8]

Tomato products have some nutritional benefits for human health which gives it some unique attributes. Tomato juice is used and could be taken to prevent blood clot. About one

cup of tomato juice per day offers the anti-clotting benefits [9]. Red raw tomatoes has the following nutritional value for-100 g; Energy 20Kcal-80 KJ; Carbohydrates- 4g; Sugar-2.6g; Dietary fiber-1g; Fat-0.2g; Proteins-1g; Vitamin C (13mg)-22% and Water 95g [8,10]. Other components include natural Lycopene which is a red fat soluble pigment found in vegetable commonly found in tomatoes which also add to its nutritive value. Tomato products also offer important nutrients like vitamin A, vitamin C, potassium and fiber [7,10]

Spoilage of canned foods can also largely result from mesophilic organisms and is indicative of under processing and is caused by species of *Bacillus*, *Clostridium*, yeast and fungi. *Clostridium butyricum* and *Clostridium pasteurianum* produce a butyric acid type of fermentation in acid or medium acid foods with swelling of the container by the production of carbondioxide (CO₂) and hydrogen (H₂). Other species of *Clostridia* may produce hydrogen sulphide and other malodorous compounds causing the can to swell. These putrefactive anaerobes generally grow in low acid foods such as peas, corn, meat, poultry and others but sometimes may also spoil medium acid foods. Some Bacilli such as *Bacillus subtilis* and *Bacillus mesentroides* have been found to grow in poorly evacuated cans of sea foods, meat and milk. *Salmonella* and other pathogens are killed at 145°C [7].

After a food has been canned it must be heated to eliminate the risk of botulism arising from *Clostridium botulinum* spores. Heat treatment is carried long enough to reduce the population of 10¹² Botulinum spores to 10⁰ (one spore) thus there is a very small chance of any can having a viable spore [11]. In this study, the microbiological quality of tomatoes was determined based on nature of microflora that populate the sample obtained in Akungba-Akoko and environs in Ondo State, Nigeria for this purpose. This is to enhance possible improvement on the availability of tomatoes because of its dietary value through determination of better storage system based on relevant information on conditions that may influence this.

2. MATERIALS AND METHODS

2.1 Sterilization of Materials and Media Preparation

The materials used for this study were sterilized by appropriate methods to free them from microbial contamination. All the media used were sterilized at 121°C for 15minutes in an autoclave and were prepared according to the manufacturers' instruction or standard methods for examination of water [12]. Culture media generally used for the study are nutrient agar and potato dextrose agar (PDA). All glass ware were sterilized in the hot air oven at 160°C for two hours. The inoculating needle and wire were sterilized by flaming in the Bunsen burner until red hot, working surface were sterilized by the application of disinfectants/antiseptic solutions (95% ethanol).

2.2 Sample Collection

Selected canned tomatoes were purchased from various areas from Ondo State. These include Ciao toma, Tasty tom, Petti and Titus. The local tomato that was used as the control sample was also purchased in a local market in Akungba-Akoko, Ondo State, Nigeria. All samples were taken to the laboratory immediately after purchase. During the collection of the samples, the observations made were that some of the canned tomatoes were rusted and others had their cans intact without rusts. The local tomatoes used were ensured spoilage free at the point of collection. The first set of the samples were analyzed

immediately on getting to the laboratory for comparative study of canned and raw tomatoes within six hours while further study on the canned tomatoes was done within 12 hours of collection in the laboratory for the first day and stored in the refrigerator at 4°C for further analysis.

2.2.1 Canned tomatoes preparation

Canned tomatoes are usually prepared under aseptic conditions by following some specific steps within one hour: This includes preparing the canning jars and two-piece caps (lids and screw bands) according to the manufacturer's instructions. Keep the jars and lids hot, wash and peel the tomatoes. Then, larger tomatoes are cut into halves or quarters. To make peeling tomatoes easier, blanch them first to loosen the skins: Dip them in boiling water for 30 seconds and then into cold water. This is followed by peeling soft-skinned tomatoes which can be done by placing tomatoes into prepared canning jars, pressing them to release their juice. (Use a canning funnel to keep the rims clean.). To each pint jar, add 1 tablespoon lemon juice or 1/4 teaspoon citric acid and, if desired, 1/2 teaspoon salt. To each quart jar, add 2 tablespoons lemon juice or 1/2 teaspoon citric acid and, if desired, 1 teaspoon salt.

If there's not enough juice to cover the tomatoes, add boiling water to the jars, leaving room for 1/2-inch of air space (headspace) below the lid. Release any air bubbles with a nonreactive utensil, adding more tomatoes as necessary to maintain the proper headspace. Wipe the jar rims; seal the jars with the two-piece caps, hand-tightening the bands. Process the filled jars in a water-bath canner for 35 minutes (pints) or 45 minutes (quarts) from the point of boiling. Remove the jars from the canner with a jar lifter. Place them on a clean kitchen towel away from drafts. After the jars cool completely, test the seals. If you find jars that haven't sealed, refrigerate them and use them within two weeks. Tomato from this kind of canning process can give per 1/2-cup serving: *Calories 44 (From fat 6); Fat 1g (Saturated 0g); Cholesterol 0mg; Sodium 19mg; Carbohydrates 10g (Dietary fiber 2g); Protein 2g* [13].

2.3 Sample Preservation

The samples were preserved in the refrigerator at 4°C after opening the can. This was meant to generally slow down biological activities and reduce chemical reaction.

2.4 Isolation of Microbes from the Study Samples

Triplicate sample sources were processed for the study through which a mean reading is taken after all. Serial dilution of each sample was carried out by pipetting 1mL of the sample into 9mL of sterile distilled water in McCartney bottles to give 10^{-1} dilution. Further dilution was made to give higher dilutions as required. For each samples after series dilution, 0.1ml and 1ml was taken from 10^{-2} , 10^{-3} , 10^{-5} dilution into a sterile plate.

The sterilized medium of nutrient agar (NA) and the potato dextrose agar (PDA) generally used during the study were poured in the plate already containing the diluents as appropriate using the method known as pour plate technique. This was then swirled evenly to make the mixture homogenized and left for few minute(s) to allow it to solidify. The inoculated plates were labeled properly for easy identification, and then the plate was incubated at 37°C for 24 hours for bacterial culture and 25°-27°C for 5 days for fungal growth.

2.5 Sub-Culturing by Streak Method

Nutrient agar and potato dextrose agar were prepared, poured into plates aseptically and allowed to solidify respectively. Distinct colonies on the samples obtained were sub-cultured using an inoculating loop by streaking on the sterile plate. These were incubated at 37°C for 24 hour for bacteria culture and 27°C for 5 days for fungi culture. Subsequent sub-culturing was carried out until pure cultures of different isolates were obtained. These pure isolates were transferred into agar slants in McCartney bottles and kept in the refrigerator at 4°C to serve as stock culture for subsequent tests during identification of organism [14, 15].

2.6 Laboratory Identification of Fungi

2.6.1 Yeast identification

Different colonial types were purified by transferring into sterile potato dextrose plate. Each pure colonial isolates was inoculated into a sterile potato dextrose agar slants and stored in refrigerator.

2.6.2 Colonial morphology

The vegetative cells of the pure culture of the yeast isolates were streaked on sterile potato dextrose agar plate. The plates were incubated at 30°C for 48 hours. The shapes, color, edge and the growth elevation were examined.

2.6.3 Cellular morphology

Smears of the pure cultures of the yeast isolates were prepared and stained with lactophenol-in-cotton blue and allowed to act for 30seconds and then washed off with distilled water. The slides were blot dried in between fold of filter paper and observed at oil immersion objective and x40 objective.

2.7 Biochemical Test and Identification of Bacterial Isolates

Standard microbiological methods were used to identify the microorganisms obtained. The bacteria isolates were identified making use of 24hours old cultures that were Gram Stained for cell morphological differentiation. Among the test carried out include, catalase test, carbohydrate fermentation test for sugars such as glucose, fructose, lactose, galactose, arabinose, maltose and sucrose according to modified methods of Cowan and Steel [16] and Forbes et al. [17]. 1% of each carbohydrate was prepared into test tubes in triplicates containing Durham tubes in an inverted position. Two (2) drops of phenol red indicator was added in a peptone medium.

These were then autoclaved at 112°C for 10minutes. Sugar test tubes were inoculated with appropriate isolate while control tubes were prepared without inoculation. The tubes were incubated and observed daily for two to ten days for ability to utilize a particular sugar as carbon source of energy leading to acid production signified by change in colour of the medium from red to yellow. This may be accompanied with gas production on the top of the inverted Durham tubes provided. The Bacterial isolates were also examined for Endospore Staining, Motility test and Starch Hydrolysis [16,17,18]

2.8 Organoleptic Test

Organoleptic refers to qualities such as appearance, colour, odour and texture [19]. These tests were performed weekly for 5 weeks. It is a major test conducted using individual sense organs of smell, odor, taste, flavor, color and other physical attributes or appearance to justify the palatability or quality of a food sample.

3. RESULTS

This study shows that various types of microorganisms were encountered in tomatoes and canned tomato product analyzed in the laboratory. The organisms that were isolated include eight bacteria species and three fungi species. Different reasons can be adduced for the presence of these organisms. It may be due to under processing, inadequate cooling, contamination of the can resulting from leakage through seams and finally, the pre-process spoilage. Since some canned foods received low heat treatment, it is to be expected that a rather large number of different types of microorganism may be found on examining such foods.

Appropriate inoculum from the tomato sample analyzed gives the bacterial load range from these sources as shown in Table 1. This range from 1×10^5 in CT3 to 38×10^5 in CT2 for canned tomato and 10 to 21×10^5 cfu/mL for the raw tomatoes. The standard error (S.E) of 18.682 obtained for canned tomatoes shows the wide variation of microbial population among various commercial canned tomatoes compared with the raw sources having 6.083 (S.E) showing relatively smaller range of tomatoes microflora possibly because of its natural lactic acid component. In Table 2, the bacteria isolates encountered were characterized for identification purposes into ten different species categorized into eight genera such as *Leuconostoc* species, *Pediococcus* species, *Staphylococcus* species, *Planococcus* species, *Micrococcus* species, *Bacillus* species, *Streptococcus* species and *Clostridium* species.

Table 1a. Bacterial load of sample on nutrient agar

Canned tomatoes		Raw tomatoes	
Sample code	Bacterial load (cfu/ml $\times 10^5$)	Sample code	Bacterial load (cfu/ml $\times 10^5$)
CT1	15	RT4	10
CT2	38	RT5	21
CT3	1	RT6	20
S.E	18.682	S.E	6.083

Legend: Canned tomatoes, CT1 –CT3; Raw tomatoes, RT4–RT6;
Standard Error: S.E

Table 1b. Bacterial load of recently exposed and long term exposed canned tomatoes

Sample code	Recently exposed can tomato (cfu/ml $\times 10^5$)	Long term exposed (5 wks) can tomatoes (cfu/ml $\times 10^5$)
CT1	18	79
CT2	35	86
CT3	2	42

Legend: Canned tomatoes, CT1 –CT3

Table 2. Biochemical characteristics of bacterial isolates encountered

Lab code	Cultural characteristics of isolates	Gram stain rxn	Motility	Catalase	Starch hydrolysis	Ornithine	Spore	Glucose	Lactose	Sucrose	Fructose	Galactose	Arabinose	Maltose	Probable identity of isolates
TA1	Raised smooth spherical entire creamy opaque	+	-	-	-	-	-	+	+	+	+	+	+	+	<i>Leuconostoc species</i>
TA2	Raised smooth Spherical entire Yellowish translucent	+	+	+	-	-	-	+	+	+	+	+		+	<i>Planococcus species</i>
TA3	Flat smooth spherical entire creamy opaque	+	-	+	+	-	-	+	+	+	-	+	-	-	<i>Micrococcus species</i>
TA4	Flat smooth long rods rhizoid creamy opaque	+	+	+	+	+	+	-	+	-	+	+	-	+	<i>Bacillus species</i>
TA5	Raised, smooth, spherical, entire creamy, translucent	+	+	-	-	+	-	+	+	+	+	+	+	+	<i>Staphylococcus species</i>
TA6	Flat rough short rods entire yellowish opaque	+	+	-	+/-	+	-	+	+	+	+	+	+	+	<i>Streptococcus species</i>
TA7	Raised smooth	+	+	-	-	-	-	+	-	+	+	+	-	-	<i>Pediococcus species</i>

	<i>spherical entire greyish white opaque</i>														
TA8	<i>Flat smooth short rods entire whitish translucent</i>	+	-	+	+	+	-	+	+	+	-	+/-	-	+	<i>Micrococcus species</i>
TA9	<i>Raised rough long rods rhizoid creamy opaque raised rough long rods rhizoid creamy opaque</i>	+	+	+	+	+	+	+	+	+	+	-	-	-	<i>Bacillus species</i>
TA10	<i>Flat rough spherical rhizoid creamy opaque</i>	+	+/ -	-	+	+	+	+	-	+	+	+	-	+	<i>Clostridium species</i>

Similarly, Table 3 shows microbiological characterization of fungi isolates. Three different species of fungi were mainly identified during the study. Changes in the physical and organoleptic properties of canned tomatoes were also determined in this study (Table 4). In general, Table 5 shows the various types of bacteria and fungi isolates encountered during the study.

Table 3. Microbiological characterization of fungi isolates encountered

Code of isolates	Colony morphology	Cell share	Ascospore	Pseudo-mycellum	Probable identity of isolates
TF 1	Cream colored, smooth edge surface and rinsed	Oval	+ve	-ve	<i>Saccharomyces species</i>
TF 2	Cream colored, smooth edge surface and rinsed	Ellipsoidal	+ve	-ve	<i>Saccharomyces species</i>
	white mold to grey in color to black color underneath plate	Mold	-ve	+ve	<i>Aspergillus species</i>
TF 4	white to orange coloration mold	Mold	-ve	+ve	<i>Neurospora species</i>

Table 4. Morphological changes in the physical and organoleptic properties of canned tomatoes

Storage days	Color	Taste	Flavour/odour	Gas production
1 st Week	Reddish brown	Slightly salty	Good and acceptable	Nil
2 nd week	Light brown	Slightly salty	Good and acceptable	Nil
3 rd week	Light brown	Slightly salty	Slightly sour-like odor	Nil
4 th week	Brownish black	Slightly salty	sour-like odor	slight Bubble formation
5 th week	Black	Unpleasant taste	Off flavor	Bubble formation

Table 5. Types of bacterial and fungal species obtained

Microbial group	Species
<i>Bacterial spp.</i> isolated	<i>Leuconostoc Species</i> <i>Pediococcus species</i> <i>Micrococcus Species</i> <i>Staphylococcus species</i> <i>Streptococcus Species</i> <i>Bacillus Species</i> <i>Clostridium species</i> <i>Planococcus species</i>
<i>Fungal spp.</i> isolated	<i>Saccharomyces species</i> <i>Aspergillus species</i> <i>Neurospora species</i>

4. DISCUSSION

The microbiological study of raw tomatoes and canned tomato products which was analyzed in the laboratory showed that various microorganisms can be isolated from both the raw and the canned tomatoes. Observations made under this context signify the presence of certain active sugar fermenters such as the lactic acid bacteria which ferments lactose sugar and the microorganisms which are present as natural microflora of tomatoes. They include organisms such as *Leuconostoc spp.* and *Saccharomyces spp.* as shown in previous investigations [20,21,22].

Various forms of microorganism were isolated from exposed sample source after some weeks of opening of the tomato can in this study. This shows the presence of some spoilage microorganisms such as *Bacillus spp.* and *Clostridium spp* that were associated with the product. This is consistent with the study of Herson and Hullard [7] which demonstrated the involvement of this group of organisms in spoilage. Similarly, various pathogens can invade tomato based on some environmental conditions. Bacterial diseases of tomatoes can be some of the most serious and destructive diseases affecting both field- and greenhouse-grown crops. Under moist field conditions they can cause localized epidemics affecting young developing fruit; in the greenhouse total crop losses can occur. The three bacterial diseases that can cause these adverse conditions are bacterial canker, bacterial speck, and bacterial spot. The third disease can also cause serious damage of peppers [23].

Based on some routine laboratory measures as prescribed by Cheesebrough [24] the sample were examined for the microbial load. The bacterial load observed during the study ranged from 1×10^5 in CT3 to 38×10^5 in CT2 which are the canned sample sources. Similar range of 10 to 21×10^5 cfu/mL was recorded for the raw tomatoes. The reason that could be adduced to this is that the lactic acid component in tomatoes can limit bacteria proliferation in this sample sources. In tomato, the lactic acid bacteria are the major natural microflora of this products due to the high acidic composition of the fruit which depicts the major reason while the product (tomato) usually ferment lactose sugar to give lactic acid. Also, yeast cells such as *Saccharomyces cerearisae* could be present due to its acidity. Other microorganisms found in tomato are not its rich composition [7]. Examples of the lactic acid bacteria in tomato are *Leuconostoc specie* and *Lactobacillus specie*. These bacterial species are its natural flora [20,22]. Under processing of canned foods result in spoilage by thermophilic bacteria and the three types of thermophiles are flat sour, thermophilic acid (TA)

spoilage and Sulphide spoilage. Some of these problems can be curtailed by adequate processing of tomato and sterilization of some facilities on industrial basis [25].

In another replicate sample of the canned tomatoes, the least bacterial count observed during the study immediately after opening of canned sample range from low of 2×10^5 cfu/ml to 18×10^5 cfu/mL. Similarly, the bacterial load after long term storage of opened canned tomatoes ranged from 42×10^5 cfu/mL to 86×10^5 cfu/mL (Table 1b). This is consistent with the range of microbial population of some processed food stuff demonstrated in previous study. However, some of these observations may negate some of the approved standard set in codex committee report by WHO and FAO [26] and Cheesebrought [27]. The highest growth was obtained after opening of can and exposure of tomatoes to air due to presence of contaminants and spoilage microorganism which shows mold formation on the surface of tomatoes and the change in color from reddish brown to brownish black.

5. CONCLUSION

The microbial load including microflora isolated from raw (local) tomatoes sources are at close range to those isolated from the canned tomatoes sources which still shows the natural protective lactic acid components of this food source. Canning is a good means of preserving food such as tomatoes, however, exposure of canned tomato to air gives room for deterioration by microorganisms as shown in this study (Table 1b). So, both raw and canned tomato should be well protected from contaminants to limit proliferation of some spoilage microbes that can use tomatoes as vehicle of infection. Also the technology of canning should be improved by taking adequate precautions to ensure safety during the process. Similarly, the nature of microorganism encountered in this study can be used to determine some food preservation systems in the study area. This will enhance nutritive consumption of this food source to promote health.

ACKNOWLEDGEMENT

I acknowledge the assistance of Prof A.I Okoh (HOD), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa for granting me the enabling environment to conclude this study at the commencement of my research programme with him in South Africa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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