

Revised paper

American Journal of Exp. Botany Agric.
2012/AJEB/2177 enviar auto al ll de octabr

NATURE OF TOMATOES MICROFLORA UNDER STORAGE

By

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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

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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, chill 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 (Jay 2000). Tomato is widely used as condiment or as food dietary supplement in various part of the world and also valuable in the food industry (Njoku, 1991). Canene-Adams *et. al.*, (2005) 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. There is emerging epidemiology data supporting 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 tomato is a native to central north and southern North America from Mexico to Argentina. There is evidence that the first domesticated tomato was a little yellow fruit ancestor of *L. ceraciforme*, grown by the Aztecs in Mexico who called it xitomat (pronounced shi-to-ma-th) meaning plump thing with a navel. 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 (Jay, 2000).

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 (Buchann, 2008). Tomato can be needed for some specialized conditions to strengthen health. According to *The Journal of San Diego History* (1978) a milpa or two of corn, a patch of wheat to sell to the presidios, a few beans, garbanzos, pumpkins, melons, squashes, tomatoes, cabbage and other vegetables were grown for personal consumption in military zones.

In areas and situations where, in season, perfectly ripe tomatoes are not available, canned tomato are of alternative to prepare dishes such as sauce or pizza. The top uses of caned 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 (Herson and Hullard, 1989). Temperature higher than 212°F 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 the boiling point of water (212°F), a pressure canner can be used. The temperature of at least 240°F 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 (240°F) or at 15 pounds of pressure (250°F). Adjustment in canner pressure made according to elevation made above sea level (Herson and Hullard, 1989).

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. Red raw tomatoes has the following values; Nutritional value for-100 g it composes, Energy, 20Kcal-80 KJ, Carbohydrates, 4g, Sugar-2.6g, Dietary fiber-1g, Fat-0.2g, Proteins-1g, Vitamin C, 13mg (22%) and Water 95g. 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. You don't have to worry about botulism in home canned tomatoes because tomatoes are too acidic for bacteria growth (Herson and Hullard, 1989). Ajayi et. al., (2010) also intensify the possibility of getting valuable enzyme like cellulose from tomatoes.

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 (Herson and Hullard, 1989).

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 (Prescott et. al., 2008).

MATERIALS AND METHODS

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 15 minutes in an autoclave and were prepared according to the manufacturers' instruction or standard methods for examination of water (APHA 1998). Culture media generally used for the study are nutrient agar and potato dextrose agar (PDA). All glass ware was 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).

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.

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 fruit and 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. *que son 28.*

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 (Jeanroy and Ward, 2012).

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

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 for 37°C for 24 hours for bacterial culture and 25°-27°C for 5 days for fungal growth. *at*

Sub-Culturing By Streak Method

Nutrient agar, *and* potato dextrose agar *was* 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 (Olutiola et.al., 1991)

Laboratory identification of fungi

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.

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.

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.

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 was used. 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 ~~the~~ 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

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