Journal of Research in Microbiology

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2021; 2(1): 01-06

www.microbiojournal.com
Received: 11-01-2021
Accepted: 02-03-2021

Aguda OY
Bioscience Department, Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

Faola DB
Bioscience Department, Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

Oyedeji MB
Federal College of Forestry, Ibadan, Nigeria

Adegboyega DA
Bioscience Department, Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

Akala AO
Bioscience Department, Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

Omotoso TP
Department of Science Laboratory Technology, Federal Polytechnic, Ile-Oluji, Nigeria

Correspondence
Aguda OY
Bioscience Department, Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

Microorganism associated with the deterioration of tomato fruits (*Lycopersicon esculentum*) in Shasha Market, Akure, Ondo State

Aguda OY, Faola DB, Oyedeji MB, Adegboyega DA, Akala AO and Omotoso TP

Abstract
Tomato is a very important fruit in Nigeria and the world as a whole, as it constitutes major world dishes. It is widely grown for home consumption and for sale. Tomato in its fresh form is highly demanded for both domestic and commercial purposes. However, tomato post-harvest losses are a threat to the harvested tomatoes as they are being subjected to harsh environmental factors as well as biological factors. From this study, it was seen that these losses are due to improper handling of the tomato fruits by man during cultivation, harvesting and even all other processes it undergoes after harvesting till it gets to the final consumer. This study was designed to validate the pathogens associated with deterioration of tomatoes fruits. The pH, proximate analysis, total bacterial and fungal counts and Pathogenicity test were carried out using laboratory standard technique. Fourteen (14) pathogens both bacteria and fungi were isolated from infected tomato samples and they varied significantly with *Aspergillus niger* being the most prevalent and *Rhizopus stolonifer* being the least prevalent for the fungal isolates while *Bacillus* sp. was the most prevalent and *Proteus mirabilis* the least prevalent from the bacterial isolates. Damage caused by the pathogens on tomato fruits also varied with *Aspergillus niger* causing about 100% rot for the fungal isolates while *Escherichia colica* used the most significant rot for the bacterial isolates. Data was analysed using SPSS and SAS One way ANOVA. This study recommends that farmers and traders should properly handle the tomato fruits right from cultivation to final consumers. The general public at large should be educated on the detrimental effects of consuming deteriorated tomato fruits.

Keywords: Tomaotes, diseases, pathogens, microorganism, post-harvest

Introduction
Tomato is a widely consumed fruit eaten in both raw and processed forms (Moneruzzaman *et al*., 2008) [15]. It has the botanical name *Lycopersicum esculentum* and belongs to the plant family solanaceae. It is rich in vitamins including vitamin A and vitamin C, carbohydrates, proteins, fats, fibres and potassium (Talvas *et al.,* 2010) [20]. It is rich in lycopene which has many beneficial health effects. Tomato is a fruit that contains the seeds and ovary of a flowering plant (Ugwu *et al*., 2014). It has much lower sugar content than other edible fruits. Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well-balanced diet which is rich in vitamins (such as vitamin A, B, C and E), carbohydrates (such as fructose and glucose), minerals (which include phosphorous, sodium, potassium, calcium, magnesium) and trace elements (like, Zinc and iron, copper) (Ugwu *et al*., 2014). The Deep red coloration of ripened tomato is due to the presence of lycopene, a form of B-carotenoid and a powerful pigment that help prevent cancer, cardiovascular disease and diabetes, thus there is an appeal and demands of the fruits by consumer as a result of their knowledge that they are healthy, tasty, convenient and fresh (Ugwu *et al*., 2014). Tomato is of juicy flesh endocarp belonging to the fruit class, berry. Naturally, it is very rich in vitamins, minerals, dietary fibre and protein (Wogu and Ofuase, 2014). Tomato fruits are not only food but as medicine, nutrient supplement, flavoring ingredient, detoxificant and human system cleanser (Abhinaba, 2009) [1].
Food spoilage refers to several changes which make the food to be toxic and less palatable to consumers, and these could be associated with alterations in appearance, texture, taste or smell (Akinmusire, 2011; Ogunbanwo *et al*., 2014) [2, 17]. This is due to contaminations with mycotoxins (naturally occurring toxic chemical usually of aromatic structure) that produces

aflatoxins in human, following inhalation or ingestion resulting to food poisoning (Muhammad, *et al*., 2004; Ogunbanwo *et al*., 2014) [17]. The objective of this study therefore focused on isolation and identification of human pathogens and spoilage microorganisms associated with tomato fruit.

Material and Method

Akure is the capital city of Ondo State dominated mainly by the Yoruba ethnic group, located in south-west Nigeria. Akure lies about 70°15 north of the equator and 50°15 east Meridian. The tomato samples used for this study were collected from Shasha market.

Sample collection

Two different samples (twenty in all) of tomato fruits ('Hausa specie') were collected randomly from different sellers at Sasha market, Akure, Ondo State, Nigeria.

Materials

Nutrient agar (NA), *Salmonella Shigella* agar (SSA), MacConkey agar (MAC), Potato Dextrose agar(PDA), cotton wool, aluminium foil paper, 70% ethanol, syringe, petri dishes, conical flask, beaker, gloves, measuring cylinder, weighing balance, microscopic slide, cover slip and a compound microscope.

Determination of pH value

The pH values of all the tomato samples was obtained using a glass electrode pH meter, which was standardized prior to use each time with a neutral buffer (distilled water of pH 7.0). The liquid obtained from the mashed tomato sample was transferred into a sterile conical flask, after which the pH meter was inserted into the liquid. The readings on the pH meter were then recorded (Fawole and Oso, 2004) [11].

Isolation of bacteria from Tomato samples

Bacteriological examinations were carried out using standard methods for aerobic bacteria.

Each tomatoes sample collected in sterile polythene bags was aseptically crushed in a mortar and pestle, aliquot (1.0 ml) was transferred into the test tube containing 9.0 ml of sterile distilled water and diluted serially in one-tenth stepwise to 10^{-7} dilution factor and 1.0 ml each of dilution $10^{-4,}$ 10^{-5}, 10^{-6} and 10^{-7} was pour plated on Nutrient agar and some selective and differential media (*Salmonella shigella* agar and MacConkey agar)the plates were inverted and incubated aerobically at 37°Cfor 24 hours after which the plates were examined for growth.

Isolation of fungi from Tomato samples

Each sample collected in sterile polythene bags was aseptically crushed in a mortar and pestle, aliquot (1.0 ml) was transferred into the test tube containing 9.0 ml of sterile distilled water and diluted serially in one-tenth stepwise to 10^{-7} dilution factor and 1.0 ml each of dilution $10^{-4,}$ 10^{-5}, 10^{-6} and 10^{-7} was pour plated on Potato dextrose agar, the plates were inverted and incubated aerobically at 28°Cfor 48 hours after which the plates were examined for growth.

Isolation and characterization of bacteria isolates

The bacteria colonies that developed on all media plates were subcultured by streaking on a freshly prepared nutrient agar plates until pure colonies were obtained accordingly to the conventional procedure as highlighted by Fawole and Oso (2004) [11]. Cultural characteristics of the discrete bacteria colonies such as colour, shape, pigmentation and opacity on nutrient agar were observed and noted; also, growth characteristic on selective and differential media was observed and recorded. Discrete bacteria colonies that developed were cultured on double strength nutrient agar slant and incubated at 37°Cfor 24 hours, growth was observed and the slants were stored in the refrigerator to preserve the bacterial isolate. The isolate on slant was sub cultured on freshly prepared nutrient agar subsequently.

Isolation and characterization of fungi isolates

The fungi colonies that developed on all media plates were subcultured by streaking on a freshly prepared potato dextrose agar plates until pure colonies were obtained accordingly to the conventional procedure as highlighted by Fawole and Oso (2004) [11]. Cultural characteristics of the discrete fungi colonies such as colour, shape and pigmentation on potato dextrose agar were observed. Discrete fungi colonies that developed were cultured on double strength potato dextrose agar slant and incubated at 28°Cfor 48 hours, growth was observed and the slants were stored in the refrigerator to preserve the fungal isolate. The isolate on slant was sub cultured on freshly prepared potato dextrose agar subsequently.

Morphological and biochemical characterization of bacteria isolates

Different tests were carried out on isolates, which include; Gram reaction, Coagulase, Motility, Oxidase, Indole production, Sugar fermentation and Catalase test described by APHA (2002) and Fawole and Oso (2004) [11].

Proximate Analysis

Proximate Composition

These were determined in terms of moisture content, crude protein, fat, ash content, crude fiber and carbohydrate according to the standard methods AOAC, (1990) [4].

Moisture content determination

5g of the tomato samples were weighed each into a Petri-dish of a known weight. It was dried in the oven set at 105^0C for 4 hours. The tomato samples were removed and cooled in desiccators. After cooling, the samples were reweighed for any weight change. It was returned to the oven at the same temperature and later cooled and weighed. These subsequent weighing was done at 30 minutes interval until a constant weight was obtained .The result was calculated thus:

$$\% \text{ Moisture Content} = \frac{\text{Moisture Loss}}{\text{Original weight of sample}} \times 100$$

100-(% Moisture + % Ash + % Fat + % Protein + % Crude fibre)

Ash content determination

Cleaned and dried crucibles were weighed using weighing balance (PM400) and the weight recorded as (W_1). 1g of tomato samples were weighed into the crucible and weight recorded as (W_2) and later placed in a muffle furnace and gently heated for combustion. The temperature of the furnace was maintained at 550^0C for 4 hours. Ashing continued until a light grey ash was obtained. The crucibles and the content was cooled in a desiccator and reweighed as (W_3).

$$\%\ ASH\ = \frac{W3\ -W2}{W2\ -W1} \times 100$$

Crude protein determination
The Kjeldahal method was used for the determination of percentage crude protein. The process is in three stages:

Digestion
0.5g of the sample was weighed into Kjelahal digestion flask and selenium catalyst and 5ml of concentrated H_2SO_4 added. At this stage, all the carbon present in the sample would be converted into carbon (IV) oxide ($C0_2$), Nitrogen to ammonium sulphate $(NH_4)_2SO_4$, sulphur and phosphorus to their oxides, which were given off as gasses. The mixture was heated on an electro-thermal heater until colourless solution obtained. Chemical reaction involved during digestion process:

$$2NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

$$C + O_2 \xrightarrow{CO_2}$$

$$S \xrightarrow{O_2} SO$$

Distillation
This involves steam distillation of the diluted digested samples which have been diluted with distilled water and made up to 50ml. After cooling, 10ml of 40% NaOH was added and the mixture distilled into a conical flask containing 5ml of 2% boric acid solution in 100ml of distilled water. To the mixture, added 4 drops of mixed indicator containing (0.01g ethyl red + 0.083g 100ml alcohol). This was placed in such a way that the delivery tube touched the boric acid level inside the conical flask while the tube was being cooled with cold water. The 40% NaOH solution was used to titrate ammonia out of the whole digest thereby making the mixture alkaline. During distillation, all other outlets were tightly closed to avoid loss of ammonia. The liberated ammonia was trapped in boric acid in the receiver flask and the mixture turned light green indicating complete reaction of ammonium with boric acid. Distillation continued until about 50ml of the distillate has been collected into the receiver flask. Chemical equations of the reactions involved at this stage are:

$$(NH_4)_2SO_4 + 40\%\ NaOH \longrightarrow 2NH_3 + Na_2SO_4 + H_2O$$

$$NH_3H_3BO_3 \longrightarrow NH_3 + H_3BO_3$$

Titration
Titration was the final stage of protein determination where the resulting solution from distillation was titrated with 0.1mHCL until the initial colour reappeared (colourless) at the end point. The chemical reaction involved:

$$NH_3 + HCL \longrightarrow NH_4CL$$

$$\%\ NITROGEN\ = \frac{T \times 0.0014 \times D}{W} \times 100$$

Where: D = Dilution factor
T = Titre value
W = Weight of sample
% Crude protein = % Nitrogen X 6.25 (conversion factor)

Carbohydrate determination
Carbohydrate was determined by difference using the method of AOAC, 1990 [4] with the formula below;

Data analysis
Data was categorized and analyzed using SPSS version 20.

Results

Table 1: pH values of spoilt and apparently healthy tomato samples

Samples	pH of spoilt tomatoes	pH of apparently healthy tomatoes
1	4.70	5.00
2	4.50	5.00
3	4.60	4.70
4	4.70	4.50
5	4.80	5.00
6	4.40	4.80
7	4.60	4.70
8	4.40	4.70
9	4.70	4.60
10	4.50	5.00
Mean pH value	4.59±0.04	4.80±0.06

The pH of the spoilt tomato samples ranges from 4.4-4.8 while the pH of the apparently healthy tomatoes ranges from 4.5-5.0. The mean pH value of the spoilt tomato (4.59± 0.04) is lower than the healthy tomato samples (4.80± 0.06)

Table 2: Total bacterial and fungal counts for spoilt tomatoes

Samples	Total bacterial counts (cfu/ml x 10^4)	Total coliform counts (cfu/ml x 10^4)	Total fungal counts (sfu/ml x 10^2)
S1	35	17	47
S2	92	61	56
S3	76	40	40
S4	45	36	18
S5	53	14	16
S6	43	22	42
S7	23	12	36
S8	26	25	19
S9	65	44	29
S10	42	29	9

Table 3: Total bacterial and fungal counts on for apparently healthy tomatoes

Samples	Total bacterial counts (cfu/ml x 10^4)	Total coliform Counts (cfu/ml x 10^4)	Total fungal Counts (sfu/ml x 10^{-2})
F1	8	1	1
F2	2	1	2
F3	4	7	6
F4	17	2	0
F5	9	12	5
F6	12	5	6
F7	15	8	0
F8	5	8	4
F9	12	7	6
F10	7	4	0

Total viable count obtained from tomato samples in Shasha market, Akure

Total bacterial viable counts are shown in Table 2 and Table 3.These tables include the total bacterial count, the coliform counts as well as the fungi colony counts from both spoilt and healthy tomatoes. Table 2 shows that the total viable bacterial count ranges from (23 x10^4 to 92 x 10 ^{4}) cfu/ml, while the coliform count ranges from (12 x10^4 to 60 x 10 ^{4})cfu/ml and the fungal colony counts ranges from (9 x10^2 to 56 x 10 ^{2}) sfu/ml for the spoilt tomatoes. Table 3 shows that the total viable bacterial count ranges from (2 x10^4to17 x 10 ^{4})cfu/ml, while the total coliform count ranges from (1 x10^4 to 12 x 10 ^{4}) cfu/ml and the total fungal colony count ranges from (0 x10^2 to 6x 10 ^{2}) sfu/ml. These results revealed that there was the total bacterial count; the total coliform count as well as the total fungal colony count was higher in the spoilt tomato samples than healthy tomato samples.

Table 7: Percentage occurrence of microorganisms isolated from the tomato samples

Microorganisms	Spoilt tomatoes (%)	Apparently healthy tomatoes (%)
Escherichia coli (20)	10 (50.00)	10 (50.00)
Bacillus sp (17)	8 (47.06)	9 (52.94)
Salmonella typhi(16)	10 (62.50)	6 (37.50)
*Shigella*sp (15)	10 (66.67)	5 (33.33)
*Klebseilla*sp (10)	10 (100.00)	0 (0.00)
Staphylococcus aureus (19)	10 (52.63)	9 (47.37)
Pseudomonas aeruginosa(10)	10 (100.00)	0 (0.00)
Proteus mirabilis (9)	9 (100.00)	0 (0.00)
Saccharomyces cerevisiae(17)	10 (58.82)	7 (41.18)
*Penicillium*sp (12)	7 (58.33)	5 (41.67)
*Mucor*sp (8)	8 (100.00)	0 (0.00)
*Fusarium*sp (12)	9 (75.00)	3 (25.00)
*Aspergillus*sp (17)	10 (58.82)	7 (41.18)
*Rhizopus*sp (9)	9 (100.00)	0 (0.00)

Percentage occurrence of microorganisms isolated from both spoilt and apparently healthy tomatoes

Table 7 shows the percentage occurrence of both bacteria and fungi isolated from the spoilt and apparently healthy tomato samples. From the result it is seen that in the spoilt tomatoes, *Bacillus subtilis* has the lowest percentage occurrence of 47.06% while *Klebseilla* sp, *Pseudomonas aeruginosa* and *Proteus mirabilis* have percentage occurrences of 100.00% each. *Penicillium* sp has a percentage occurrence of 58.82% while *Mucor* sp and *Rhizopus* sp have percentage occurrences of 100.00% each. The apparently healthy tomatoes have *Bacillus subtilis* with a percentage occurrence of 52.94% as its highest while *Klebseilla* sp, *Pseudomonas aeruginosa* and *Proteus mirabilis* have 0.00% occurrence as its lowest. The percentage occurrence of *Mucor* sp and *Rhizopus* sp is lowest with 0.00% each and highest with the percentage occurrence 41.18% of *Penicillium* sp.

Table 8: Result for proximate analysis

Samples	Moisture (%)	Fat (%)	Ash (%)	Fibre (%)	Protein (%)	Carbohydrate (%)
Apparently healthy tomato	93.16	0.80	0.37	1.51	3.16	1.00
	92.75	0.83	0.35	1.54	3.12	1.41
Spoilt tomato	92.73	0.65	0.56	0.95	2.79	2.32
	92.41	0.68	0.56	0.98	2.79	2.58

Table 8 shows the percentage amount of moisture, fat, ash, fiber, protein and carbohydrate in both spoilt and apparently healthy tomato samples. The result shows that the percentage moisture (93.16%), fat (0.80%), fiber (1.51%) and protein (3.16%) content are higher in the apparently healthy tomato samples compared to the spoilt tomato samples. The result also revealed that the percentage ash (0.56%) and carbohydrate (2.36%) content are higher in the spoilt tomato samples.

Discussion

Tomato fruit as well as other fruits and fresh vegetables have epidermal layer which serve as a natural protective cover that effectively guide against most pathogenic

microbes and plant spoilage. However, this protective cover can be tampered with during cultivation and harvesting of the produce by improper handling by man and interference with certain environmental factors which are subject to change.

The pH of the spoilt tomato samples ranges from 4.40-4.80 as shown in Table 1 which is different from 4.2-4.3 obtained by Jay (2000) [13], this slight increase in pH may be as a result of the neutralizing effect of the microbial metabolic products, produced by the initial contaminants of the tomato fruit. This statement is supported by ICMSF (2005) [12] and Bartz *et al*. (2009) [6].

The presence of coliforms such as *Escherichia coli*, *Klebseilla* sp in the tomato samples indicate that other pathogenic organisms of faecal origin may be present. These pathogenic organisms may have been opportunistic pathogens via the use of manure as fertilizer for growing of tomato. *Escherichia coli* is a normal flora of the human gut as well as the intestines of humans and animals. Although most types of *E. coli* are harmless, some types can cause diseases. The worst type of *E. coli*, known as *E. coli O157:H7*, causes bloody diarrhea and can sometimes cause kidney failure and even death. *E. coli* O157:H7 makes a toxin called Shiga toxin and is known as a Shiga toxin-producing *E. coli* (STEC) (CDC, 2012). There are many other types of STEC, and some can make you just as sick as *E. coli* O157:H7 (CDC, 2012). One severe complication associated with *E. coli* infection is hemolytic uremic syndrome (HUS) (CDC, 2012). The infection produces toxic substances that destroy red blood cells, causing kidney injury. HUS can require intensive care, kidney dialysis, and transfusions. The application of these fetilizers to tomato plant can infect the fruits with *Escherichia coli* which causes severe diarrhoea and can also cause pneumonia, and other respiratory illnesses and urinary tract infections (CDC, 2012). *Salmonella typhi* and *Shigella* sp isolated from these tomato samples can also be as result of manure used as fertilzers or through irrigation with water contaminated with faeces or poor hygiene habits of farmers and traders. The health implications of these organisms are Typhoid fever and Shigellosis respectively. The *Shigella* germ is a family of bacteria that can cause diarrhea in humans (CDC, 2012). People with shigellosis shed the bacteria in their faeces (CDC, 2012).

The soil is most intensively inhabited by *Fusarium*, *Aspergillus* and *Penicillium*. The presence of these organisms in the tomato samples is attributed to the cultivation process where the tomato plant in contact with the soil and obtains its nutrients from the soil through its roots.

The result of the proximate analysis showed that the apparently healthy tomatoes have higher percentage values of moisture content (93.16%), fat (0.80%), fibre (1.51%) and protein (3.16%) than the spoilt tomatoes. This shows that the apparently healthy tomato samples are more beneficial to humans than the spoilt tomato samples because it contains higher amount of protein which is needed for building and repair of tissues, it serves as an important building block of bones, cartilage, skin and blood (Duyff, 2012) [17]. The apparently healthy tomato is richer in fibre than the spoilt one with percentage values of 1.51 and 0.95 respectively. This shows that the healthy tomato which is richer in fibre helps to prevent cancer, maintain bowel health as well as it normalizes its movement, it lowers cholesterol levels and helps to control sugar levels (Duyff, 2012) [7]. The apparently healthy tomatoes are also rich in fat. In fact, the beneficial effects of this cannot be over-emphasized. Fat is important in human health as it helps in vitamin absorption as well as it serves as a source of energy. Fat cells, stored in adipose tissue, insulate the body and help sustain a normal core body temperature. (Duyff, 2012) [7]. However, in the spoilt tomato samples, the percentage of carbohydrate is high. The major function of carbohydrate is that it serves as a source of energy which fat can also be akin to in the apparently healthy tomato fruit. The amount of Carbohydrate in the apparently tomato sample is complemented by the fat content, which ideally should give the necessary daily energy requirement to man.

Conclusion

Tomato fruits have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other importance cannot be over emphasized, as its spoilage often result to wastage of economic resources as well as food poisoning. Their spoilage by fungi results in loss of economic resources as well as food poisoning. The result in this study has shed light into the gaining of entrance of food borne pathogens as well as some spoilage microorganisms (mostly fungi) during selling, harvesting and cultivation which may result in food poisoning. In this study, it was revealed that tomatoes are of great benefits to human health. The only constraint in the consumption of tomato is the presence of spoilage and pathogenic organisms. In this study, it was revealed from the occurrence of the isolated organisms and the pathogenicity test that *Aspergillusniger*, *Escherichia coli*, *Mucor* sp and *Proteus mirabilis* are the main spoilage organisms of tomato fruit.

References

1. Abhinaba G. Identification of microorganisms responsible for spoilage of tomato (*Solanum lycopersicum* L) fruit. J Phytopath 2009;1(6):414-416.
2. Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri, Northern Eastern Nigeria. Advanced Environmental. Biology 2011;5(1):157161.
3. Akinyele BJ, Akinkunmi CO. Fungi associated with the spoilage of berry andtheir reaction to magnetic field. Journal Yeast and Fungal Resource 2012;3(4):49-57.
4. AOAC. Official Methods of Analysis of Association of Analytical chemists (AOAC).International, 12th Edition, Arlington, Texas, USA 1990;4:56-90.
5. Bartz M, Hankinson TR, Zhuang H, Breidt F. Microbiological Spoilage of Fruits and Vegetables. W.H. Sperber, M.P. Doyle (eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety.C Springer Science Business Media, LLC 2009, 135-183.
6. Bartz JA, Sargent SA, Mahovic M. Guides to Identifying and Controlling Post Harvest Tomato Diseasein Florida 2009. http://www.edis.ifas.ufl.edu/topictoma diseases.
7. Duyff RL. Carbs: sugar, starches, fiber, In:American Dietetic Association Complete Food and Nutrition Guide. 4th ed. Hoboken, N.J: Wiley& sons 2012.
8. Eni AO, Ibokunoluwa O, Oranusi U. Microbial quality of fruits and vegetables. Africa Journal Food Science 2010;4(5):291-296.

9. Fajola AO. The Post Harvest Fruit Rot of Tomato (*Lycopersicon esculentum*) in Nigeria. Molecular Nutrition and Food Research 2006;23(2):105-109.
10. Fatima D, Mebrouk K, Miloud H. Biopreservation of tomato paste and sauce with *Leuconostoc* spp. metabolites. Africa journal of Food Science 2015;1(6):359-366
11. Fawole MO, Oso BA. Laboratory Manual of Microbiology.4th edition. Spectrum Books Limited 2004, 1-127.
12. ICMSF. Microorganisms in Foods In: Microbial ecology of Food Commodities. Kluwer Academic/ Plenum Publishers, New York, USA 2005, 23-56.
13. Jay JM. Modern Food Microbiology.6th Edition. Gaithesburg CMD: Aspen 2000, 679.
14. Marikawa M. Beneficial Biofilm Formation by Industrial Bacteria B. subtilis sand related Species. Journal of Bioscience and Bioengineering 2009;101(1):1-8.
15. Moneruzzaman KM, Hossain ABMS, Sani W, Saiffudin M. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. American Journal of Biochemistry and Biotechnology, 2008;4(4):336-344.
16. Moser MJ, Christensen DR, Norwood O, Prudent JR. Multiplexed Detection of Anthrax Related Toxin Genes. Journal of Molecular Diagnosis 2006;8:89-96.
17. Ogunbanwo ST, Fadahunsi IF, Molokwu AJ. Thermal stability of lactic acid bacteria metabolites and its application in preservation of tomato pastes. Malaysia. Journal Microbiologyl 2014;10(1):15-23.
18. Oladipo IC, Adeleke DT, Adebiyi AO. The effect of pH and Chemical Preservatives on the Growth of Bacteria Isolates from some Nigerian packaged Fruit Juices. Pakistan Journal of Pharmaceutical Science 2010;13:16-21.
19. Ryu JH, Beuchat LB. Influence of acid Tolerance Responses on Survival, growth and Thermal Cross-Protection of E. coli 0157:H7 in Acidified Media and Fruit Juices. International Journal of Food Microbiology 1998;45:185-193.
20. Talvas J, Caris-veyrat C, Guy L, Rambeau M, Lyan B, Minet-Quinard R *et al*. Differential effects of lycopene consumed in tomato paste and lycopene in the form of a purified extract on target genes of cancer prostatic cells. American Journal of Clinical Nutrition 2010;91(6):1716-1724.