Nigerian Food Journal

Official Journal of Nigerian Institute of Food Science and Techonology www.nifst.org

NIFOJ Vol. 30 No. 2, pages 106 – 113, 2012

Incidence of Bacteria with Potential Public Health Implications in Raw *Lycopersicon esculentum* (Tomato) Sold in Lagos State, Nigeria

* Ogundipe, F.O.[1], Bamidele, F.A.[1], Adebayo-Oyetoro, A.O.[2], Ogundipe, O.O.[2] and Tajudeen, O.K.[1]

ABSTRACT

Lycopersicon esculentum (tomato) is a fleshy berry that is highly perishable and prone to microbial spoilage. The presence of some microorganisms of public health significance makes it a potential health hazard to consumers. There is therefore the need to determine the food safety and public health implications of some of the microorganisms present in it. The aim of the study was to isolate, identify and compare the incidence of the organisms that could be potential pathogens among the four grades of tomato samples used in the investigation. Seven batches of tomato samples were obtained from two (2) major produce markets in Lagos Nigeria. A total of 40 isolates were obtained from the tomato samples. *Staphylococcus aureus* (22.5%), *Escherichia coli* (15%), *Bacillus* species (20%) and *Salmonella* species (5%) were among the bacteria isolated from the tomato samples. The average pH values of the tomato samples ranged from 4.34 to 4.60 while the average moisture content of the tomato samples was between 12% and 95.1%. The results showed that the freshness and hardness of tomato samples were important factors which determine the types of bacteria found on them. As some of the bacterial genera identified are potential food-borne pathogens that could pose some public health challenges, proper handling and adequate cooking before consumption is suggested.

Keywords: Tomato, public health, potential pathogen, Nigeria.

Introduction

Food-borne pathogens are the leading cause of illness and death in developing countries and they create a great financial burden on medical care and social cost (Fratamico *et al.*, 2005). A number of studies have reported outbreaks of food-borne diseases associated with tomato (Hedberg *et al.*, 1999; FDA, 2009 and CDC, 2011). These outbreaks are usually due to the consumption of contaminated produce and poor hygiene. Contamination may arise as a consequence of treating soil with organic fertilisers, such as sewage sludge and manure, and from the irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables (Hamilton *et al.*, 2006; Tyler and Triplett, 2008; Heaton and Jones, 2008).

Traditionally, fresh fruits and vegetables have not been considered high risk foods in terms of causing food-borne illnesses, especially when compared with foods of animal origin such as meat, dairy products and sea foods (Madden, 2002). However, the assumption of the lower risk of fresh produce has been somewhat modified as a result of recent epidemiological data and the adverse publicity associated with outbreaks of food-borne illnesses at national and international levels due to fresh produce (Olsen *et al.*, 2000).

Fresh vegetables normally carry natural non-pathogenic epiphytic microorganisms, but during growth, harvest, transportation and further handling the

[1] Department of Biological Science, Yaba College of Technology, Lagos, Nigeria.

[2] Department of Food Technology, Yaba College of Technology, Lagos, Nigeria.

* corresponding author: floraogundipe@yahoo.com

produce can be contaminated with pathogens from animal and human sources. As most of these produce are eaten without further processing, their microbial content may represent a risk factor for the consumer's health and therefore a food safety problem (Brandl, 2006). Therefore, it is important that food safety should be the concern of food inspectors and food vendors since pathogenic organisms can easily enter and grow in the poorly handled food (Orazulike and Osunaiye, 2003).

Tomato is a fruit/vegetable that is highly perishable due to its high water content and hence prone to spoilage by microorganisms. The activities of these microorganisms bring about high levels of post-harvest losses especially after harvesting (Aworh *et al.*, 1983). Being perishable, tomato is more susceptible to injury because of its shape and structure and its relative soft texture which is associated with high moisture content, and these lead to deterioration in transit and storage which is more rapid under conditions of high temperature and humidity, hence, heavy losses are encountered (Idah *et al.*, 2007).

These damaged tomatoes locally called '*Esa'* by the Yoruba-speaking people of Nigeria are not thrown away but sold to consumers in large measures in order for the produce seller to make maximum profit. The proliferation of microorganisms, more especially in damaged tomatoes could be considered to be potentially harmful when such contaminated tomatoes are consumed in improperly cooked foods or eaten raw.

Hence, the objective of this study was to isolate and identify potentially pathogenic bacteria associated with four different grades of raw tomato as well as to compare the frequency/distribution of the isolated organisms among the tomato samples.

Materials and Methods

Seven (7) batches of tomato samples in four grades based on their freshness and hardness were purchased from two (2) markets in Lagos State, Nigeria. Samples were collected in sterile containers, labelled appropriately and taken to the laboratory for immediate analysis. The first grade of the samples consisted of tomatoes that were firm, hard and undamaged (Figure 1), the next grade had soft and slightly damaged tomatoes (Figure 2), third grade comprised soft and badly damaged tomatoes (Figure 3) while the fourth grade of sample were dried tomatoes (Figure 4).

Each of the samples was homogenized in 100 ml of sterile distilled water; this was filtered and used to determine the pH values of the samples by the method described by Mbugua and Njenya (1991). The moisture content of the samples was also determined using the recommended method of AOAC (2000) immediately the samples were brought to the laboratory.

Fig. 1: Firm undamaged tomato sample (GA)

Fig. 2: Slightly damaged tomato sample (GB)

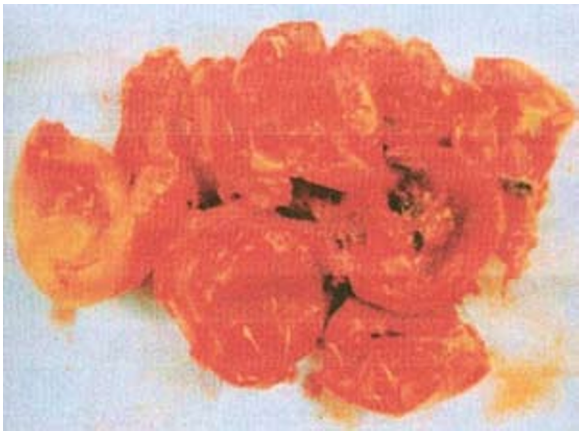

Fig. 3: Soft and badly damaged tomato sample (GC)

Fig. 4: Dried tomato sample (GD)

Ten (10) g of each sample was weighed and homogenized into 90 ml of sterile distilled water using a sterile Warring blender. One ml from Tenfold dilution of the homogenized samples was inoculated in Nutrient Agar (Lab M) for total aerobic counts and MacConkey agar (Lab M) for coliform counts using the pour plate method. All the plates were incubated aerobically at 37°C for 24 – 48 h. After the incubation period, counts were made, colonies were selected randomly and each isolated colony was purified by successive streaking. Discrete pure colonies were thereafter placed on Nutrient Agar slants and stored at 4°C.

The pure colonies obtained were later characterized and identified using various morphological and biochemical tests such as gram stain, spore stain, motility, catalase, coagulase, indole, MR-VP, urease, citrate, oxidase and sugar fermentation tests.

Results

The average moisture content of the various tomato samples indicated that the soft and slightly damaged tomato samples (GB) had the highest value (95.1%) while the dried tomato samples (GD) had the lowest (12%). The average pH values also shown in Table 1 indicate that the range was between 4.34 and 4.60.

Table 1: Average values of pH and moisture content obtained from the tomato samples

Sample code	pH Value	Moisture Content (%)
GA	4.60	95.0
GB	4.34	95.1
GC	4.41	94.0
GD	4.42	12.0

GA: Firm undamaged tomato, GB: Soft and slightly damaged tomato, GC: Soft and badly damaged tomato, GD: Dried tomato

The microbial load of the samples (Table 2) investigated showed that the first batch of the soft and badly damaged sample (GC_1) had the highest coliform count while the second batch of firm undamaged sample (GA_2) had the least count. It was also observed that the first batch of the firm undamaged sample (GA_1) had coliform count higher than the second batch (GA_2) despite the fact that both samples were firm undamaged tomatoes. The total aerobic count ranged from 5.12×10^6 cfu/g to 9.2×10^5 cfu/g. The result also shows that the dried sample (GD) had the highest total aerobic count despite the low moisture content.

Our findings show that fourteen (14) bacteria genera were isolated and identified. A total of forty (40) bacterial isolates were obtained with the highest incidence of occurrence recorded for *Staphylococcus*

spp (22.5%), followed by *Bacillus spp* (20%) and *Escherichia coli* (15%) while the least incidence was obtained for *Proteus spp* (2.5%) and *Citrobacter spp* (2.5%) (Table 3).

Table 2: Microbial load of the tomato sample

Type of analysis	GA_1	GA_2	GB_1	GB_2	GC_1	GC_2	GD
Total aerobic count	1.8×10^6	9.2×10^6	3.4×10^6	3.12×10^6	4.4×10^6	4.2×10^6	5.12×10^6
Coliform count	2.6×10^6	1.5×10^6	3.6×10^6	3.5×10^6	7.6×10^6	6.68×10^6	5.8×10^6

GA: Firm undamaged tomato, GB: Soft and slightly damaged tomato, GC: Soft and badly damaged tomato, GD: Dried tomato

Table 3: Frequency of occurrence of identified bacteria isolates from the tomato samples

Identified genera	Number of isolates	Frequency (%)
Staphylococcus aureus	9	22.5
Bacillus spp	8	20.0
Escherichia coli	6	15.0
Enterobacter spp	5	12.5
Alcaligenes faecalis	4	10.0
Acinetobacter spp	3	7.5
Salmonella spp	2	5.0
Corynebacterium spp	1	2.5
Proteus mirabilis	1	2.5
Citrobacter spp	1	2.5
Total	40	100

The study also revealed, as shown in Figure 5, that *Bacillus spp*, *Salmonella spp* and *Enterobacter spp* were not isolated from the firm undamaged samples (GA); however, the soft damaged samples (GC) were the only ones positive for all the organisms isolated and the only ones from which *Salmonella spp* was obtained. It is significant to note that all the samples used had incidences of contamination with *Staphylococcus aureus* while the dried tomato samples had the highest incidence for *Bacillus spp*.

Discussion

The epidemiology of food-borne disease has changed rapidly over the last decades as fresh vegetables have emerged as new vehicles for the transmission of these infectious diseases. Since the early 1990s, awareness of the potential of fresh produce to cause food-borne disease has increased and reported outbreaks associated with consumption of fresh vegetables have grown steadily (Falomir *et al.*, 2010).

Tomato is a highly perishable fruit/vegetable that may be eaten raw. Its high water activity and high solute concentration enhance microbial growth and multiplication (Uria and Izuaghe, 1990; Charley, 2002). The average moisture content obtained in the study agrees with Sohail *et al.* (2011) who reported that the moisture content of fresh tomatoes was 94.4%; however, it is observed to be higher than the recommended 90% for ripe tomato (Adebanwo *et al.*, 2002).

Fruits and vegetables are dried to enhance storage stability, minimize packaging requirement and reduce transport weight (Sugar and Suresh, 2010). Because not all fresh tomatoes can be consumed at the time of harvest, preservation provides a larger market, allowing consumers to buy the product on a year-round basis (Sohail *et al.*, 2011). Drying is a suitable alternative for post-harvest management especially in developing countries where there exist poorly established low temperature distribution and handling facilities.

Despite the low moisture content of the dried tomato samples (GD), bacteria such as *S. aureus*, *Bacillus spp* and *Enterobacter spp* were isolated. This

is because reduction of moisture content alone is not sufficient to protect the food from microbial contamination. This could be as a result of pest contamination and prolonged exposure of the tomato during the drying process (Jideani *et al.*, 1995).

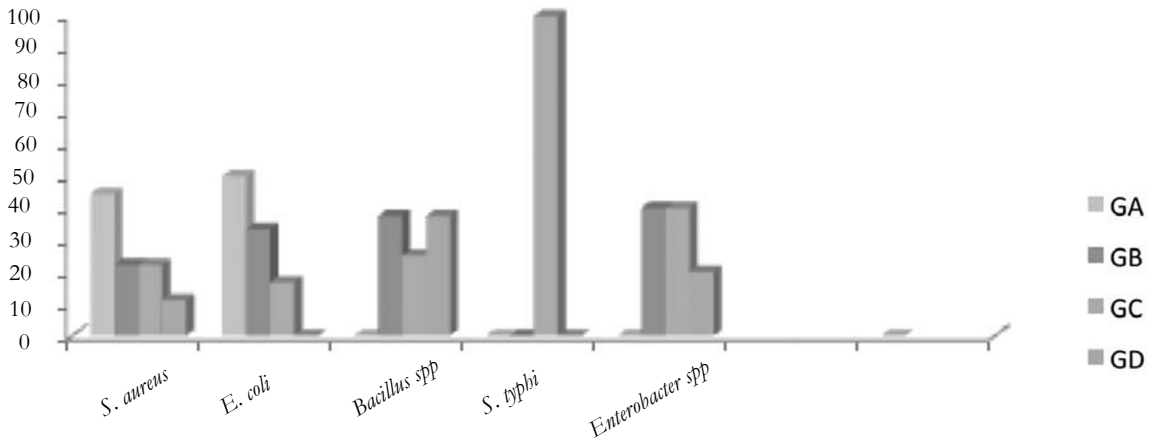

Fig. 5: Distribution of potentially pathogenic bacteria among the four tomato grades

There are a lot of human health consequences in consuming contaminated foods, ranging from protracted illness to death; also, patients with impaired immunity are at greater risks (Iroha *et al.*, 2011). Most bacteria do not grow at low pH especially below 3.5, however, the pH recorded in this study was higher than 3.5. Thus it can be assumed that this created a conducive environment for the proliferation of the bacteria species found in the tomato samples.

The International Commission on Microbiological Specification for Food (ICMF, 1974) recommends that the limit of bacterial contaminants for food should be in the range of 102 – 103 cfu/g for coliform organisms and less than 105 cfu/g for total aerobic count. The result revealed that all the tomato samples had total aerobic counts and coliform counts that were very high above the acceptable limits. This may be attributed to the fact that the produce has normal microbial flora present. However, the samples investigated were microbiologically unacceptable to be consumed without adequate cleaning and cooking.

This study has shown that all the tomato samples bought from the selected retail markets in Lagos Nigeria were contaminated with at least two bacteria species, indicating a potential risk to public health. Studies by Ashenafi, 1989 and Obieze *et al.*, 2011 confirm that all the identified bacteria obtained in this study can be found in tomato. The presence of these organisms indicates that raw tomato may represent a risk factor for infection and more especially to immune-compromised individuals. This agrees with the studies of Brandl (2006) and Lynch *et al.* (2009).

It is known that microbial contamination is enhanced when the food structure is disorganised and the protective natural barrier damaged or weakened. The results from this study corroborate this; it was noted that the microbial population of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus spp* and *Enterobacter spp*

on the soft and slightly damaged samples (GB) was higher compared to that of the firm undamaged samples (GA). This can be attributed to the gradual loss of firmness and disorganisation of the protective covering of the GB samples.

As further loss of the integrity of the protective covering continued, more nutrients and moisture were provided for adequate growth of the contaminating organisms. This is made obvious by the result obtained from the soft and badly damaged samples (GC) which was the only one that was positive for all the organisms obtained and also the only one from which *Salmonella typhi* was significantly isolated.

The high incidences of *S. aureus*, *E. coli*, *S. typhi* and *Enterobacter spp* indicate poor handling by sellers, unhygienic market environment and faecal contamination. Oftentimes, these contaminations are further increased by cross-contamination with contaminated surfaces, vessels and hands (Adebanwo *et al.*, 2002). *E. coli* contamination may also arise from faecal contamination from farm-yards and manure.

The problem of food safety in the industrialized world differ considerably from those faced by developing countries. Whereas traditional methods are used for marketing fresh produce in the latter countries, food processing and packaging are the norm in industrialized countries (Mensah *et al.*, 2002). Traditional methods are often used for marketing of fresh produce in Nigeria, one of which is the sprinkling of water on produce such as tomatoes. This is to give it a fresh appearance and to delay decomposition. However, this spraying method provides moist environment which encourages the growth of microorganisms on the produce (Schwab *et al.*, 2008).

Untreated or contaminated water seems to be a particularly likely source of contamination. This is because tomato has been said to absorb water and associated bacteria if washed in water colder than it is (Zhuang *et al.*, 1995; Rushing *et al.*, 1996). Hence, water used for spraying and maintaining the appearance of produce must be microbiologically safe (Tauxe, 1997).

Another traditional method is in the manner in which the produce is displayed for sale. Oftentimes, the tomato is portioned into baskets or plastic containers and displayed on a low platform very close to the ground or placed directly on the bare floor in the market. This encourages sand and run-off from waste water generated in the market or from rainfall to contaminate the produce.

Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with bacterial contamination, particularly with members of the *Enterobacteriaceae* family (DuPont, 2007). In addition, the presence of antibiotic resistances in both normal flora and pathogenic microorganisms in fresh vegetable may contribute to horizontal spreading of resistance between different isolates, species and genera (De La Cruz and Davies, 2000; Tenovar, 2006; Heuer and Smalla, 2007).

Bacillus spp has been implicated as one of the common contaminants of vegetables (Banwart, 2001). The high incidence of the organism in the study may be due to its spores. These spores could resist killing by high temperature of ultraviolet sun rays which has the ability to kill and reduce the bacterial load in vegetable during exposure and display for sale (Obieze *et al.*, 2011).

S. aureus produces enterotoxin which could also be resistant to heat; this can be a major risk especially when such contaminated tomato is eaten raw or undercooked. *S. typhi* is the primary aetiological agent of typhoid fever which is responsible for significant morbidity and mortality particularly in developing countries (Kumar *et al.*, 2005). The incidence of these organisms poses a great risk to public health.

From the result it can be noted that that the most significant source of the *S. typhi* was from the soft and badly damaged sample (GC) which is commonly called *'Esa'*. *Esa* is basically a grade of

tomato that is damaged and has lost some market value; the sellers therefore display large quantities for less value in order not to lose their capital entirely. The large portion is often a big attraction to a large number of the poor populace who are oftentimes ignorant of the potential health risk of the produce.

Conclusion

Due to the fact that fresh produce is usually widely distributed, most produce related outbreaks can result in widespread epidemics across communities. Thus, to prevent an outbreak of disease, tomato fruits should be thoroughly washed, and if damaged, they should be thoroughly cooked before consumption. Tomato/produce sellers should be educated or enlightened to sanitize their containers and to handle their produce hygienically. The government should also ensure that markets are clean, wastes generated are properly disposed and the traders encouraged to display their tomatoes on elevated platforms rather than on bare ground.

References

Adebanwo, O.O., Osiles, O., Olagunju, J.A., Odunuga, O. and Makinde, Y.O. (2002). National nutrition and food policy for Nigeria: Hazards and safety. *Journal of Nutritional Science* 13 (2): 8 – 10.

AOAC (Association of Official Analytical Chemists) (2000). *Official Methods of Analysis of the Association of Official Analytical Chemists*. 17th Edition, Washington D.C. p. 25.

Ashenafi, M. (1989). Microbial load, incidence and antibiotic resistance of some disease causing microorganisms on raw food items consumed in Ethiopia. *World Journal of Microbiology and Biotechnology* 5 (3): 313 – 319.

Aworh, O.C., Olorunda, A.O. and Akuemonkhan, I.A. (1983). Effects of post-harvest handling on quality attributes of tomatoes in Nigerian marketing system. *Food Chemistry* 10: 225 – 230.

Banwart, G.J. (2001). Bacteria as food spoilage organisms. In: *Basic Food Microbiology.* Avi Publishing Co. Westport Conn: pp. 119 – 125.

Brandl, M.T. (2006). Fitness of human enteric pathogen on plants and implications for food safety. *Annual Review of Phytopathology* 44: 367 – 392.

CDC (Center for Disease Control and Prevention). (2011). Foodborne outbreak online database (FOOD). Data retrieved February 1, 2012 from http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx.

Charley, H. (2002). *Vegetables: Food Science* 2nd Edition. John Willey and Sons Inc. New York, 487pp.

De La Cruz, F. and Davies, J. (2000). Horizontal gene transfer and the origin of species: Lessons from bacteria. *Trends in Microbiology* 8: 128 – 133.

DuPont, H.L. (2007). The growing threat of foodborne bacterial enteropathogens of animal origin. *Clinical Infectious Diseases* 45: 1353 – 1361.

Falomir, M.P., Gozalbo, D. and Rico, H. (2010). Coliform bacteria in fresh vegetables: From cultivated lands to consumers. *Applied Microbiology and Microbial Biotechnology* 1175 – 1181.

FDA (US Food and Drug Administration). (2009). Safe Practices for Food Processes, Chapter IV: Outbreaks Tables, Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh Cut Produce. Retrieved February 1, 2012, from http://www.fda.gov/Food/Science Research/Research Areas/Safe Practices for Food Processes/ucm091270. htm.

Fratamico, P.M., Bhunia, A.K. and Smith, J.L. (2005). *Foodborne Pathogens in Microbiology and Molecular Biology*. Norfolk: Caister Academic Press, Wymondham, UK, pp. 270 – 275.

Hamilton, A.J., Stagnitti, F., Premier, R., Boland, A.M. and Hall, G. (2006). Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and Environmental Microbiology* 72: 3284 – 3290.

Heaton, J.C. and Jones, K. (2008). Microbial contamination of fruits and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology* 104: 613 – 626.

Hedberg, C.W., Angulo, F.J., White, K.E., Langkop, C.W., Schell W.L., Stobierski, M.G., Schuchat, A., Besser, J.M., Dietrich, S., Helsel, L., Griffin, P.M., McFarland, J.W. and Osterholm, M.T. (1999). Outbreaks of salmonellosis associated with eating uncooked tomato: Implication for public health. *Epidemiology and Infection* 122: 385 – 393.

Heuer, H. and Smalla, K. (2007). Horizontal gene transfer between bacteria. *Environmental Biosafety* 6: 3 – 13.

ICMSF (International Commission on Microbiological Specification for Food) (1974). Sampling Plans for Dried Foods. In: *Microorganisms in Foods*. University of Toronto Press, Toronto, Can. 2: 110 – 118.

Idah, P.A. Ajisegiri, E.S.A. and Yisa, M.G. (2007). Fruits and vegetables handling and transportation in Nigeria. *AU J.T.* 10 (3): 175 – 183.

Iroha, I.R., Ugbo, E.C., Ilang, D.C., Oji, A.E. and Ayogu, T.E. (2011). Bacterial contamination of raw meat sold in Abakaliki, Ebonyi State. *Nigeria Journal of Public Health and Epidemiology* 3 (2): 49 – 53.

Jideani, V.A., Durojaye, O.O. and Jideani, I.A. (1995). Moisture sorption isotherm of intermediate moisture millet dough. *Nigerian Food Journal* 13: 94.

Kumar, S., Balakrishna, K., Singh, G.P. and Batra, H.V. (2005). Rapid detection of salmonella typhi in food by combination of immunogenic separation and polymerase chain reaction. *World Journal of Microbiology and Biotechnology* 21: 625 – 628.

Lynch, M.F., Tauxe, R.V. and Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: Risks and opportunities. *Epidemiology and Infection* 137: 317 – 315.

Madden, P. (2002). Microbial pathogens in fresh produce: The regulatory perspectives. *Journal of Food Protection* 55: 821 – 823.

Mbuga, S.K. and Njenya, J. (1991). Antimicrobial properties of the fermented 'Iyi' as a weaning food. In: Westby, A. and Reilly, S. (eds.) Proceedings of a Regional Workshop on Traditional and African Foods: Quality and Nutrition. *International Nutrition of Science*.

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. and Ablordey, A. (2002). Street foods in Accra, Ghana: How safe are they. B*ulletin of World Health Organisation* 80 (7).

Obieze, K.O., Obuagu, C.N., Asikong, B.E., Onyido, A.E., and Ogolo, B.A. (2011). Bacteriological study of vegetables from markets of Calabar Cross-River State South Eastern Nigeria. *The Internet Journal of Public Health*. 1(2).

Olsen, S.J., MacKinnon, L.C., Goulding, J.S., Bean, N.H. and Slutsker, L. (2000). Surveillance for foodborne disease outbreaks. McGraw Hill Company, USA, p. 517.

Orazulike, R.E. and Osunnaiye, E. (2003). Evaluation of the microbiological quality of street foods in Bauchi metropolis. Proceeding of 27th Annual NIFST Conference. 94 – 95.

Rushing, J.W., Angulo, F.J. and Beuchat, L.R. (1996) Implementation of a HACCP program in a commercial fresh market tomato packaging house: A model for the industry. *Dairy, Food and Environmental Sanitation* 16: 549 – 553.

Schwab, A.H., Duran, A.P., Barnard, R.J. and Read, R.B. (2008). Microbial quality of some spices and herbs in retail markets. *Journal Applied and Environmental Microbiology* 24 (3): 627 – 630.

Sohail, M., Ayub, M., Ahmad, I., Ali, B. and Dad, F. (2011). Physicochemical and microbiological evaluation of sun dried tomatoes in comparison with fresh tomatoes. *Pakistan Journal of Biochemistry and Molecular Biology* 44 (3): 106 – 109.

Sugar, V.R. and Suresh, K.P. (2010). Recent advances in drying and dehydration of fruits and vegetables. *Journal of Food Science and Technology* 47: 15 – 26.

Tauxe, R.V. (1997). Emerging foodborne diseases: An evolving public health challenge. *Emerging Infectious Diseases* 3 (4): 425 – 434.

Tenovar, F.C. (2006). Mechanism of antimicrobial resistance in bacteria. *American Journal of Infection Control* 34 (Suppl 1): 3 – 10.

Tyler, H.L. and Triplett, E.N. (2008). Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annual Review of Phytopathology* 46: 53 – 63.

Uria, N. and Izuaghe (1990). *Public Health: Food and Industrial Microbiology*. Uniben Press, Nigeria, p. 256.

Zhuang, R.Y., Beuchat, L.R. and Angulo, F.J. (1995) Fate of salmonella montevideo on and in raw tomato as affected by temperature and treatment with chlorine. *Applied Environmental Microbiology* 61: 2127 – 2131.