BACTERIOLOGICAL QUALITY OF FRESHLY-CUT AND READY TO EAT VEGETABLES SOLD IN DUTSE MARKET NORTH WEST, NIGERIA

Supported by tetfund TERTIARY EDUCATION TRUST FUND

A. O. Adeleye[1], B. Yalwaji[1]*, G. P. Shiaka[2], A. A. Amoo[1] and C. C. Udochukwu[3]
[1]Department of Environmental Sciences, Federal University Dutse, Jigawa State, Nigeria
[2]Department of Microbiology & Biotechnology, Federal University Dutse, Jigawa State, Nigeria
[3]University Clinic, Federal University Dutse, Jigawa State, Nigeria
*Correspondent author: yalwajib@gmail.com

Received: November 16, 2018 **Accepted:** February 14, 2019

Abstract: Despite the enormous health benefits attributable to the consumption of vegetables, contamination by pathogenic microorganisms inevitably leads to public health problems. This study was conducted to assess the bacteriological quality of freshly-cut and ready to eat lettuce and cabbage sold to the public by Dutse old and ultramodern markets' food vendors. *Escherichia coli*, *Salmonella* species and *Shigella* species were isolated from ten sampled vegetables using plate count method. The three organisms were detected in all the ten samples analysed except two cabbage samples that presented <1 cfu/ml of *Salmonella* sp. The mean microbial contamination of Lettuce detected from the three organisms ranged from $2.7x10^8$ to $1.04x10^9$ cfu/ml in the old market and $7.0x10^7$ to $1.01x10^9$ cfu/ml in the new market. Meanwhile, Cabbage contamination ranged from $1.8x10^8$ to $1.8x10^9$ cfu/ml in the old market and $8.0x10^7$ to $1.4x10^9$ cfu/ml in the new market. A statistically significant difference in *Salmonella* contamination ($2.0x10^8$ cfu/ml) and *Shigella* contamination ($3.3x10^8$ cfu/ml) of Lettuce between the old and new markets was revealed while the mean difference in *E. coli* contamination ($3.0x10^7$ cfu/ml) was found not to be significant ($p>0.05$). It is therefore recommended that food vendors should ensure top notch hygiene practices to attain public health safety in the consumption of vegetables.

Keywords: Cabbage, lettuce, colony count, food vendors, public health

Introduction

Food is indispensable to the maintenance of life, but can also be responsible for ill health. The foods we eat are hardly sterile as they contain a lot of microorganisms if poorly prepared or handled. Some microorganisms in food are natural microflora while others are introduced into food materials through the process of harvesting, transportation, storage, processing, preparation, distribution and selling (Adams and Moss, 2008). Despite the increased knowledge and industrialization obtainable these days, food borne diseases are perhaps the most widespread health problems and important causes of reduced economic productivity (WHO, 1992) with an incidence in every person at least once a year (Whiteney *et al.*, 1990). The available evidence indicates that biological contaminants are the major cause of food borne diseases (Adams and Moss, 2008). It is however concluded that food quality and safety are central issues in today's food economy (Grunert, 2005). World Health Organisation recognises food as one of the environmental factors that affects human health and responsible for diarrheal diseases, poisonings, cancer and other infections (Santra, 2014). A number of assessments on the relative significance of hazards associated with food have concluded that microorganisms are of paramount importance. A study conducted in the United State found out that although the attention given to different food hazards by the media, pressure groups and authorities might differ, as far as food industry was concerned, microbial hazards were the highest priority and similarly, it has been estimated that the risk of becoming ill as a result of microbial contamination of food was hundred thousand times greater than the risk from pesticide contaminations (Adams and Moss, 2008).

However, fruits and vegetables are amongst the highly nutritious foods providing vitamins and minerals and are highly recommended foods that are commonly consumed (Gupta and Rana, 2003; Isa *et al.*, 2014). Increasing health awareness has led to consumption of raw foods in recent years, these foods get polluted through the production activities and vegetables get contaminated at each step from cultivation to consumers (Shobha, 2014). The presence of numerous genera of spoilage bacteria, yeast and moulds and occasional pathogens on fresh produce has been recognised for many years (Benchat, 1996). A wide variety of fresh fruits and vegetables have been associated with diseases caused by microbial pathogens they harbour (Linda, 1997; Eni *et al.*, 2010).

The safety of food and fresh produce is a global issue covering both the countries that import and supply them (Muhammad *et al.*, 2016). Among the vegetable group of highest concern from microbiological safety identified by Mritunjay and Kumar (2015) are salad, cabbage and spinach. A study conducted by Heaton and Jones (2008) on microbial contamination identified fruits and vegetables consumption as a risk factor for infection with enteric pathogens. It is against this backdrop that this study was conducted with a view to assessing the bacteriological quality of freshly cut and ready to eat vegetables sold by food vendors and consumed by the public in Dutse markets.

Materials and Methods

Study area

Dutse is the capital city of Jigawa State and also the headquarters of the 28 local government areas that make up the state. Dutse is located at 11.76° North latitude, 9.34° East longitude and 460 meters elevation above the sea level. It is a small city that has about 17,129 inhabitants (Map, 2011). Dutse ultra-modern (new) and old market (famously known as Yantifa) were used for this study. The Yantifa market which was a farmland covered with grasses was established after the birth of Jigawa state in 1991, and effort to upgrade and relocate the Yantifa market resulted to the establishment of the Dutse ultra-modern market in 2011. Arrays of food products are sold in these markets. However, the ultra-modern market is located just by the side of the main waste disposal site in Dutse.

Sampling and sampling technique

A simple random sampling technique described by Abadias *et al.* (2007); Meldrum *et al.* (2009); Kothari and Garg (2014) was adopted to collect (purchase) samples of freshly-cut and ready to eat cabbage and lettuce from food vendors in each market. Each vegetable sample was collected in duplicate giving a total of ten (10) vegetable samples collected from the two markets. As established by United State Food and Drug Administration (2018), sterile containers were used for collecting each sample so as to ensure the aseptic technique reported by FSSAI (2012); Viswanathan and Kaur (2001).

The sampled vegetables were handled with sterile disposable gloves through the employment of a fresh glove for each sample. Samples were collected at the beginning of the week as done by CFIA (2014) and were appropriately labeled with masking tape and permanent marker.

Preparation of samples

Twenty five (25) grammes of each vegetable sample were weighed using a sensitive weighing balance machine and subsequently rinsed thoroughly with distilled water to prepare the sample homogenate as done by Shobha (2014) and Eni *et al.* (2010). From the sample homogenate, the original sample was serially diluted further into seven (7) fold sterile tubes to decrease the population of the organism sufficiently as done by Waithaka *et al.* (2014) and Begum *et al.* (1986). Using the six fold dilution, the first tube for each organism was labeled in respect of bacteria meant for assessment and original bacterial culture (OBC). The labeled tubes were then placed in a test tube rack as done by Cappuccino and Sherman (2002). Using a sterile pipette, one (1) ml of the sample was subsequently inoculated into the dilution tube by employing aseptic techniques, and mixed thoroughly to obtain 10^1 to 10^7 serial decimal dilution range of the test sample as reported by Prescott (2002); Gopal and Agrawal (2010). The highest decimal dilution (10^7) was then employed for the pour plate process.

Dissolution and sterilization of culture media

A sensitive weighing balance machine was employed to measure MacConkey's and SS agar which were subsequently dissolved and prepared according to manufacturer's instructions. Both media were successively sterilized immediately after preparation to ensure rapid multiplication of the contaminating organisms and prevent the composition from being altered as done by Yadav (2012). MacConkey's agar was sterilized in an autoclave at 121^0C for 15 min and SS agar that did not require autoclaving was sterilized in a water bath at 100^0C for 15 min as done by Eni *et al.* (2010) and Yadav (2012).

Isolation and identification of bacteria in the vegetable samples

The culture media that were used in this study depended on the targeted and enumerated bacteria (*Escherichia Coli, Salmonella sp and Shigella sp*). However, plate count method (pour plate method) was adopted in this study. MacConkey's agar was employed to isolate and identify *Escherichia Coli* following the method prescribed by Cappuccino and Sherman (2002); Baveja (2013); Nishith and Chakraborty (2014). *Salmonella* and *Shigella* agar (SS Agar) was used for the isolation and identification of *Salmonella* sp and *Shigella* sp present in the samples of the vegetables as done by Eni *et al.* (2010). Colonies of *Shigella* sp on the medium were differentiated by their colourless appearance with no blackening while those of *Salmonella* sp were identified by their colourless appearance with black centers as done by Baveja (2013).

Enumeration of bacterial colonies

Having incubated the culture plates at appropriate and acceptable temperature ranges, they were brought out with a view to counting the colonies in each petri dish. Counting was done with the help of a colony counting machine as prescribed by Gopal and Agrawal (2010); Eni *et al.* (2010). Since solid media were used for the bacterial culture, colony forming unit (CFU) was adopted in reporting the results as prescribed by Tortora *et al.* (2014). Bacterial counts between 30 and 300 colonies per plate were reported as cfu/ml. Plates with no colonies were reported as <1cfu/ml and those with colonies >300 were reported as too numerous to count (TNC) as done by Santra (2014).

Data collection and analysis

Data were generated from the results of the colony enumerations carried out after the bacteria had been cultured and incubated. Data collected from this study were analyzed using general descriptive statistics. IBM SPSS Statistics 19 software was employed for the statistical analysis where T-test statistical tool at 95% probability level of significance was used to determine the level of significance.

Results and Discussion

The vegetables targeted in this study comprised of six (6) lettuce samples and four (4) cabbage samples. Each sample was analysed for the presence and concentration of *Escherichia coli* (faecal coliform), *Salmonella* species and *Shigella* species. Table 1(a) and (b) depict the microbial counts obtained in each sample across the two markets having subjected it to microbial analysis. The results showed that freshly-cut and ready to eat lettuce and cabbage harboured microbial pathogens as reported by Eni *et al.* (2010). The fact that the bacteria detected were isolated from freshly cut and ready to eat vegetables confirms the description of Adams and Moss (2008); Abadias *et al.* (2007) that microbial contaminants are introduced into food materials at each stage including preparation and selling.

Bacterial contaminations estimated in this study could be from contaminated equipment or the workers handling the vegetables as reported by Nguz *et al.* (2005) and might originate from the influence of insect vectors (flies). From the results, two lettuce samples, one from old market and the other from new market recorded the highest *Escherichia coli* count ($1.5x10^9$ cfu/ml) while a Lettuce sample from the new market recorded the lowest *E. coli* count ($6.8x10^8$ cfu/ml). *Shigella* species were also isolated from all the samples analysed. A Cabbage sample from the new market had the highest *Shigella* count ($2.6x10^9$ cfu/ml) while the lowest *Shigella* count ($2.8x10^8$ cfu/ml) was detected in a Lettuce sample from the new market. However, it can be seen from the results that not all the vegetable samples were contaminated with *Salmonella* species as two (2) Cabbage samples recorded <1 cfu/ml *Salmonella* colony in the new market and the old market, respectively. This result reiterates that vegetable samples recorded to have got zero *Salmonella* count in 25 grams as reported by Meldrum *et al.* (2009) constitutes microbial safety.

Table 1 (a): Bacterial counts in ready to eat sampled lettuce sold at Dutse old and new markets

Organisms	Vegetable Samples					
	OM (cfu/ml)			NM (cfu/ml)		
	LT1	LT2	LT3	LT1	LT2	LT3
E. coli	$1.5x10^9$	$9.1x10^8$	$7.1x10^8$	$8.4x10^8$	$6.8x10^8$	$1.5x10^9$
Salmonella sp.	$2.3x10^8$	$3.5x10^8$	$2.2x10^8$	$4.0x10^7$	$5.0x10^7$	$1.2x10^8$
Shigella sp.	$5.4x10^8$	$7.9x10^8$	$6.3x10^8$	$3.7x10^8$	$2.8x10^8$	$3.0x10^8$

(reciprocal of dilution = 10^7)

OM= Old market NM= New market LT= Lettuce sample

Table 1 (b): Bacterial count in ready to eat sampled cabbage sold at Dutse old and new markets

Organisms	Vegetable Samples			
	OM (cfu/ml)		NM (cfu/ml)	
	CB1	CB2	CB1	CB2
E. coli	$1.3x10^9$	$1.5x10^9$	$1.1x10^9$	$1.5x10^9$
Salmonella sp.	$3.6x10^8$	<1	<1	$1.6x10^8$
Shigella sp.	$2.6x10^9$	$1.0x10^9$	$3.5x10^8$	$2.4x10^9$

(reciprocal of dilution = 10^7)

OM: Old market NM: New market CB: Cabbage sample

A cabbage sample from the old market recorded the highest *Salmonella* colony count ($3.6x10^8$ cfu/ml) while a lettuce sample from the new market had the lowest *Salmonella* count ($4x10^7$ cfu/ml). As presented in Table 2, the results of the statistical analysis for Lettuce across the two markets depicted that *E. coli* contamination dominated the samples from the old market ($1.04x10^9$ cfu/ml) followed by *Shigella* contamination ($6.5x10^8$ cfu/ml) then *Salmonella* contamination ($2.7x10^8$ cfu/ml). Similarly, this trend is synonymous with the Lettuce samples from the new market as they were more contaminated with faecal coliform ($1.01x10^9$ cfu/ml) and *Shigella* species ($3.2x10^8$ cfu/ml) than *Salmonella* sp. ($7.0x10^7$ cfu/ml). *E. coli* contamination also dominated the samples from the new market. Also, Table 3 shows the results of the microbial load in cabbage across the two markets. The pattern transposes compared to that of Lettuce. The results indicate that Cabbage samples from the old market were highly contaminated with *Shigella* species ($1.8x10^9$ cfu/ml).

Table 2: Microbial load of enteric microorganisms enumerated in sampled lettuce across the two markets

Variables	OM Mean (cfu/ml)	NM Mean (cfu/ml)	Mean diff. (cfu/ml)	t-value	Sig.	Remark
Escherichia coli	$1.04x10^9$	$1.01x10^9$	$3.0x10^7$	1.88	0.134	**N S**
***Salmonella* Species**	$2.7x10^8$	$7.0x10^7$	$2.0x10^8$	2.91	0.044	**S**
***Shigella* Species**	$6.5x10^8$	$3.2x10^8$	$3.3x10^8$	4.32	0.013	**S**

OM= Old Market NM= New Market NS= Not significant S= Significant

Table 3: Microbial load of enteric microorganisms enumerated in cabbage across the two markets

Variables	OM Mean (cfu/ml)	NM Mean (cfu/ml)	Mean diff. (cfu/ml)	t-value	Sig.	Remark
Escherichia coli	$1.4x10^9$	$1.3x10^9$	$1.0x10^8$	0.558	0.633	**NS**
***Salmonella* Species**	$1.8x10^8$	$8.0x10^7$	$1.0x10^8$	0.558	0.633	**NS**
***Shigella* Species**	$1.8x10^9$	$14x10^9$	$4.0x10^8$	0.345	0.763	**NS**

OM= Old Market NM= New Market NS= Not significant

This is similar to the Cabbage samples from the new market which were also highly contaminated with *Shigella* sp. ($1.4x10^9$ cfu/ml). The faecal contamination of Cabbage in both markets ($1.4x10^9$ and $1.3x10^9$ cfu/ml) as revealed by the results was higher than *Salmonella* sp contamination ($1.8x10^8$ and $8.0x10^7$ cfu/ml). In the Cabbage samples obtained from both markets, *Shigella* contamination preponderated. As reported by Meldrum *et al.* (2009), all the vegetables analysed for *E. coli* contamination in this study were not satisfactory according to microbial criteria for ready to eat vegetables as they presented way above the recommended count ($>10^2$cfu/ml). As reported by Adams and Moss (2008); Linda (1997), infection may result from ingestion of such contamination if the infectious dose falls between 2-1000 cells. Also, in this study, all the *Shigella* counts recorded did exceed the infectious dose and as such sampled vegetables harbouring the microorganism are not safe for human consumption. Generally, the results generated in this study are in conformity with the research studies of Abadias *et al.* (2007); Eni *et al.* (2010) which detected higher microbial contamination range in vegetables (10^5 to 10^8 cfu/ml), Lettuce ($1.7x10^7$ cfu/ml) and Cabbage ($1.8x10^7$ cfu/ml) respectively. Uzeh *et al.* (2009) also discovered a higher bacteriological load of $10^8 – 10^9$ cfu/ml in commonly eaten freshly fruits and vegetables which is in conformity with the result obtained in this study.

The bacterial contamination of Lettuce detected from the three bacteria ranged from $2.7x10^8$ to $1.04x10^9$ cfu/ml in the old market and $7.0x10^7$ to $1.01x10^9$ cfu/ml in the new market. Meanwhile, Cabbage contamination ranged from $1.8x10^8$ to $1.8x10^9$ cfu/ml in the old market and $8.0x10^7$ to $1.4x10^9$ cfu/ml in the new market. However, it is worthy to point out that in Tables 2 and 3, T-test failed to reveal a statistically significant difference between the mean numbers of *Escherichia coli* contamination in the sampled Lettuce and Cabbage coupled with *Salmonella* and *Shigella* contaminations in the Cabbage sampled across the two markets. However, as depicted in the Tables (2 and 3), the difference between *Salmonella* and *Shigella* contaminations of lettuce in the two markets were significant.

Conclusion

The results obtained from the bacteriological analysis carried out in this study indicated that all the vegetable samples examined were highly contaminated with *Escherichia coli*, *Salmonella* species and *Shigella* species. No sample from either market was devoid of the three bacteria isolated and identified in this study. The bacterial loads detected in the vegetables assessed in this study had the possibility of causing infections attributable to all the bacteria isolated. The fact that *E. coli* contamination was detected in the vegetables directly suggests that faecal contamination could be present and, consumers may be at risk of contacting faeco-oral diseases such as Typhoid fever, Gastroenteritis and Bacillary dysentery that in turn pose a public health threats. Also, among the different vegetable samples analysed, *Shigella* sp had the highest concentration while *Salmonella* sp had the lowest concentration. In addition, the presence of these bacteria in such concentrations indicates that these freshly-cut and ready to eat vegetables might have been contaminated from unhygienic containers or contaminated water used for their preparation, the handlers or flies that carry disease agents from faecal matter and other contamination sources onto uncovered ready to eat foods.

Recommendation

It is recommended that food handlers and vendors must be educated on the health effects associated with food contamination, personal hygiene and good food handling practices. Equipment to be used for food preparation should be thoroughly washed and the water used for washing vegetables should be potable. Also, freshly-cut and ready to eat vegetables should be covered always unless during serving to avert contamination by flies and airborne pathogens. Food handlers must wash their hands frequently most especially after handling money or visiting conveniences. However, it is important to indicate that this study was limited to the isolation and identification of the enteric organisms present in the vegetables investigated. It is therefore recommended that there is a need for further identification of other pathogenic organisms that could be present and the specific species and strains of the enteric organisms isolated in this study through

the employment of biochemical tests and most importantly molecular identification.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abadias M, Usall J, Anguera M, Solsona C & Vinas I 2007. Microbiological quality of fresh, minimally-processed fruit and vegetables,and sprouts from retail establishments. *Int. J. Food Microbio.*, 123: 121–129.

Adams R & Moss O 2008. Food Microbiology, Third Edition. UK: The Royal Society of Chemistry.

Baveja C 2013. Text Book of Microbiology Fourth Edition. New Delhi: Arya Publications.

Begum M, Ali Z & Bari A 1986. Quantitative and qualitative microbial load determination from meat samples effected by time and temperature. *J. Pak. Med. Assoc.*, 36(90): 90-93.

Benchat C 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Protection*, 59: 204-216.

Canadian Food Inspection Agency 2014. Procedure for Sampling Fresh Fruits and Vegetables. Available at http://www.inspection.gc.ca/food/fresh-fruits-and-vegetables /food-safety/sampling-fresh-fruit-and-vegetables/eng. Retrieved on 22nd May, 2017.

Cappuccino J & Sherman N 2002. Microbiology: A Laboratory Manual, Addison– Wesley.

Eni AO, Oluwawemitan IA & Solomon OU 2010. Microbial quality of fruits and vegetables sold in Sango, Ota Nigeria. *Afr. J. Food Sci.*, 4(5). Available at http://www.academicjournals.org/ajfs. Retrieved on 22nd May, 2017.

Food Safety and Standard Authority of India 2015. Manual on General Guideline on Sampling. New Delhi, New Delhi, India: Ministry of Health and Family Welfare, Government of India. Available at http://old.fssai.gov.in/Portals/0/Pdf/Draft_Manuals/GENERAL_GUIDELINES_ON_SAMPLING.pdf. Retrieved on August 2nd 2018.

Gopal K & Agrawal A 2010. Principles of Toxicology. Delhi: IBDC Publishers.

Grunert K 2005. Food quality and safety: Consumer perception and emand. *Oxford University Press Journal*, 32(3): 369-391.

Gupta K & Rana M 2003. Salad Crops Dietary Importance: Encyclopedia Of Food Science and Nutrition. 2nd Edition. UK: Academic Press.

Heaton J & Jones K 2008. Microbial contamination of fruits and vegetables and the behaviour of enteropathogens in the phyllosphere. *J. Appl. Microbio.*, 613-626.

Isa A, Isa M, Bukar A & Muhammad I 2014. Bacteriological quality of fruits and vegetables sold at Maiduguri Metropolis, Nigeria. *Research Desk*, 3(2): 471-475.

Kothari C & Garg G 2014. Research Methodology: Methods and Techniques Third Edition. New Delhi: New Age International Publishers.

Linda J 1997. Microbial pathogens associated with produce. *Perishables Handling*, 91: 2-3.

Maps 2011. Dutse Maps - road map, satellite view and street view. Available at https://www.maps-streetview.com/Nigeria/Dutse/. Retrieved on March 30, 2017.

Meldrum R, Little C, Sagoo S, Mithani V, McLauchlin J & Pinna E 2009. Assessment of the microbiological safety of Salad Vegetables and Sauces from Kebab take-away Restaurants in the United Kingdom. *Food Microbiology*, 26: 573–577.

Mritunjay K & Kumar V 2015. Fresh Farm Produce As A Source Of Pathogens. *Res. J. Envtal. Toxicol.*, 9: 59-70.

Muhammad R, Yaro C, Balarabe M, Zainab J & Adedayo M 2016. Assessment of bacteria associated with ready-to-eat food sold at Federal University Dutse, Jigawa State, Nigeria. *Int. J. Current Res. Biosci. and Plant Bio.*, 3(4): 2349-8080.

Nguz K, Shindano J, Samapundo S & Huyghebaert A 2005. Microbial evaluation of fresh-cut organic vegetables produced in Zambia. *Food control*, 16: 623-628.

Nishith K & Chakraborty P 2014. Manual of Practical Microbiology and Parasitology. New Delhi: New Central Book Agency.

Prescott H 2002. Laboratory Excercise in Microbiology Fifth Edition. New York: Mc-Graw Hill publishers.

Santra S 2014. Environmental Science. New Delhi: New Central Book Agency.

Shobha S 2014. Bacteriological analysis of fresh vegetables and fruits of local market. *Int. Res. J. Bio. Sci.*, 3(11): 15-17.

Tortora J, Funke R & Case L 2014. Microbiology: An Introduction. *9th Edition.* New Delhi: Dorling Kindersely Publishers.

United State Food & Drug Administration 2018. Bacteriological Analytical Manual (BAM) Chapter One: Food Sampling and Preparation of Sample Homogenate. Available at https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm063335.htm. Retrieved on 26th August 2018.

Uzeh RE, Alade FA & Bankole M 2009. The Microbial Quality of Pre-packed Mixed Vegetable Salad in Some Retail Outlets in Lagos, Nigeria. *Afr. J. Food Sci.*, 3(9): 270-272.

Viswanathan P & Kaur R 2001. Prevalence and growth of pathogens on Salad Vegetables, Fruits and Sprouts. *Int. J. Hygeine and Envtal. Health*, 203(3): 205-213.

Waithaka P, Nyamache A & Maingi J 2014. Microbial Contamination of Environmental, Food and treated Water samples in Nakuru North Sub-county, Kenya. *Int. J. Microbio. and Allied Sci.*, 1(2): 78-91.

Whiteney E, Hamilton E & Rolfes S 1990. Understanding Nutrition. Fifth Edition. New York: West Publishing Company.

World Health Organisation 1992. World Health Organisation Report Of The Panel On Food And Agriculture. Available at www.who.int.iris/handle/10665/61427. Retrieved May 09, 2016.

Yadav M 2012. Microbiology. New Delhi: Discovery Publishing House.