Journal of Advances in Microbiology

8(4): 1-9, 2018; Article no.JAMB.38965
ISSN: 2456-7116

Effect of Blanching and Oil Marination on Bacteriological Quality of Tomato

S. G. Ibrahim[1*], A. S. Isa[1] and A. Yusuf[1]

[1]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author SGI designed the study, performed the data analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ASI supervised the study and reviewed the manuscript and author AY managed the literature searches and participated in the laboratory analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/38965
Editor(s):
(1) Adekunle Sanyaolu, Epidemiology Division, Nigeria Center for Disease Control, Federal Ministry of Health, Abuja, Nigeria.
Reviewers:
(1) Mustapha Umar, Nigerian Institute of Leather and Science Technology, Nigeria.
(2) M. Pandimadevi, SRM University, India.
(3) Selma Gomes Ferreira Leite, Federal University of Rio de Janeiro, Brasil.
(4) Neeta Bhagat, Amity University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/23405

Original Research Article

Received 22nd December 2017
Accepted 7th February 2018
Published 3rd March 2018

ABSTRACT

Tomato has suffered a great yield loss owing to microbial contaminations, especially fungi and bacteria. This study was carried out to know the effect of blanching and oil marination on the total bacterial count and isolates present in tomato. Fresh tomato samples were collected from Kasuwan Daji, Sokoto state. The tomato was divided into three groups; two groups were processed into blanched and un-blanched marinated into oil. The bacteria analyses of both the fresh and preserved stored samples were carried out at day 1 for fresh and after 2 days interval for preserved tomatoes for a period of 14 days. The bacterial load of the fresh sample was 5.06 ± 0.900Log_{10}cfu/g and that of the blanched sample ranges from 4.63 ± 0.702 to 5.31 ± 0.915 Log_{10}cfu/g while that of the un-blanched sample ranges from 5.31 ± 0.950 to 5.66 ± 0.960 Log_{10}cfu/g. There is no significant difference in the total bacterial load of both the blanched and un-blanched samples with that of the fresh P>0.05, but load increases with time while the bacterial load of the un-blanched sample is higher compared to the fresh and also increases with time. The pH value of the blanched sample over the 14 days ranges from 7.00 to 6.52 while that of the un-blanched sample ranges from 6.90 to

**Corresponding author: E-mail: ishafaatu@rocketmail.com;*

5.92. A total of three bacteria were isolated from the samples, they are *Bacillus lacterosporus, Listeria monocytogenes and Staphylococcus hominis*. *Bacillus lacterosporus* and *Listeria monocytogenes* were isolated from the fresh sample, *Listeria monocytogenes* was isolated from the blanched sample while *Staphylococcus hominis* and *Bacillus lacterosporus* were isolated from the un-blanched sample. Blanching is effective in reducing microbial load and increase the shelf life of tomato coupled with oil marination.

Keywords: Bacteriological; blanch; marination; preserved; tomato.

1. INTRODUCTION

Tomato (*Lycopersicum esculentum*) is a berry plant in the *solanaceae* family. It is a short lived perennial plant grown as an annual plant, typically growing about 3-5 m approximately in height. The fruit is edible and brightly red colour [1]. It is an important vegetable crop across the world, originated in West South America [2]. China is the largest producer followed by United States and Turkey [3] and Nigeria ranked 16th on the global tomato production scale, accounting for 10.79% of Africa and 1.2% of total world production of tomatoes [4]. Seymour et al. [5]; Ajayi and Olasehinde [6] indicate that tomato fruit contributes to a healthy, well balanced diet. It is rich in vitamin A, B, C and E, minerals which include phosphorus, sodium, potassium, calcium, magnesium and trace elements like iron, copper, zinc, it is also rich in essential amino acids, sugars, and dietary fibers. Tomato fruits are not only food but as medicine, nutrient supplement, flavour, detoxificant and human system cleanser [7]. The deep red coloration of ripened tomato is due to the presence of lycopene, a form of β-carotenoid pigment and a powerful antioxidant that help to protect against prostate cancer, cardiovascular disease and diabetes [8]. 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 [9].

Blanching is a thermal treatment that is usually performed prior to food processes such as drying, freezing, frying, and canning [10]. It is essential to preserve product quality during long-term storage because it inactivates enzymes and destroys microorganisms that might contaminate raw vegetables and fruits during production, harvesting and transportation [11,12]. Marination is commonly used to improve the functional and sensory properties of fruit, vegetables, meat and fish by soaking, injecting or tumbling with an aqueous solutions composed of different ingredients [13]. Marination inhibits microbial growth in beef and the development of bacteria remains below 10^3 cfu/g [14].

Tomato however, has serious challenges to their existence due to negative effects of temperature/climatic condition, pest, bacteria and fungi [15]. Therefore, during amply production of tomato there is the need to process and preserve, inorder to increase the shelf life and minimize microbial load of the fruit. The objective of the present study was to process fresh tomato into blanched and un-blanched oil marinated tomato, determine the bacterial load of fresh and oil marinated tomatoes, isolate and identify the bacteria present and to determine the pH of the oil marinated tomatoes.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh tomato samples were collected at Kasuwan Daji in Sokoto metropolis in a sterile polythene bag.

2.2 Sample Preparation

The tomato samples were divided into three groups; one group was processed into oil marinated blanched another group into oil marinated un-blanched tomatoes while the last group was taken to the Microbiology laboratory of Usmanu Danfodiyo University Sokoto for bacterial analysis. For the oil marinated blanched sample; the tomatoes were sliced into 2.5 cm size using a sterile knife and were blanched using steam blanching method as described by Xiao et al. [16]. The oil was bleached at a very high temperature to remove impurities and allowed to cool to bring down the temperature to around 85 to 90ºC, 50 g of the blanched tomato was then weighed into an empty container and 200ml of the bleached oil was added to submerge the tomato and the container was sealed. For the oil marinated un-blanched sample; the tomatoes were sliced into equal size using a sterile knife and 50 g was weighed into empty container and 200 ml of the bleached oil was added to submerge the tomato and the container was sealed. The sealed containers

were then pasteurised at 95ºC for 5 minutes and kept, bacterial analysis and pH determination of the oil marinated blanched and un-blanched tomatoes were then taken after two days' interval for 14 days.

2.3 pH Determination

The pH was measured as described by Ibrahim et al. [17] and a digital pH meter HARC SENS ION was used for the analysis. The meter was switched on and was allowed to warm for 5 minutes. It was then standardized with basic, acidic and neutral buffers solution. The meter was then immediately introduced into the sample and reading was recorded. After the pH of the first sample was recorded, the electrode was re-washed with distilled water, calibrated using the different standard solutions before been dipped into the second and subsequent samples until all the pH of the samples were taken and recorded.

2.4 Bacteriological Analysis of Samples

All materials used for the analysis were sterilized using a hot air oven at 160^{o}C for 1 hour. Media were prepared according to the manufacturers instruction. Serial dilution was made with 9ml of distilled water and 1g of homogenized tomato and the diluted samples were inoculated on nutrient agar and incubated for 24 hours at 37ºC. The colony formed after 24 hours was counted and recorded and the plates were sub-cultured as described by Ibrahim et al. [17].

2.5 Gram Staining

The isolated organisms were fixed separately on a slide using Bunsen burner. The slide (with the bacteria mounted on it) was passed through the interface between the blue flame and the yellow flame 5 times for a second. After fixing, the slides were placed on a slide holder. The entire mounting region was flood with crystal violet. The crystal violet was allowed to stand for about 60 seconds. When the time elapsed, the slides were washed for 5 seconds with the distilled water. The specimen appeared blue-violet when observed with the naked eye. The slides were then flood with the iodine solution; it was allowed to stand for about a minute as well. When time has expired, the slides were rinsed with distilled water for 5 seconds and immediately decolourizer (ethanol) was added drop wise and allowed to stand for a minute. The slides were rinsed with distilled water for 5 seconds. The counter-stain (saffranin) was added and was allowed for 60 seconds for the bacterial to incorporate the saffranin. Gram positive cells will incorporate little or no counter-stain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink colour and are easily distinguishable from the Gram positives. Again, the slides were rinsed with distilled water for 5 seconds to remove any excess dye [18]. Microscopy was conducted as described by Cheesbrough [19].

2.6 Biochemical Tests

Biochemical tests including motility, indole, catalase, citrate utilization, Methyl Red (MR), Voges- Proskauer (VP), Triple-Sugar Iron agar (TSI), starch hydrolysis, urease and hydrogen sulfide production were carried out in accordance with standard methods described by Oyeleke and Manga [20]. The bacterial isolates were characterized and identified on the basis of their cultural, morphological and biochemical properties as described by Cheesbrough [19].

2.7 Data Analysis

The results obtained were analysed using one-way ANOVA by instat3 software (Version 2.0: San Diego, USA) and presented as Mean ± Standard Error of Mean. Difference between Means are considered significant at $P<0.05$.

3. RESULTS

Fig. 1 shows the pH value of the oil marinated tomatoes. There was decrease in the pH value with time. The mean of the pH values for the blanched sample over the 14 days ranges from 6.52 to 7.00 while that of the un-blanched sample ranges from 5.92 to 6.90. There was significant decrease ($P<0.05$) in the pH of the blanched samples during the storage period from day 8, 10 and 12. There was significant decrease ($P<0.05$) in the pH of un-blanched from day 8, 10, 12 and 14. No significant difference in the pH of blanched and un-blanched marinated tomato at the respective days.

Fig. 2 shows the total bacterial count of the fresh, blanched and un-blanched samples. The mean of the total bacterial count of the fresh tomato was 5.06 Log_{10}cfu/g. After blanching there was a significant decrease in the microbial load but it increases with time and the mean of the total bacterial count of the blanched sample ranges from 4.63 to 5.31 Log_{10}cfu/g while that of the un-blanched sample ranges from 5.31 to 5.66

Log_{10}cfu/g. No significant difference (P>0.05) in the blanched and un-blanched sample compared to the fresh sample.

Table 1 present the morphological and biochemical characteristics of isolated bacteria. A total of three bacteria were isolated, they include *Listeria monocytogenes*, *Bacillus laterosporus* and *Staphylococcus hominis*. *Listeria monocytogenes* and *Bacillus laterosporus* are both Gram positive bacteria that are motile while *Staphylococcus hominis* is also a Gram positive bacteria but non motile. All the isolates were negative for starch hydrolysis, indole test, MR (Methyl Red) test except *Listeria monocytogenes which was positive for MR*, citrate test, gas production, hydrogen sulfide production while positive for VP (Voges-Proskauer) test and catalase test except *B. Laterosporus* which was negative for VP. *Staphylococcus hominis* was positive for urease test while *Bacillus laterosporus and Listeria monocytogenes* were negative for urease test. *Staphylococcus hominis* was positive for glucose, lactose, and sucrose utilization while *Bacillus laterosporus* was only positive for glucose utilization and *Listeria monocytogenes* was only positive for lactose utilization.

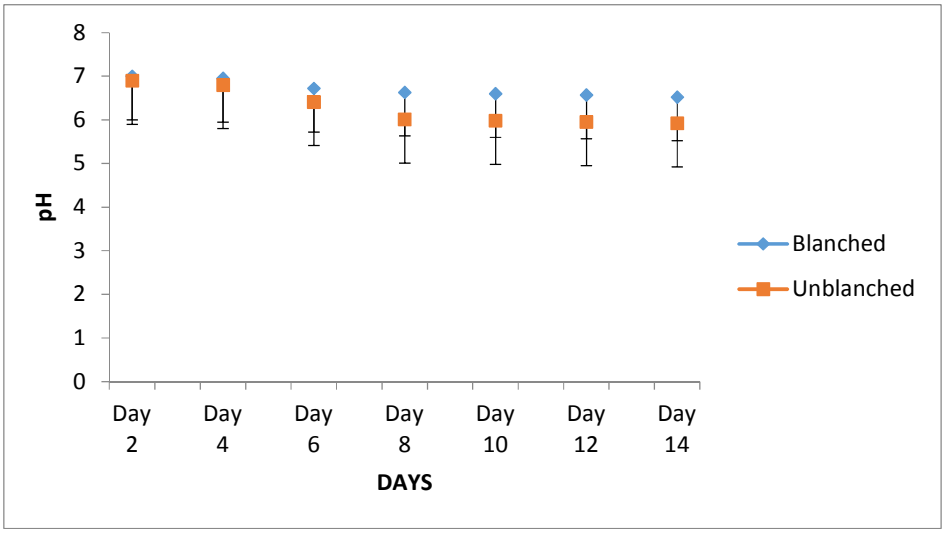

Fig. 1. pH values of oil marinated blanched and un-blanched tomatoes after 2 days interval

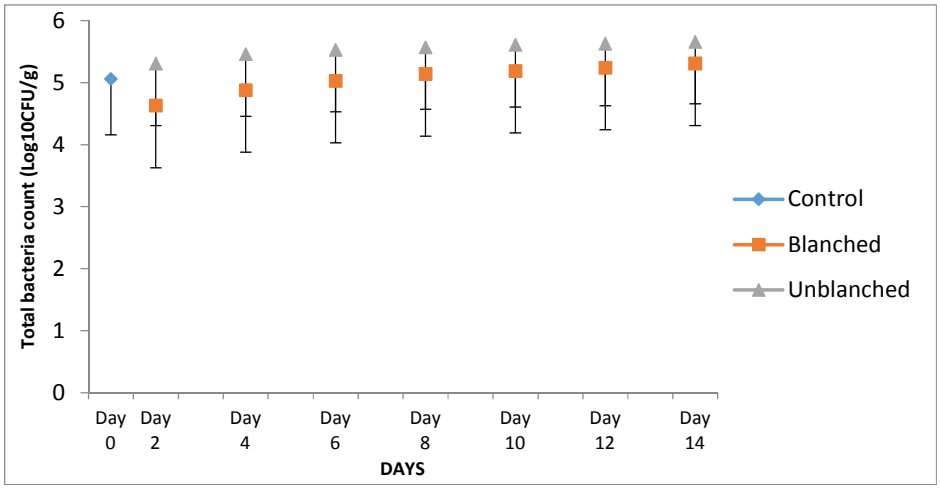

Fig. 2. Total bacterial count of fresh and processed (oil marinated blanched and un-blanched) tomatoes after 2 days interval

Table 1. Morphological and biochemical characteristics of isolated bacteria

	Biochemical Tests													
Gram reaction	**Glu**	**Lac**	**Suc**	**Mot**	**H_2S**	**Gas**	**Cat**	**Cit**	**Ure**	**MR**	**VP**	**SH**	**Indole**	**Confirmed isolates**
Gp short rod / coccobacilli in single / chain	-	+	-	+	-	-	+	-	-	+	+	-	-	*Listeria monocytogenes*
GV long rod with spores	+	-	-	+	-	-	+	-	-	-	-	-	-	*Bacillus laterosporus*
Gp cocci in single / paired / tetrad	+	+	+	-	-	-	+	-	+	-	+	-	-	*Staphylococcus hominis*

Key: Glu = Glucose, SH = Starch hydrolysis, Lac = Lactose, Gp = Gram positive, Suc = Sucrose, + = Present, Mot = Motility, - = Absent, Cat = Catalase, VP – Voges-ProskaÜer, Cit = Citrate, Ure = Urease, MR = Methyl red. GV=Gram variable.

Table 2. Bacteria isolated from fresh, blanched and un-blanched tomatoes

	Bacillus laterosporus	*Listeria monocytogenes*	*Staphylococcus hominis*
Fresh	+	-	+
Blanched	+	-	-
Un-blanched	-	+	+

Key: + = Present, - = Absent.

Table 2 shows the bacteria isolated from fresh tomato sample, blanched and un-blanched oil marinated tomato samples. *Staphylococcus hominis* and *Bacillus laterosporus* were found to be present in the fresh sample, *Bacillus laterosporus* was found in the blanched sample while *Staphylococcus hominis* and *Listeria monocytogenes* were found in the un-blanched sample.

4. DISCUSSION

4.1 Effect of Processing and Preservation on pH

Marinates are solutions, that can include sugar, spices, oil, acids (from vinegar, fruit juice, wine), and they are used to improve tenderness, juiciness, flavor, and aroma and to extend shelf life of meat, poultry, seafood, and vegetables [21]. Marination preserves meat, poultry, fish, and vegetables through the simultaneous action of salt and organic acids. It involves an increase in ionic strength and a decrease in pH [22]. According to Campos et al. [23], pH is a key element in tomato quality and for tomato paste, pH below 4.5 is appropriate for tomato paste, but above 4.5 is undesirable trait, because it will not halt the proliferation of microorganism in the final product. pH is one of the main quality characteristics that describes the stability of bioactive compounds in fruit juice [24]. Capaccioni et al. [25] reported decrease in the pH of oil marinated sample with time and that decrease in pH suppress microbial growth, which comply with the result of this study as there was decrease in the pH value of the blanched and un-blanched sample as the day progresses, which in turn decrease the number of bacteria in the samples. The pH value of marinated products should not be more than 4.8 [26] and this disagrees with the results of this study, this might be due to differences in variety of tomato used, different methods of processing and environmental differences. Schwartz et al. [27] also indicated a decrease in pH during the storage of an olive paste, indicating that this was probably due to the presence of microorganisms which produce lactic acid and lower the pH. Luna-Guevara et al. [28] reported decrease in the pH of blanched tomato with time which is in accordance with the result of this study.

4.2 Effect of Processing and Preservation on Microbial Load

Blanching reduces microbial load by inactivating micro-organisms present in a food sample and therefore improves the quality of the food. Fresh vegetable fruits including tomato have natural protective cover (epidermal layer) that effectively guide against most pathogenic microbes and plant spoilage. This protection however could be hindered and the fruits may be contaminated during field cultivation, harvesting, post-harvest handling and distribution [29]. High pH allows easy proliferation of microorganism and therefore increase microbial load while low pH inhibits proliferation of micro-organism and therefore decrease microbial load [30]. Increase in the microbial load observe in this study might be due to the high pH of the tomato. The increase in the microbial load observe in this study was not significant from the fresh and all the micro-organisms isolated can survive within the pH of the sample throughout the study.

All the bacterial species isolated can survive at high pH. Wakil and Daodu [31] reported a decrease in microbial population as the pH of fermented Ogi tends acidic. *Staphylococcus hominis* is part of normal skin flora, *Listeria monocytogenes* are found in milk, meat and their products while *Bacillus laterosporus* are found in the soil. The isolated organisms have also been isolated by Bello et al. [32] who isolated *Staphylococcus* and *Bacillus spp.* from tomato. The current result is in accordance with Wogu and Ofuase [33] who also isolated *Bacillus laterosporus* from tomato, this result also agrees with previous work by Duffy [34] who isolated *Listeria monocytogenese.* Duman et al. [35] reported increase in microbial load of marinated sample with time which agrees with the result of the current work. Maktabi et al. [36] also reported increase in microbial load of marinated samples

with time in fish fillets. The absence of oxygen usually favours the growth of gram-positive bacteria [37] which agrees with the current result, all the isolated bacteria are gram positive bacteria. *Listeria monocytogenes* are deadly microorganism and are the cause of listeriosis [38], the presence of this micro-organism in the un-blanched sample might be because this sample was not blanched. The presence of *L. monocytogenes* is surprising as this organism was not isolated from the fresh tomato, this organism could be killed by cooking and pasteurization [39] and these might be the reason for it absent in the blanched tomato samples. Likewise, as reported by Larry et al. [40] that tomatoes are not a good growth substrate for the organism. Although, *L. monocytogenes* is known to occur in the environment on a wide range of vegetation [41].

Staphylococcus hominis causes nocosomial infections [42] but it presence does not cause threat because it is a skin normal flora but pose threat to individual with compromise immunity. *Bacillus laterosporus* has been considered as a safe and beneficial bacterium for gut wellness, it has also been shown to accelerate the natural enzymatic activities of the body, thus ameliorating metabolic rates [43]. It has antibacteria and immune support properties [43], hence, it present in both fresh and marinated blanched tomatoes is of health benefits.

5. CONCLUSION

Based on the bacteriological assessment of fresh and processed (oil marinated blanched and un-blanched) tomatoes, it can be concluded that blanching is effective in reducing microbial load and increase the shelf life of tomato coupled with oil marination.

6. RECOMMENDATION

It is recommended that during bounty production of tomato, blanching being an effective method of reducing microbial load and increase shelf life should be employed in pre-processing of tomato for preservation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. ACMSF, (Advisory Committee on the Microbiological Safety of Food), 2005. Microbiological status of ready to eat fruit and vegetables.
   Available:http://www.food.gov.uk/multimedia
   (Retrieved on April 12, 2017)
2. Kimura S, Sinha N. Tomato (*Solanum lycopersicum*): A model fruit Bearing crop. CSH Protoc; 2008.
   DOI: 10.1101/pdb.emo 105.
3. Denton VS. Africa Symposium on Horticultural Crops: Tomato Cultivation and Its Potential in Nigeria.
   Available:http://www.ishs.org/tomato_cultivation_nd_its_potential_in_Nigeria
   (Retrieved April 12, 2017)
4. Weinberger K, Lumpkin TA. Diversificatioon into Holticulture and poverty Reduction: A research agenda; 2007.
   Available:http://dx.doi.org/10.1016/j.worlddev
   (Retrieved on April 12, 2017)
5. Seymour IJ, Appleton H. Foodborne viruses and fresh produce. J. of App. Microbiol. 2001;91:759-773.
6. Ajayi AA, Olasehinde IG. Studies on the pH and Protein Content of Tomato (*Lycopersicon esculentum*) Fruits Deteriorated by *Aspergillus niger*. Sci. Res. and Ess. 2009;4(3):185-187.
7. Abhinaba G. Identification of microorganisms responsible for spoilage of tomato (*Solanum lycopersicum*) fruit. J. Phytopath. 2009;1(6):414-416.
8. Amusa NA, Kehinde IA, Ashaye OA. Biodeterioration of Bread, fruit in storage and its effects on the Nutrient Composition. Afr. J. of Biotech. 2002;1(2):57-60.
9. Barton BC, Blaney D, Bidol SA, Solivia S, Daly ER, Taylor T. Multistate outbreaks of *Salmonella typhmurium* infections associated with consumption of restaurant tomatoes. U.S.A. J.I of Epidem. and Infec. 2006;1-9.
10. Arroqui C, Lopez A, Esnoz A, Virseda P. Mathematical model of heat transfer and enzyme inactivation in an integrated blancher cooler. J. Food Eng. 2003;58:215–225.
11. Cruz RMS, Vieira MC, Silva CLM. Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in

watercress (*Nasturtium officinale*). J. Food Eng. 2006;72:8–15.
12. Mukherjee S, Chattopadhyay PK. Whirling bed blanching of potato cubes and its effects on product quality. J. Food Eng. 2007;78:52–60.
13. Latif S. Effect of marination on the quality characteristics and microstructure of chicken breast meat cooked by different methods. Seria Zootehnie Lucrative Ştiinţifice. 2011;54:314-324.
14. Knöchel S, Mach N, Karlsson A. Microbial and physical changes in marinated beef of high and normal pH during storage in different atmospheres. In: 53rd International Congress of Meat Science and Technology. 2009;1:37-38.
15. Oyemaechi CU, Chukwuezi FO, Ozougwu VEO. Microbial agents of tomato spoilage in onitsha metropolis. Adv. in Bio. Res. 2014;8(2):87-93.
16. Xiao HW, Zhongli P, Deng LZ, Hamed MEM, Yang XH, Arun SM, Gao ZJ, Qian Z. Recent developments and trends in thermal blanching – A comprehensive review. Info. Proc. Agric. 2017;4:101-127.
17. Ibrahim SG, Umar RA, Isa AS, Farouq AA. Influence of Preservation Methods on the pH and Microbiological Quality of Tiger Nut (*Cyperus esculentus*) Milk. Bayer. J. of Pur and Appl. Sci. 2016;9(2):234-242. ISSN: 2006-6996.
18. Tortora GJ, Funke BR, Case CL. Microbiology an Introduction. (6th Edition), McGraw Hill, New York. 2003;210-212.
19. Cheesbrough M. Biochemical Tests to Identify Bacteria. Laboratory Practices in Tropical Countries. (3rd Edition), Cambridge Publisher, U.K. 2002;63-70.
20. Oyeleke, Manga. Essentials of Laboratory practical in microbiology. Tobest Publisher Minna, Nigeria. 2008;20-80.
21. Cadun A, Cakli S, Kisla D. A study of marination of deep water pink shrimp (*Parapenaeus longirostris*) and its shelf life. Food Chem. 2005;90:53-59.
22. Poligne I, Collignan A. Quick marination of anchovies (*Engraulis enchrasicolus*) using acetic and gluconic acids. Quality and stability of the end product. Lebensmittel Wissenschaft und Technologie. 2000;33:202-209.
23. Campos CAB, Fernandes PD, Gheyi HR, Blanco FF, Goncalve CB, Campos SAF. Yield and fruit quality of industrial tomato under saline irrigation. J. Sci. Agric. 2006;2:63-69.
24. Sanchez-Moreno C, Plaza L, De Ancos B, Cano MP. Nutritional characterization of commercial traditional pasteurized tomato juices: Carotenoids, vitamin C and radical scavenging capacity. Food Chemistry. 2006;98:749-756.
25. Capaccioni ME, Casales MR, Yeannes MI. Acid and salt uptake during the marinatig process of *Engraulis anchoita* fillets influence of the solution: fish ratio and agitation Ciênc. Tecnol. Aliment., Campinas. 2011;31(4):884-890.
26. Rehbein H, Oehlenschlager J. Fische und fischerzeugnisse, krebs und weichtiere. In: Allgemeines lehrbuch der lebensmittelchemie, Franzke, C. (Ed.) Hamburg: Behr Verlag. 1996;395–411.
27. Schwartz M, Quitral V, Daccarett C, Callejas J. Desarrollo de pasta untable de aceituna variedad Sevillana. Grasas Aceites. 2009;60:451-457.
28. Luna-Guevara ML, Hernández-Carranza1 P, Luna-Guevara JJ, Flores-Sánchez R, Ochoa-Velasco CE. Effect of sonication and blanching postharvest treatments on physicochemical characteristics and antioxidant compounds of tomatoes: Fresh and refrigerated. Acad. J. of Agric. Res. 2015;3(8):127-131.
29. Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbial. Infect. 2002;4:413-423.
30. James MJ. Modern Food Microbiology. (6th Edition), Aspen Publishers, Inc, Goithersburg Maryland. 2000;34-60:251-363.
31. Wakil SM, Daodu AA. Physiological Properties of a microbial community in spontaneous fermentation of maize (*Zea mays*) for Ogi production. Int. Res. J. Microbiol. 2011;2:109-115.
32. Bello OB, Olawuyi OJ, Azeez AH, Adebisi, OS, Owoade TA. Microorganisms causing post-harvest tomato (*Solanum lycopersicum*) fruit decay in Nigeria. Sci. Agric. 2016;13(2):93-96.
33. Wogu MD, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits (Lycopersicum esculentum) sold in markets in Benin City, Southern Nigeria. Sch. Acad. J. Biosci. 2014;2(7):459-466.
34. Duffy G. Verocytoxigenic *Escherichia coli* in animal faeces, manures and slurries. Journal of Associated with semi-dried

tomatoes. England App. Microb. 2003;4:94-103.
35. Duman M, Çoban ÖE, Özpolat E.. The natural preservative effect of rosemary and thyme essential oils on marinated crayfish (*Astacus leptodactylus*). The J. of Ani. & Plant Sci. 2015;25(6):1771-1778.
36. Maktabi S, Zarei M, Chadorbaf M. Effect of traditional marinating on bacterial and chemical characteristics in frozen rainbow trout fillet. J. of Food Qual. and Haz. Contrl. 2015;2:128-133.
37. Abadias M, Usall J, Anguera M, Solsona C, Viñas I. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. of Food Microbiol. 2008;123:121-129.
38. Available:https://www.webmd.com/food-recipes/food-poisoning/tc/listeriosis-topic-overview
39. Available:https://www.foodsafety.gov/poisoning/causes/bacteriaviruses/listeria/index.html
40. Larry RB, Robert EB. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. J. of App. and Environ. Microbiol. 1991;57(5):1367-1371.
41. Welshimer HJ. Isolation of *Listeria monocytogenes* from vegetation. J. Bacteriol. 1968;95:300-320.
42. Szczuka E, Trawczynski K, Kaznowski A. Clonal analysis of *Staphylococcus hominis* strains isolated from hospitalized patients. Polish J. of Microb. 2014;63:349-354.
43. Maya C. Probiotics and Bacillus laterosporus; 2016.
Available:https://probioticsamerica.com/bacillus-laterosporus/

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23405