http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (17): 857-861, 2010
ISSN 1028-8880

Internalisation of Microbes in Vegetables: Microbial Load of Ghanaian Vegetables and the Relationship with Different Water Sources of Irrigation

[1,4]Eric S. Donkor, [2]R. Lanyo, [3]Boniface B. Kayang, [3]Jonathan Quaye and [2]Dominic A. Edoh
[1]Department of Microbiology, University of Ghana Medical School, Accra, Ghana
[2]Department of Environmental Science, Faculty of Science, University of Ghana, Accra, Ghana
[3]Department of Animal Science, School of Agriculture, University of Ghana, Accra, Ghana
[4]Department of Infectious and Tropical Diseases,
London School of Hygiene and Tropical Medicine, London, UK

Abstract: The occurrence of pathogens in the internal parts of vegetables is usually associated with irrigation water or contaminated soil and could pose risk to consumers as the internalised pathogens are unaffected by external washing. This study was carried out to assess the rate of internalisation of microbes in common Ghanaian vegetables. Standard microbiological methods were employed in microbial enumeration of vegetables collected at the market and farm levels, as well as irrigation water and soil samples. The overall mean counts of vegetables were 4.0×10^3 cfu g^{-1}; 8.1×10^2 cfu g^{-1}; 2.0×10^2 cfu g^{-1}; 3.5×10^2 cfu g^{-1} for total bacteria, coliform counts, faecal coliform counts and yeast counts, respectively. The rate of internalisation of coliforms in vegetables irrigated with stream/well water was 2.7 times higher than those irrigated with pipe water. The mean coliform counts (4.7×10^7 cfu g^{-1}) and faecal coliform counts (1.8×10^6 cfu g^{-1}) of soil samples were similar to those of stream water suggesting both sources exerted similar contamination rates on the vegetables. Generally, there were no significant variations between the rates of internalisation of microbes at the market and farm levels at $p<05$, indicating that internalisation of microbes in the vegetables mainly occurred at the farm level. The study has shown that microbial contamination of vegetables in Ghana is not limited to the external surface, but internal vegetable parts could harbour high microbial loads and pose risk to consumers. Safety practices associated with the commodity should therefore not be limited to external washing only. There is the additional need of heating vegetables to eliminate microbes both externally and internally before consumption.

Key words: Food, safety, consumers, outbreaks, microbial

INTRODUCTION

Microbial risk is the most important form of risk associated with food and recent epidemiological information has shown an increment in outbreaks with several foods including vegetables (Tood, 1997; Doyle, 1991; Mensah *et al*., 2002; Greig *et al*., 2007). The microbial load of food measures the extent of food contamination, the likelihood of the presence of pathogens and the keeping quality of the food. Microbial contamination of vegetables is usually associated with the external surface of vegetables. However, internalisations of microbes including pathogenic forms in vegetables have been documented (Bernstein *et al*., 2007; Solomon *et al*., 2002). Microorganisms may enter vegetables through damage to the natural structure, such as punctures and cuts, which can occur during maturation, harvesting or processing (Ryall and Pentzer, 1982). The occurrence of pathogens in the internal parts of vegetables could pose risk to consumers as the internalised pathogens are unaffected by external washing.

The hygienic safety of vegetables is threatened by various factors including poor quality irrigation water, as such water could result in internal and external contaminations of vegetables (Wachtel *et al*., 2002a, b). Pipe water, groundwater, surface water and human wastewater are commonly used for irrigation. Pipe and ground waters are generally of good microbial quality, unless ground water is contaminated with surface runoff; human wastewater is usually of very poor microbial quality and requires extensive treatment before it can be used safely to irrigate crops; surface water is of variable microbial quality (Steele and Odumeru, 2004). In Accra, the capital city of Ghana where this study was carried out, the various water sources mentioned are used in irrigation

Corresponding Author: Eric Sampane-Donkor, Department of Infectious and Tropical Diseases,
London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK Tel: +442079272032

of vegetables. Pipe-borne water was one of the most commonly used water sources, however, owing to cost and availability, most farmers seem to have shifted from the use of pipe water to other water sources especially, streams and wells which are usually subjected to high rate of environmental pollution

In Ghana, studies on various vegetables had shown association of the commodity with high microbial risk (Mensah *et al*., 2001; Obeng *et al*., 2007). However, it is unclear whether this is limited to external contamination of vegetables from handling and other sources, or their internal parts could also pose substantial risk to consumers. We previously studied sweet green pepper (*Capsicum annuum grossum*) sold in Accra and observed high microbial load internally in the vegetable (Donkor *et al*., 2009). This prompted us to evaluate other vegetables especially, the types that are usually consumed without heat treatment and therefore consumer risk may be higher. The present study reports on the internal microbial load of some exotic vegetables (lettuce, cabbage, pepper, tomatoes) and the relationship with pipe, stream and well water sources of irrigation.

MATERIALS AND METHODS

Study area and sample collection: The study was conducted from September, 2006 to August, 2007 in Accra, the capital city of Ghana. The entire area of study is located within the Dahomian ecological zone of West Africa and the vegetation is coastal savanna grassland. The climate is hot and humid and there is a bimodal rainfall pattern with a mean annual rainfall of about 1300 mm. The mean daily temperature is 26°C with a range of 18-35°C. The relative humidity can be as high as 97% in the mornings of wet seasons and as low as 20% in the afternoon of the dry seasons. Some of the main types of vegetables found in the various markets are tomatoes (*Lycopersicum* sp.), garden eggs (*Solanum* sp.), green pepper (*Capsicum* sp.) and cabbage (*Brassica oleracia* var. *capitata*).

The study was carried out at both the market and farm levels. At the market level, a total of 272 vegetable samples comprising 68 each of lettuce, cabbage, pepper, tomatoes were collected from four major markets. At the farm level, a total of 30 cabbage samples were collected from three vegetables stations where either pipe water or stream/well water were used in irrigation. A total of 30 samples each of pipe water, stream water and well water used for irrigations were collected. The soil at each vegetable station was also sampled and this included 4 samples at each station. All the samples were collected aseptically and transported to the laboratory for analyses.

Laboratory analyses

Microbial enumeration of vegetable samples: The internal part of all the vegetable samples were analysed for Yeast Counts (YC), Total Plate Counts (TPC), Coliform Plate Counts (CPC) and Faecal Coliform Plate Counts (FCPC) using direct culture methods (Marshall, 1992). The procedure followed is described as follows: whole vegetable samples were decontaminated adequately with hypochloride solution of 200 ppm. The vegetables were then opened up aseptically using a sterile scalpel. One gram of the inner tissues of vegetables was taken from an area that could not possibly have been touched by external items. The anatomical structure of pepper, tomato and cabbage made it easy to obtain internal samples of these vegetables. However, in the case of lettuce, it was difficult to obtain internal samples due to the thinness of this vegetable. Internal lettuce samples were therefore taken from the mid-rib of the lettuce leaves. The internal samples of the various vegetables were transferred into MacContey bottles containing 1 mL saline solution. The mixture was macerated and 1 mL used to prepare a ten fold serial dilution to obtain a dilution range of 10^{-1}-10^{-6} for each sample. One milliliter of the dilutions of each sample was pipetted into a petri dish and then mixed with about 20 mL of culture medium. The culture media used, included Standard Plate Count Agar (SPCA) for enumeration of total bacteria; MacConkey Agar (MA) for enumeration of coliforms; Eosin Methylene Blue Agar (EMBA) for enumeration of faecal coliforms; and Potato Dextrose Agar (PDA) for enumeration of yeasts. After cooling and solidification of the media, all the plates were incubated: SPA and MA plates were incubated at 37°C for 18-24 h; EMBA plates were incubated at 44°C for 18-24 h; PDA plates were incubated at 37°C for 48 h. After the incubation periods, plates with colonies ranging from 20-200 colony forming units (cfu) were counted with a colony counter and used to compute microbial counts of the vegetable samples (Speck, 1984; Marshall, 1992).

Coliform and faecal coliform enumeration of water and soil samples: One millilitre of the water sample was diluted in 9 mL of the standard saline solution and subsequently used to prepare a ten fold serial dilutions of 10^{-1}-10^{-6}. A soil sample was analysed by weighing 20 g (dry weight) into 180 mL standard saline solution and, subsequently used to prepare a ten fold serial dilutions of 10^{-1}-10^{-6}. One milliliter of the diluents of each sample was used to inoculate MA and EMB plates and incubated at a 37 and 44°C, respectively for 18-24 h. Following this period, plates with colonies ranging from 20-200 colony forming units (cfu) were counted with a colony counter and used to compute CPC and FCPC (Marshall, 1992).

Data analyses: Laboratory analyses results were entered in MS-EXCEL and analysed in SPSS to address the objectives of the study. The strategies taken to analyse the data involved descriptive statistics, including geometric means, frequencies, ranges and prevalence rates of the study variables. Significant differences, associations and interrelationships of the variables were also assessed. Specific analyses were carried out to:

- Evaluate internal microbial load of various vegetables
- Compare internal microbial quality of vegetables irrigated with pipe water and vegetables irrigated with stream/well water
- Assess the possible effects of manured soil on internal microbial load of vegetables
- Estimate the risk of internalisation of microbes in vegetables to consumers

RESULTS

Microbial load of soil and irrigation water samples: The geometric means of microbial counts of water and soil samples analysed are shown in Table 1, the geometric means of CPC for the various irrigation water sampled were, Pipe-borne water (1.1×10^{1} cfu mL^{-1}); Well water (1.6×10^{6} cfu mL^{-1}) and Stream water (5.8×10^{7} cfu mL^{-1}). The geometric means of FCPC for the various irrigation water were Pipe-borne water (0.2×10^{1} cfu mL^{-1}); Well water (2.3×10^{5} cfu mL^{-1}) and Stream water (1.6×10^{7} cfu mL^{-1}). The mean CPC and FCPC of the soil samples were 4.7×10^{7} and 1.8×10^{6} cfu g^{-1}, respectively. Significant differences were observed among CPC and FCPC of the various water sources at $p<0.05$. However no significant differences were observed among CPC and FCPC of the various soil samples at $p<0.05$.

Microbial load of farm-level vegetables (cabbage) irrigated with different types of water: The proportion of cabbage samples contaminated with coliforms was 40% (pipe water) and 53.3% (stream/well water). The mean CPC of pipe water and stream/well water irrigated cabbage were 6.2×10^{1} and 1.7×10^{2} cfu g^{-1}, respectively. The mean FCPC of pipe water and stream/well water irrigated cabbage were 3.7×10^{1} and 1.0×10^{2} cfu g^{-1}, respectively. Variations were observed in microbial counts of vegetables irrigated with pipe water and stream/well water and this was significant at $p<0.05$ for CPC and FCPC.

Microbial load of vegetables sampled at the market-level: The overall mean counts of the vegetables were 4.0×10^{3}, 8.1×10^{2}, 2.0×10^{2} and 3.5×10^{2} cfu g^{-1} for total bacteria, coliform counts, faecal coliform counts and yeast counts, respectively. A proportion of 27.5% of all the vegetables sampled at the market level were not contaminated internally with bacteria or fungi. Table 2 reports on the internal microbial contaminations of the various vegetables sampled including, lettuce, cabbage, pepper and tomatoes. Overall, the proportion of samples contaminated were 16.2-55.9% (TPC); 30.9-54.4% (CPC); 11.8-39.7% (FCPC); and 33.8-63.2% (YC). Pepper had the highest proportion of contaminated samples for TPC, CPC, FCPC, while tomatoes had the highest proportion of contaminated samples for YC. Tomatoes had the lowest proportion of contaminated samples for TPC, CPC, FCPC, while cabbage had the lowest proportion of contaminated samples for YC. The mean counts for TPC, CPC, FCPC and YC of the various vegetables are reported in Table 3. Generally, lettuce and cabbage showed similar microbial counts, while tomatoes and pepper also had similar counts; the former pair of vegetables had higher counts and significant variations were observed in FCPC between the two pairs of vegetables at $p<0.05$. Overall, 42.6% of the vegetable samples had unacceptable CPC (counts higher than 1×10^{3} 100 g^{-1} fresh weight).

Table 1: Coliform and faecal coliform content of soil and irrigation water samples

		Total coliform		Faecal coliform	
Source	N	Mean	SD	Mean	SD
Pipe-borne water	30	1.1×10^{1}	3.7×10^{1}	0.2×10^{1}	0.6×10^{1}
Stream water	30	5.8×10^{7}	1.7×10^{8}	1.6×10^{7}	3.8×10^{7}
Well water	20	1.6×10^{6}	3.4×10^{6}	2.3×10^{5}	5.9×10^{5}
Soil	12	3.3×10^{7}	3.5×10^{7}	5.0×10^{6}	2.8×10^{6}

N indicates number of samples analysed. Total coliform and faecal coliform of water and soil samples are expressed as cfu mL^{-1} and cfu g^{-1}, respectively

Table 2: Proportions of contaminated vegetable sampled at the market level

Type of vegetable	TPC (%)	CPC (%)	FCPC (%)	YC (%)
Pepper	47.1	54.4	39.7	58.8
Tomatoes	55.9	30.9	11.8	63.2
Lettuce	16.2	50.0	36.8	47.1
Cabbage	45.6	33.8	26.5	33.8

68 samples were analysed for each vegetable type

Table 3: Mean microbial counts of internal parts of vegetables sampled at the market level

Type of vegetable	TPC	CPC	FCPC	YC
	----------(cfu g^{-1})----------			
Pepper	8.9×10^{3}	4.4×10^{2}	4.8×10^{1}	2.0×10^{2}
	(3.0×10^{4})	(1.2×10^{3})	(8.6×10^{1})	(5.1×10^{2})
Tomatoes	1.5×10^{3}	2.2×10^{2}	5.7×10^{1}	2.0×10^{2}
	(4.9×10^{3})	(6.1×10^{2})	(4.1×10^{2})	(5.1×10^{3})
Lettuce	4.0×10^{3}	8.3×10^{2}	2.0×10^{2}	3.5×10^{2}
	(9.1×10^{3})	(1.2×10^{4})	(6.7×10^{2})	(7.0×10^{2})
Cabbage	2.0×10^{3}	3.3×10^{2}	1.5×10^{2}	1.8×10^{2}
	(6.8×10^{3})	(8.6×10^{2})	(6.4×10^{2})	(4.0×10^{2})

Mean counts are indicated without brackets, while the corresponding standard deviations are indicated in brackets

DISCUSSION

Wide variations were observed in the microbial quality of the three types of water samples with pipe borne water being of good quality and stream/well water showing poor quality. This observation is due to the differences in purification treatment of the various water types and their susceptibility to contamination. While pipe borne water receives a thorough purification treatment, well and stream water may not be treated and are also highly susceptible to environmental pollution. Similar findings have been reported by several other studies especially regarding stream and well water and the results of the microbiological quality of irrigation water confirm earlier reports that low quality water is being used for urban vegetable production in most Ghanaian cities (Mensah *et al*., 2001; Keraita *et al*., 2002, 2003).

The inner tissues of fruits and vegetables are usually regarded as sterile (Lund, 1992). However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures and if these waters are contaminated with human pathogens these may also be introduced (European Commission, 2002). Based on the necessary computations made, it was observed that internalisation of coliform or faecal coliforms in vegetables using stream/well water could occur 2.7 higher than using pipe water. Though vegetables irrigated with pipe-borne water showed lower microbial contaminations than vegetables irrigated with stream/well water, these differences are far less than the differences observed in microbial contaminations between pipe-borne water and stream/well water. This suggests other sources of contaminations of the vegetables apart from irrigation water, which could include several factors such as soil, green or inadequately composted manure, air (dust), wild and domestic animals, insects and human handling (Beuchat, 1996). As both pipe borne water and stream/well water irrigated vegetables were grown on soil (that received poultry manure) which also had high coliform and faecal coliform content, it is very likely the manured soil may have contributed significantly to the contamination of the vegetables. As matter of fact the coliform/faecal coliform content of the manured soil (4.7×10^{7} cfu g^{-1}/1.8×10^{6} cfu g^{-1}), were about similar to that of stream water (5.8×10^{7} cfu mL^{-1}/1.6×10^{7} cfu mL^{-1}) and well water (1.6×10^{6} cfu mL^{-1} /2.3×10^{5} cfu mL^{-1}) and is likely both the water and soil sources may have exerted similar contamination rates on the vegetables.

Comparing vegetables (cabbage) at the farm and market levels, it was observed that, generally, there were no significant variations between the rates of internalisation of microbes at the market and farm levels. This indicates that internalisation of microbes in the vegetables occurred mainly at the farm level, from vegetable cultivation practices such as irrigation.

Though the inner tissues of vegetables are normally sterile, in this study, only 27.5% of the vegetables were sterile as judged by the absence of bacteria and fungi. Similarly, internalisation of microbes in vegetables has been reported by several other workers (Samish and Etinger-Tulczynska, 1963; Solomon *et al*., 2002; Ibarra-Sánchez *et al*., 2004; Zhuang *et al*., 1995; Johannessen *et al*., 2005; Penteado *et al*., 2007). In a study by Penteado *et al*. (2007), it was observed that exposing mangoes to 10^{7} cfu mL^{-1} *Salmonella enteritidis* resulted in *Salmonella* internalisation at a frequency of 80 and 87%, respectively for immature and ripened mangoes. The most important microbial indicator of food hygiene is bacteria coliform, especially those of faecal origin (WHO, 1993; Edberg *et al*., 2000). In this study, the level of coliforms counts of a high proportion of the samples (42.6%) were higher than the recommended level of 1×10^{3} 100 g^{-1} fresh weight (ICMSF, 1974). Similarly, the overall mean coliform counts of the vegetable samples (8.1×10^{2} cfu g^{-1}) were also far higher than the recommended level. The high microbial load of the vegetables internally, indicates that even with adequate washing of the external surface of vegetables, consumers are still exposed to high microbial loads. In this regard, vegetables consumed raw, without any form of heat treatment pose the greatest risk to consumers. We did not investigate the presence of specific pathogens in the vegetable samples, however, the appreciably, high microbial counts especially, coliform and faecal coliform counts indicate the likely presence of pathogens (Edberg *et al*., 2000).

The study has shown that microbial contamination of vegetables consumed in the study area is not limited to the external surface, but the internal vegetable parts could pose risk to consumers. Safety practices associated with vegetables should therefore not be limited to external washing only. There is the additional need of heating vegetables where possible to eliminate microbes both externally and internally before consumption. Additionally, since internalisation of microbes in vegetables is mainly associated with irrigation water, there is the need to use safe water in irrigation.

The main limitation of this study is that decontamination of the surface of vegetables may not have rendered the external vegetable surface sterile and hence the possible introduction of some microbes into the internal parts of the vegetable samples. However, the aseptic conditions employed in the experiments should have eliminated or minimised this.

REFERENCES

Bernstein, N., S. Sela and S. Neder-Lavon, 2007. Assessment of contamination potential of lettuce by Salmonella enterica serovar Newport added to the plant growing medium. J. Food Prot., 70: 1717-1722.

Beuchat, L.R., 1996. Pathogenic microorganism associated with fresh procedure. J. Food Prot., 59: 204-206.

Donkor, E.S., B.B. Kayang, J. Quaye, D.A. Edoh and G. Owusu-Okyere, 2009. Microbial contamination of green pepper sold in Accra. GJAHS, 2: 22-28.

Doyle, M.P., 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol., 12: 289-301.

Edberg, S.C., E.W. Rice, M.J. Karlin and J. Allen, 2000. *Escherichia coli*: The best biological drinking water indicator for public health protection. J. Applied Microbiol., 88: 106S-116S.

European Commission, 2002. Risk profile on the microbial contamination of vegetables and fruits eaten raw. Report of the Scientific Committee on Food. Health and Consumer Protection Directorate-General, European Commission Brussel, Belgium. http://ec.europa.eu/food/fs/sc/scf/out125_en.pdf.

Greig, J.D., E.C. Todd, C.A. Bartleson and B.S. Michaels, 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease: description of the problem, methods and agents involved. J. Food Prot., 70: 1752-1761.

ICMSF, 1974. An Evaluation of the Role of Microbiological Criteria for Foods and Food. National Academies Press, Washington, D.C.

Ibarra-Sánchez, L.S., S. Alvarado-Casillas, M.O. Rodríguez-García, N.E. Martínez-Gonzáles and A. Castillo, 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. J. Food Prot., 67: 1353-1358.

Johannessen, G.S., B.G. Bengtsson, B.T. Heier, S. Bredholt, Y. Wasteson and L.M. Rørvik, 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. Applied Environ. Microbiol., 71: 2221-2225.

Keraita, B., P. Dreschsel, F. Huibers and L. Raschid-Sally, 2002. Waste water use in informal irrigation in urban and peri-urban areas of Kumasi, Ghana. Urban Agric. Mang., 8: 11-13.

Keraita, B., P. Dreschsel and P. Amoah, 2003. Influence of urban wastewater on stream water quality and agriculture in and around Kumasi, Ghana. Environ. Urbanisation, 15: 171-178.

Lund, B.M., 1992. Ecosystems in vegetable foods. J. Applied Bacteriol. Symp. Ser., 21: 115S-126S.

Marshall, R.T., 1992. Standard Methods for the Examination of Dairy and Vegetable Products. 16th Edn., American Public Health Association, Washington DC.

Mensah, P., D. Yeboah-Manu, K. Owusu-Darko and A. Ablordey, 2002. Street foods in Accra, Ghana: How safe are they?. Bull. WHO, 80: 546-554.

Mensah, P., M. Armar-Klemesu, A.S. Hammond, A. Haruna and R. Nyaku, 2001. Bacteria contaminants of lettuce, tomatoes, beef and goat meat from the Accra Metropolis. Ghana Med. J., 35: 1-6.

Obeng, A.S., A.G. Kwakye-Nuako, R.H. Asmah and P.F. Ayeh-Kumi, 2007. Pathogenic parasitic microbes associated with fresh vegetables consumed in Accra. GJAHS, 1: 11-15.

Penteado, A.L., B.S Eblen and A.J. Miller, 2007. Evidence of salmonella internalization into fresh mangos during simulated postharvest insect disinfestation procedures. J. Food Prot., 67: 181-184.

Ryall, A.L. and W.T. Pentzer, 1982. Handling Transportation and Storage of Fruit and Vegetables. AVI. Publishing Co. Inc., Connecticut, pp: 610.

Samish, Z. and R. Etinger-Tulczynska, 1963. Distribution of bacteria within the tissue of healthy tomatoes. Applied Microbiol., 11: 7-10.

Solomon, E.B., S. Yaron and K.R. Mathews, 2002. Transmission of *Escherichia coli* 0157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied Environ. Microbiol., 68: 397-400.

Speck, M.L., 1984. Compendium of Methods for the Microbiological Examination of Foods. 2nd Edn., American Public Health Association, Washington, DC. USA.

Steele, M. and J. Odumeru, 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. J. Food Prot., 67: 2839-2849.

Tood, E.C., 1997. Epidemiology of foodborne diseases. World Health Stat. Q., 50: 30-50.

Wachtel, M.R., L.C. Whitehand and R.E. Mandrell, 2002a. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. J. Food Prot., 65: 18-25.

Wachtel, M.R., L.C. Whitehand and R.E. Mandrell, 2002b. Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. J. Food Prot., 65: 471-475.

WHO, 1993. Guidelines for Drinking Water Quality. 2nd Edn., WHO, Geneva.

Zhuang, R.Y., L.R. Beuchat and F.J. Angulo, 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Applied Environ. Microbiol., 61: 2127-2131.