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ORIGINAL RESEARCH

Isolation and identification of some isolates of some canned tomatoes in Egypt

Mohamed ATIA[1], Hoda MAHROUS[1*] , Hanafy A. HAMZA[2]
[1]Industrial Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt.
[2]Microbial Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt.
*Corresponding Authors email: hmahrous7@yahoo.com

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ABSTRACT
Tomato products are essential source of some nutrients such as vitamin A, vitamin C, fiber, potassium, and very important herbaceous plant (*Solanum lycopersicum* L.) in all countries. The objective of the paper was to evaluate microbial analysis in three popularly consumed canned tomato products from the market in Sadat City, Menofya Government, Egypt. The results indicated that the total count ranged from $2.0\times 10^3\pm0.10$ to $9.7\times10^4\pm0.01$.Twelve isolates were isolated and identified as : 6 gram negative bacteria identified as (2 *Enterobacter aerogenes; Enterobacter sakazakii; Escherichia coli; Pseudomonas aeruginosa and Pseudomonas fluorescens*); 2 gram positive bacteria identified as (*B. brevis* and *B.pasteurii*) and 4 cocci identified as *Micrococcus* sp. *Aspergillus flavus*; *Fusarium oxysporum* and *Macrophomina phaseolina* and *Saccharomyces cerevisiae* were isolated from canned tomato products. However, the opening of tin tomato products allows easy colonization of fungi and this has health implications on human being, Therefore, tin tomato products should be used immediately after opening.

KEY WORDS: *canned tomatoes, Pathogens, microbial count in canned tomatoes*

INTRODUCTION

Tomato (*Lycopersicon esculentum*, commonly referred to as vegetable is grown throughout the tropical and temperate regions of the world (Okorie *et al*., 2004). Tomato is an important herbaceous perrenial vegetable grown for its edible fruit and as an annual vegetable in temperate regions. This fruit vegetable can raise the standard quality and acceptance of other diets and are consumed both as raw and/or processed products. Fresh tomatoes are the fifth most popular vegetable consumed in the United States (16.6 pounds per capita) (USDA, 2000). They are a reasonably good source of vitamins and minerals. It is also very high in moisture and cellulose but low in protein, most of which is in the seed. Although tomato production in Nigeria has more than double in the last decade with the production in 2001 alone reaching 879,000 metric tones (FAO, 2007), and presently up to 1million tones (FAO, 2007), the market continues to decline because of problems which bother on substantial losses during post-harvest transit of this perishable fruit. Tomatoes are now consumed world over. Tomatoes also contain calystegine alkaloids (polyhydroxylated nortropane alkaloids) (Asano et al. 1997, 2001). Tomato products make a significant contribution to human nutrition due to the concentration and availability of several nutrients in these products and to their widespread consumption (Sahlin *et al.* 2004).

Tomato is widely used as condiment or as food dietary supplement in various part of the world and valuable in the food industry (Olorunda and Tung, 1985). It is associated with a reduced risk of chronic degenerable diseases (Agarwa & Aai 2000). Tomato seeds contain high quality plant

proteins that can be supplemented into various food products (Sogi *et al*. 2005). In recent years, tomato has received a considerable increment in its horizontal and vertical total annual production (FAO 2007). A very valuable constituent of tomato is the red pigment carotenoid lycopene, an exceptionally efficient quencher of singlet oxygen and therefore an important anti-oxidant. Lycopene, as well as other valuable substances such as beta-carotene, alphacarotene, alpha-tocopherol, gamma-tocopherol and delta-tocopherol can be effectively extracted from tomato skins, seeds, and other by-products using supercritical fluid extraction technology (Baysal et al. 2000, Rozzi et al. 2002)

Canned tomato pastes are packed in tin or steel cans, an air-tight container for distribution, storage, or preservation. In this study, the aim was to evaluate microbial analysis in three popularly consumed canned tomato products from the market in Sadat City, Menofya Government, Egypt.

RESULTS AND DISCUSSION

Standard plate count (SPC).

The detection and enumeration of microorganisms in food are an essential part of any quality control or food safety plan. decreased the pH of products and increased also the CO_2 production; these conditions inhibit the growth of contaminants in the tomato paste (Benkeblia, 2004). Table 1 showed the microbial content in the tomato cans samples. Absence of *Salmonella; Shigella; S. aureus,* and *Clostridium* in the tomatoes samples (Table 1) showed that thermal treatment and cold storage of tomato inhibit the growth and developments of pathogens and spoilage microorganisms. However, thermal treatments applied during industrial preparation of tomato products (Chanforan *et al*., 2010a and b).

Pre-identification results of strains isolated from samples

A total of 12 isolates were obtained from tomato cans identified as 8 rods:(2 Gram-positive and 6 Gram-negative bacteria) and 4 cocci (Table 2).

Pre-identification test using API System

The conformation of identified isolates was accomplished using API 20E test kit strep for the *Enterobacteriaceae* and API staph for the identification of *Staphylococcus* and *Micrococcus* spp. in table (3).

The Fungi content

Fungi may be found in canned tomato paste due to corrosion and leakage of the metals or from tin foils used in packaging. Different fungi were isolated from the samles of canned tomato products. *Aspergillus flavus*; *Fusarium oxysporum* and *Macrophomina phaseolina* were isolated from canned tomato products. *Saccharomyces cerevisiae* was also isolated from canned tomato products. There might be two factors such as packaging and processing method that influence growth of fungi and the proximate composition of tomatoes. This result was in accordance with the report made by Alabi & Esan (2013) who identified *Aspergillus flavus*, *A. fumigatus Fresenius, A. niger van Tieghem* and *Fusarium* sp. associated with the spoilage of the industrial tomato paste. The processing stages are more influential factors than production method. *Aspergillus* sp. is very common and is involved in spoilage of food items. This work is also in line with the work of Kolawole *et al.* (2010) who reported the presence of *Aspergillus* sp., *Aspergillus niger, Rhizopus stolonifera* and *Penicillium chrysogenum* in dried tomato products.

MATERIALS AND METHODS

Collection of samples

Three different brands of canned tomatoes were purchased from the market in Sadat City, Menofya Government, Egypt. The samples were brought to the laboratory and analyzed immediately. However, products of canned tomato purchased were labeled and immediately transported to the laboratory in an ice-box where they were processed immediately.

Standard plate count (SPC)

Numerous selective and differential tests used to determine the specific types of bacteria in samples. The canned tomato products were aseptically opened using a sterile tin cutter in a microbial free environment. Serial dilutions of tomato were used for microbial enumeration as: 5 ml of the canned

Table 1: Standard plate count (SPC) of samples.

Samples / Count	Can 1	Can 2	Can 3
PCA	2.0× 10^3±0.10	6.1×10^4±0.03	9.7×10^4±0.01
V. R (*E. coli*)	1×10^2±0.02	2×10^2±0.10	3×10^2±0.11
V.R (*Enterobacter*)	-	-	3×10^3±0.13
Vogel Johnson (*Staph.aureus*)	-	-	-
XLD (*Salmonella &* *Shigella*)	-	-	-
Spore forming bacilli	1.6×10^2±0.01	1.3×10^2±0.05	1.7×10^2±0.16
S F (*Enterococcus*)	-	-	2×10^2±0.12
PDA	50 ±0.01	35 ±0.03	72 ±0.04

Note: +, - indicates present and not present respectively, Data are presented as mean ± SD

tomato products was measured into each of the sterilized bottles labeled accordingly. This was vigorously shaken, and 1 ml of sample was pipette into a sterile tube containing 9 ml of distilled water. The sample was serially diluted and 1 ml each of aliquots of 10^6 and 10^7 were added plated on different types of selective media for total count bacteria using plate count agar (P.C.A, biolife. Italy), coliform and *Enterobacter* sp. count using Violet Red Bile Glucose Agar (V.R.A, biolife. Italy), *Staphylococcus aureus* count using Vogel and Johnson Agar (V.J.A. biolife. Italy), thermophilic spore forming bacilli using Nutrient Agar*, Entercoccus* spp. using *Streptococcus faecalis* Medium (S.F.A biolife. Italy) and *Salmonella* spp. using Xylose lysine deoxycholate agar (X.L.D.A. biolife. Italy) and potato dextrose agar (PDA) for fungal content. The plates could solidify and incubated at 30°C for 3–5 days for PDA and the other plates for 37^{o}C for 24 h. The fungal colonies were counted every 24 hours. Successive hyphae tip was transferred until pure cultures of each of fungus was obtained. Pure culture was obtained by picking distinct colonies of fungi from the pour plate using inoculating needle and subculture into freshly prepared plates of PDA. The plates were incubated at room temperature. After which the pure culture was transferred into slant for fungal. Different colonies were isolated in Nutrient broth media and incubated at 37˚C for 48 hours. After the incubation period, Gram staining examined cultures and biochemical testes.

Microbiological identification tests

Isolates were identified according to Bergey's Manual Analyses for biochemical and microbiological characteristics and AOAC (2005).

Identification of Gram Negative Bacilli—IMViC

The IMViC tests are a group of individual tests used in microbiology lab testing to identify an organism in the coliform group also; these tests are useful for differentiating the family *Enterobacteriaceae* Each of the letters in "IMViC" stands for one of these tests. "I" is for indole; "M" is for methyl red; "V" is for Voges-Proskauer, and "iC" is for citrate.

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Table 2: Pre-identification results of strains isolated from samples

No.	Shape	Ox.	Cat.	Salt%			Starch	Nit.	Indol	MR.	V/P	Cit.	Gelatin	Urease	Motil.	Pre-identified Strain
				4	6	10										
1	Rod-	-	+	+	-	-	-	+	-	+	-	-	-	-	+	*E.adecarboxylata*
2	Rod-	-	+	+	+	+	-	+	+	-	+	-	-	-	+	*E.agglomerans*
3	Rod+	+	+	+	+	-	-	+	+	+	-	+	-	-	+	*B.fastidiosus*
4	Rod-	+	+	+	+	-	-							-		*K.oxytoca*
5	Rod-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	*Xenorhabdus luminescens*
6	Cocci	-	+	+	-	-	-	+	-	+	-	+	-	-	+	*Planococcus*
7	Cocci	-	+	+	-	-	-	-	-	+	-	+	-	-	-	*M. roseus*
8	Rod-	-	+	+	+	-	±	+	+	+	-	+	-	-	±	*Providencia alcalifaciens*
9	Cocci	-	-	+	-	-	-	+	-	+	±	+	-	-	-	*M. varians*
10	Cocci	-	-	+	-	-	-	-	-	+	±	-	-	-	+	*M. agilis*
11	Rod-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	*Xenorhabdus luminescens*
12	Rod+	-	+	+	+	+	-	+	-	±	-	-	-	-	+	*B.pasteurii*

Nitrate Test

The nitrate reduction test is based on the detection of nitrite in the medium after incubation with an organism If present in the medium, nitrite will react with sulfanilic acid (Nitrate reagent A) to form a colorless complex (nitrite-sulfanilic acid). This complex will then yield red precipitate when nitrate reagent B (alpha-naphthylamine) is then added to the test. A red color will be produced in the medium only when nitrite is present in the medium. Lack of a red color in the medium (after the addition of sulfanilic acid and alpha-naphthylamine) means only that nitrite is not present in the medium. If the nitrate is unreduced and still in its original form, this would be a negative nitrate reduction result.

However, it is possible that the nitrate was reduced to nitrite but has been further reduced to ammonia or nitrogen gas. This would be recorded as a positive nitrate reduction result. To distinguish between these two reactions, zinc dust must be added. Zinc reduces nitrate to nitrite. If the test organism did not reduce the nitrate to nitrite, the zinc will change the nitrate to nitrite. The tube will turn red because alpha-naphthylamine and sulfanilic acid are already present in the tube. Thus, a red color indicates the zinc found the nitrate unchanged. This is recorded as a negative nitrate reduction test.

Urease Reaction

Urease hydrolyzes urea releasing ammonia which alkalinizes the medium by forming ammonium carbonate, and the pH indicator

phenol red becomes red **.***Proteus*, *Morganella,* and *Providencia* are strong urease producers, *Klebsiella* is a weak urease producer, and *Yersinia enterocolitica* frequently a urease producer.

Gelatin hydrolyses

Cultures were stabbed on nutrient gelatin tubes and incubated at 20˚C for 7 days. After 7 days the tubes were placed in refrigerator for 15 minutes. After refrigeration time, if the gelatin remains liquid, the result is positive. If gelatin is solid the result is negative.

Starch hydrolysis

Flood the surface of a 48-hour culture on Starch Agar that consists of 10 gm/L of Trypton, 10gm/L yeast extract, 5gm/L of K_2HPO_4, 3gm/L of soluble starch, 15gm/L of Agar Agar with Gram Iodine. Starch hydrolysis (+) is indicated by a colorless zone surrounding colonies. A blue or purple zone indicates that starch has not been hydrolyzed (-).

Growth in the presence of 4, 6.5, 10% salt

Salt tolerance was assessed after 2 days of incubation at concentrations of 4, 6.5, 10% NaCl in nutrient broth medium.

Table 3: Pre-identification results of Gram Negative rods isolates using API20E

Identification using API System	**Number of isolates**
Enterobacter aerogenes 99.7%	**2**
Enterobacter sakazakii 98.4%	**1**
Echerichia coli 99.9%	**1**
Pseudomonas aeruginosa	**1**
Pseudomonas fluorescens	**1**
B. brevis	**1**
B.pasteurii	**1**
Micrococcus spp.	**4**

Oxidase test

The Oxidase test was performed according to filter paper method that described below: soak a piece of filter paper in the reagent solution. Scrap some growth from the culture plate with a disposable loop or stick and rub onto the filter paper or touch a colony with edge of paper and examine the blue color within 10 seconds.

Catalase test

This test is used for studying the ability to break down toxic O_2 by catalase of peroxidase (catalyzes the destruction of hydrogen peroxide).

$$2\ H_2O_2 + \text{catalase} \longrightarrow 2\ H_2O + O_2$$

Motility Test

Motility Test Medium is used for the detection of motility of gram-negative enteric bacilli. Bacterial motility can be observed directly from examination of the tubes following the incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of nonmotile organisms only occurs along the stab line.

Apparatus and Procedure of Identification (API) system

The API-20E test kit, and API staph (BioMérieux, Marcy-l'Etoile, France) provide an easy way to inoculate and read tests relevant to members of the Family *Enterobacteriaceae* and associated organisms, and staphylococcus cultures, respectively. The API strip consists of microtubes containing dehydrated substrates for the demonstration of enzymatic activity and carbohydrate (CHO) fermentation. The substrates are reconstituted by adding a bacterial suspension. After incubation, the metabolic end products are detected by indicator systems or the addition of reagents. CHO fermentation is detected by colour change in the pH indicator. The gallery was read after 24 and 48 hours of incubation at 37 °C and compared to a standard profile using API LAB plus software version 1.2.1.

Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of samples from the tomato cans and the control.

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