International Food Research Journal 22(4): 1681-1686 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Incidence of multiple pathogenic bacteria in green chilli and cabbage in Dhaka city

[1]Swagato, N. J., [1]Parvin, N., [2]Ahsan, S. and [1*]Kabir, M. S.

[1]*Department of Microbiology, Stamford University Bangladesh*
[2]*Department of Microbiology, University of Dhaka, Bangladesh*

Article history

Received: 10 April 2014
Received in revised form: 21 December 2014
Accepted: 25 December 2014

Keywords

Green chilli
Cabbage
Microbiological quality

Abstract

The present study was carried out to investigate the microbiological quality and incidence of pathogenic bacteria found in green chilli and cabbage from different shops in Dhaka city. A total of 20 samples, 10 green chillies *(Capsicum annuum)* and 10 cabbages (*Brassica oleracea* var. *capitata*) were randomly collected from 20 sampling sites in central Dhaka. The numbers of total heterotrophic bacteria, total coliform and total *Staphylococcus aureus* were enumerated for each sample by dilution and spread plate technique. The ranges of total heterotrophic bacterial counts (log_{10}cfu/g) in chilli and cabbage samples were 8.30 – 11.43 and 10.27 – 11.83, respectively. Total coliform counts (log_{10}cfu/g) ranged between 5.30 – 8.39 and 6.38 – 8.57 in chilli and cabbage samples, respectively. The level of contamination by *Staphylococcus aureus* was lower than that of coliforms and demonstrated counts (log_{10}cfu/g) between 4.25 – 7.91 and 5.61 – 7.77 in chilli and cabbage, respectively. The presence of *Salmonella, Shigella* and *Vibrio* species were determined following appropriate enrichment culture technique. More than 40% of both types of samples were contaminated with one or more of the pathogens (*Salmonella* spp., *Vibrio cholerae* or *V. parahaemolyticus*). It can be concluded that high level of bacterial contamination of green chilli and cabbage poses serious threat to public health if they were consumed raw or after inadequate processing.

Introduction

Cabbage and green chilli are generally consumed raw as salad items in Bangladesh and in many other parts of the world. Vegetables are cheap sources of important proteins, vitamins and essential amino acids (USDA, 2011; Liu, 2013). Green chilli is an integral component of Bangladeshi, Indian, Thai and Mexican cuisines. It is used in cooking for almost all savoury dishes as well as spices. Green chilli is low in fat and cholesterol but rich in fibre, vitamins and minerals. The spicy and hot flavour of green chilli is imparted by a chemical, capsaicin. On the other hand, cabbage is one of the popular winter vegetables in Bangladesh. It is widely grown in both tropical and temperate regions of the world with positive increase in production.

The growth of different vegetables varies in different seasons of the year that suit their growth conditions. Vegetables can be contaminated from different sources, such as soil, water, insects, air, birds, animals and from equipment during cultivation and marketing them. Generally vegetables have 10^3 to 10^5 microorganisms/cm^3 or 10^4 to 10^7 microorganisms/g. Some of the predominant bacterial types are lactic acid bacteria, *Corynebacterium*, *Enterobacter, Proteus, Micrococcus, Enterocoocus, Pseudomonas* and spore-formers. They may also have different types of molds, such as *Alternaria, Fusarium*, and *Aspergillus* growing on their surface. Vegetables can be contaminated by enteric pathogens if animal or human wastes and polluted water are used for fertilization and irrigation which may cause illness in human (Soriano *et al*., 2001; Amoah *et al*., 2009). *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Clostridium botulinum, Clostridium perfringens* and *Escherichia coli* are important pathogens reported to cause food borne infections associated with different vegetables (Soriano *et al*., 2001; Harris *et al*., 2003; Polcovnicu *et al*., 2008). Pathogens present in contaminated foods may harbour virulence genes, toxins and enzymes, which aids in pathogenesis (Fremaux *et al*., 2007; Sahilah *et al*., 2010). Raw vegetables may be bruised during processing and distribution resulting in the release of plant nutrients which may serve as the potential organic and inorganic substrates for microorganisms (Zhao *et al*., 1997; Soriano *et al*., 2001). As a consequence, outbreaks of food borne diseases are often associated with vegetables in many countries

*Corresponding author.
Email: *mskabir@yahoo.com*

of the world (CDC, 2000, 2010).

Food borne outbreaks are likely to occur worldwide because of the consumption of contaminated ready to eat salad vegetables (Ponka *et al*., 1999; Tauxe *et al*., 1997). Global incidence of cholera was estimated to be 2.8 million cases and 91,000 deaths every year (Ali *et al*., 2012). It is important to establish critical control points to reduce contamination to safe levels by applying the principles of Hazard Analysis and Critical Control Point (HACCP) (Michalowska and Korczak, 2008). This study was conducted to determine the level of microbial contamination of green chilli and cabbage in samples collected from different selling spots in Dhaka city.

Materials and Methods

Sampling sites

Two types of vegetables (green chilli and cabbage) were collected randomly from 10 different selling spots (grocery shops) and from street vans from Moghbazar and surrounding areas in central Dhaka city between August 2013 and December 2013. Samples were collected early in the morning in a sterile plastic bag and transported to the laboratory as soon as possible for further processing. Sample collection sites for these two vegetables are shown in Table 1.

Table 1. Sample collection sites of green chilli and cabbage in Dhaka city

Chilli		Cabbage	
Sample no.	**Sampling site**	**Sample no.**	**Sampling site**
1.	Mowchak	1.	Baily Road
2.	Malibagh Rail gate	2.	Poribagh
3.	Shantinagar Bazar	3.	Rajarbagh
4.	Shantibagh	4.	Boro Moghbazar
5.	Chamelibagh	5.	Moghbazar Rail Gate
6.	Shahidbagh	6.	New Eskaton
7.	Gulbagh	7.	Doctor's Lane
8.	Malibagh Wireless	8.	Kakrail
9.	Gopibagh	9.	Haji Para
10.	Chowdhury Para	10.	Kazi Para

Sample processing

All samples were processed following standard methods (APHA, 2012). Briefly, 25 g of each sample was mixed with 250 ml sterile normal saline and homogenized in a blender. Homogenized sample was serially diluted to 10^{-9} following 10-fold serial dilution and spread on different types of media for enumeration of bacterial load (Cappuccino and Sherman, 1996).

Microbiological analysis

All microbiological analyses were done in triplicate to determine the mean of the bacterial count.

Enumeration of total heterotrophic bacteria

A total of 0.1 ml of diluted homogenate was spread on nutrient agar (NA, Oxoid, UK) for enumerating total heterotrophic bacteria (THB). After incubation at 37°C for 24 hours, plates were examined to enumerate total number of colonies.

Quantitation of total coliforms

Diluted homogenates were plated onto membrane Faecal Coliform (mFC, Oxoid, UK) agar and incubated at 37°C overnight. Characteristic blue coloured colonies were counted to estimate the number of total coliforms.

Estimation of total Staphylococcus aureus

Diluted homogenates (0.1 ml) was spread onto Mannitol Salt Agar (MSA, Himedia Laboratories Ltd., India) and incubated for 24-48 hours at 37°C. Typical yellow mannitol fermenting colonies were counted to quantitate the total number of *Staphylococcus aureus* in the sample.

Isolation of Salmonella spp. and Shigella spp.

A total of 1 ml of homogenized suspension was added to 9 ml of lactose broth and incubated for 4 hours at 37°C. 0.1 ml of suspension was transferred to 10 ml of Selenite Cystine broth (Himedia Laboratories Ltd., India) and incubated for 4-6 hours at 37°C before streaking onto *Salmonella Shigella* (SS) agar. Plates were incubated at 37°C overnight to determine the presence of any *Shigella* and *Salmonella* like colonies. A single representative colony was identified by biochemical tests to determine the particular type of bacteria.

Isolation of Vibrio spp.

One ml of homogenized sample was added to 9 ml of Alkaline Peptone Water (APW) and incubated at 37°C for 4-6 hours. Enriched APW was streaked onto Thiosulfate Citrate Bile salts Sucrose (TCBS) agar media. Plates were further incubated at 37°C overnight. One representative colony was subcultured for biochemical identification of the pathogens.

Table 2. Isolation of pathogenic bacteria from green chilli and cabbage

Green chilli				Cabbage			
ample o.	*Salmonella* spp.	*Vibrio cholerae*	*Vibrio parahaemolyticus*	Sample no.	*Salmonella* spp.	*Vibrio cholerae*	*Vibrio parahaemolyticus*
1	-	-	-	1	+	-	+
2	-	+	+	2	+	-	-
3	+	+	+	3	-	+	+
4	-	-	-	4	-	-	-
5	+	-	-	5	+	-	-
6	-	+	+	6	-	+	+
7	-	-	-	7	+	+	+
8	+	+	+	8	-	-	-
9	+	-	+	9	-	+	+
10	-	-	-	10	+	-	-
Total % + ve	**40%**	**40%**	**50%**		**50%**	**40%**	**50%**

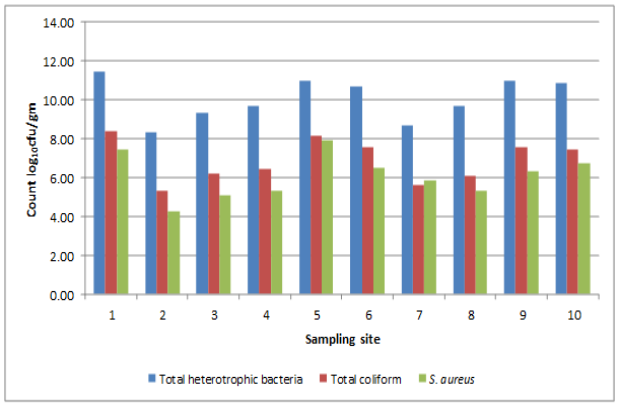

Figure 1. Quantitation of total heterotrophic bacteria, total coliforms and *Staphylococcus aureus* in green chilli

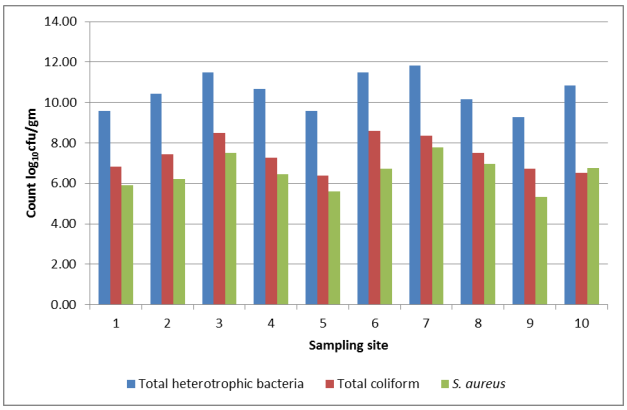

Figure 2. Distribution of total heterotrophic bacteria, total coliforms and *Staphylococcus aureus* in cabbage sample

Biochemical tests for identification of pathogenic bacteria

Standard biochemical tests were performed to confirm the identification of pathogenic bacteria isolated from both types of vegetable samples. KIA, MIU, citrate, catalase, oxidase, MR and VP tests were done for identification of the pathogens (Cappuccino and Sherman, 1996).

Results

This study focuses on the quantitation of bacterial load, indicator bacteria and presence of potential pathogenic bacteria in green chilli and cabbage samples collected from different shops in Dhaka city. Total heterotrophic bacterial counts (HPC) were determined using Nutrient agar, total coliform count was determined on mFC agar and *S. aureus* counts were determined using Mannitol Salt agar. The respective counts for different groups of bacteria for different study sites are shown in Figures 1 and 2, respectively. All bacterial counts were calculated as $\log_{10}$cfu/g. The range of total HPC ranged between 8.30-11.43 and 9.28-11.86 for green chilli and cabbage, respectively.

Total coliform count was determined in both green chilli and cabbage samples (Figure 1 and 2). The highest count for green chilli was found in site 1 (8.39) and the lowest count was found in site 2 (5.30). Total coliform counts for cabbage samples ranged between 8.58 and 6.38 in site 6 and 5, respectively. Total count of *Staphylococcus aureus* was determined in 10 sites for both green chilli and cabbage samples. Site 5 showed the highest count (7.91) and site 2 showed the lowest count (4.26) of *S. aureus*. Total count of *S. aureus* in cabbage samples ranged between 5.34 and 7.78 in sites 9 and site 7, respectively (Figures 1 and 2).

Selected pathogenic bacteria including *Salmonella, Shigella* and *Vibrio* species were found to be present in some of the samples studied (Table 2). Samples 1, 4, 7 and 10 of green chilli were not found to be contaminated with any of the above pathogens. Samples 3 and 8 showed the presence of

all of the above mentioned pathogens. For cabbage samples only sample 7 showed the presence of the above mentioned pathogens and samples 4 and 8 did not show presence of any of the pathogens.

Discussion

Over the past decade outbreaks of human disease associated with the consumption of raw fruit and vegetables have occurred in developing countries and have now become more frequent in developed countries (Moy, 2004). In Bangladesh, vegetable products are the most common food items. Vegetable borne diseases may put the overall public health at a serious risk. Freshly consumed vegetables especially those used in salad mixtures, have been implicated in food poisoning and thus hazardous to the health of the consumers. This could be linked to the fact that most of these vegetables are consumed without any thermal process or even thorough washing (Lund, 1992; Bowen *et al*., 2006; Campos *et al*., 2011). The present study portrays the pathogenic load of two salad vegetables (green chilli and cabbage) consumed in Bangladesh.

Khan *et al.* (1992) reported that bacterial contamination results from various unsanitary cultivation and marketing practices. In another study Tambekar and Mundhada (2006) reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any heat treatment. These vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling, harvesting equipment, transport containers, wild and domestic animals. Pathogens from human and animal reservoirs as well as other environmental pathogens can be found at the time of consumption. Although spoilage bacteria, yeasts and mold dominate the microflora on raw fruits and vegetables, the occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has also been documented (Hasan *et al*., 2006).

The presence of *E. coli* in salad vegetables analysed in the present study was indicative of fecal contamination. It is a well-known fact that *E. coli* are part of the normal flora of the human intestines. Some strains of *E. coli* have been linked to diarrhoea, gastro-enteritis and urinary tract infections (Hasan *et al*., 2006). *E. coli* and *Klebsiella* spp. are well known in the environment and can be cultured from soil, water, leafy green vegetables and salads (Ibrahim, 1996). Khan *et al.* (1992) also isolated *E. coli, Klebsiella* spp. and *Enterobacter* spp. from salad vegetables. In a study done by Tambekar and Mundhada (2006), *E. coli* was found to be predominant in some salad vegetables which included coriander followed by carrot, radish, spinach, fenugreek and cucumber. The present study showed that *E. coli, Salmonella* spp., *S. aureus, Vibrio* spp. and *Pseudomonas* spp. were common in chilli and cabbage samples which were similar to other findings conducted previously in Bangladesh (Rahman and Noor, 2012).

The detection of *S. aureus* was of serious public health importance because of its ability to cause a wide range of infections especially food-borne intoxication (Tambekar and Mundhada, 2006). Contamination with *S. aureus* has been linked to carriage in nasal passages of food handlers or by infected workers. The presence of *S. aureus* and some Gram negative rods have been reported to contaminate some salad vegetables such as carrots, cucumber, tomato and radishes (Beuchat, 1995). The association of *S. aureus* with some of the samples investigated in the present study was an alarming finding from a public health standpoint.

Salmonella spp. and *Shigella* spp. are non-lactose fermenters usually associated with water contamination. Contamination with these organisms could arise from washing vegetables with contaminated water or handling of vegetables by infected workers. Pathogens such as *E. coli, Salmonella* spp., *S. aureus* and *Vibrio* spp. were found to be present in 40% of the green chilli and cabbage samples. This is a serious threat to public health and may cause an outbreak of food borne infection and spread of diseases. In this study, *Salmonella* spp. were isolated from green chilli and cabbage. Their presence in food is of serious concern to food safety and major public health concern worldwide (WHO 2002, 2008). Ibrahim (1996) reported the presence of *Salmonella* spp. from lettuce, cucumber and parsley. *Salmonella* spp. has also been isolated from carrots, cucumber and lettuce collected from different markets and vendors in Nigeria (Itohan *et. al*., 2011).

The presence of microorganisms in salad vegetables is a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (Beuchat, 1995). Other workers also reported isolation of similar loads and types of bacterial contamination in fruits and vegetables in another study in India (Tambekar and Mundhada, 2006). Survival of microorganisms is linked to their capacity to obtain nutrients from vegetables having intact and bruised skin (Odhav, 2007). It was found that in healthy plant tissues bacteria can enter through natural apertures such as stomata, lenticels, broken trichomes and stem scars and may survive (Bartz and Wei, 2003).

In a separate study the shelf life quality of green bell peppers was improved by using edible coating formulations. Three different biopolymers (pectin, arabic, and xanthan gums) were evaluated in mixtures with candelilla wax as hydrophobic phase, jojoba oil as plasticizer and a crude extract of polyphenols as source of bioactive compounds. Use of mixtures of biopolymers, candelilla wax, jojoba oil and polyphenols to develop edible and functionalized coatings significantly extended shelf life of green bell pepper (Ochoa-Reyes *et al*., 2013). Similar findings were reported on lime fruits coated with coconut oil; it may be explained by closure of opening of the stomata, reducing transpiration and respiration rate and also reducing microbial activity (Bisen *et al*., 2008 and 2012).

Overall, this study revealed the presence of a high load of microorganisms in the commonly consumed vegetable items. The pathogens might be introduced from the crop land, organic fertilizers, irrigating water, packaging materials, transport vehicles etc. Besides, unhygienic personnel handling and processing of the vegetables and their storage in such a condition which favours microbial growth might also account for such spoilage of vegetables. The contaminating pathogens are responsible for various types of enteric diseases as well as serious intoxications in human health. Further study on detection of the virulent genes in the isolated pathogenic bacteria would be useful for determining the cause of outbreak of diseases. It is necessary to create awareness among the producers, processors, handlers and consumers of raw vegetables in order to reduce the bacterial contamination and reduce the risk of illness associated with the consumption of salad vegetable items. Improvement in the health and hygiene condition of the vegetable handlers, appropriate preservation, maintenance and processing will be necessary to control any outbreak associated with the consumption of raw and unprocessed vegetables.

Conclusion

The high bacterial load and presence of indicator organism especially *E. coli* serve as an indicator of contamination of vegetable items from faecal sources. It is now important to promote awareness on different sources of contamination, transmission chain and their link with food borne disease. It is necessary to authorize regulatory bodies to monitor the microbiological quality and standards established and practiced by farmers and sales people for the handling and distribution vegetable items.

Acknowledgment

We thank the Department of Microbiology, Stamford University Bangladesh for providing laboratory support.

Reference

Ali, M., Lopez, A.L., Ae You, Y., Eun Kim, Y., Sah, B.., Maskerya, B. and Clemens, J. 2012. The global burden of cholera. Bulletin of World Health Organization 90: 209–218.

American Public Health Association (APHA), 2012. 22nd Ed., Standard Methods for the Examination of Water & Wastewater, American Water Works Association (AWWA) & Water Environment Federation (WEF).

Amoah, P., Drechsel, P., Abidoo, R.C. and Abraham, E.M. 2009. Improving food hygiene in Africa where vegetables are irrigated with polluted water. Regional Sanitation and Hygiene Symposium, 3-5 Nov. 2009, Accra, Ghana.

Bartz, J.A. and Wei, C.I. 2003. The influence of bacteria postharvest physiology and pathology of vegetables. 2nd ed., Marcel Dekker, Inc, New York. 519-541.

Beuchat, C. R. 1995. Pathogenic microorganisms associated with fresh produces. Journal of Food Protection 59: 204-216.

Bisen, A. and Pandey, S.K. 2008. Effect of post-harvest treatments on biochemical and organoleptic constitutes of Kagzi lime fruits during storage. Journal of Horticultural Science 3: 53–56.

Bisen, A., Pandey, S.K. and Patel, N. 2012. Effect of skin coating on prolonging shelf life of kagzi lime fruits (*Citrus aurantifolia*, Swingle). Journal of Food Science and Technology 49: 753-759.

Bowen ,A., Fry, A., Richards, G. and Beuchat, L. 2006. Infections associated with cantaloupe consumption: a public health concern. Epidemiology and Infection 134: 675-685.

Campos, J., Peixe, L., Mourão, J., Pires, J., Silva, A., Costa, C., Nunes, H., Pestana, N., Novais, C. and Antunes, P. 2011. Are ready-to-eat salads an important vehicle of pathogenic and commensal bacteria resistant to antibiotics? Clinical Microbiology and Infection 17(S4): R 2357.

Cappuccino, J.G. and Sherman, N. 1996. Microbiology-A Laboratory Manual, 4th ed., pp. 13-182. The Benjamin/Cummings Publishing Co., Inc., Menlo Park, California.

Centre for Disease Control and Prevention (CDC). 2000. Surveillance for foodborne-disease outbreaks-United States, 1993-1997. Morbidity and Mortality Weekly Report 49(SS01):1-51.

Centre for Disease Control and Prevention (CDC). 2010. Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded romaine lettuce from a single processing facility. Available from: *http://www.cdc.gov/ecoli/ 2010/ecoli_o145/ index.html.*

Fremaux, B., Delignette-Muller, M.L., Prigent-combaret, C., Gleizal, A. and vernozy-Rozand, C. 2007. Growth and Survival of non-O157: H7 shigatoxin-producing *E. coli* in cow manure. Journal of Applied Microbiology 102: 89-99.

Harris, L.J., Faber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.B., Garret, E.H. and Busta, F.F. 2003. Outbreaks associated with fresh produce: incidence, growth and survival of pathogens in fresh and fresh cut produce. Comprehensive Review of Food Science and Food Safety 2: 115-119.

Hasan, A., Utku, O. and Koray, K. 2006. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. International Journal of Hygiene Environ and Health 209: 197-201.

Ibrahim, S. A. 1996. Microbiological studies on some salad vegetable in local markets. Journal of King Saud University 8: 99-106.

Itohan, A. M., Peters, O. and Kolo, I. 2011. Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malaysian Journal of Microbiology 7(2): 111-114.

Khan, M.R., Saha, M.L. and Kibria, A.M. 1992. A bacteriological profile of salad vegetables in Bangladesh with special reference to coliforms. Applied Microbiology 14: 88-90.

Liu, R.H. 2013. Health-promoting components of fruits and vegetables in the diet. Advances in Nutrition 4(3): 384S-92S.

Lund, B.M. 1992. Ecosystems in vegetable foods. Journal of Applied Bacteriology 73: 115-135.

Michalowska, G.A. and Korczak, J. 2008. Vegitable Products as HACCP System Subject in Modern Gastronomy. Acta Scientiarum Polonorum Technologia Alimentaria 7(3): 47-53.

Moy, G. 2004. Report of Joint FAO/WHO Workshop on Fruit and Vegetables for Health: Held on 1-3 September, 2004, Kobe, Japan.

Ochoa-Reyes, E., Martínez-Vazquez, G., Saucedo-Pompa, S., Montañez, J., Rojas-Molina, R., Miguel, A., de Leon-Zapata, Rodríguez-Herrera, R. and Aguilar, C. N. 2013. Improvement of shelf life quality of green bell peppers using edible coating formulations. Journal of Microbiology Biotechnology and Food Science 2(6): 2448-2451.

Odhav, B., Beekrum, S., Akula, U. and Baijnath, H. 2007. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. Journal of Food Comparative Analysis 20: 430-435.

Polcovnicu, C., Ionescu, L. and Bahrim, G. 2008. Confirmation and identification of *Listeria* species from fresh lettuce. Roumanian Biotechnological Letters 13(6): 32-36.

Ponka, A. L., Maunula, C. H., von Bonsdorff, and Lyytikainen, O. 1999. An outbreak of calicivirus associated with the consumption of frozen raspberries. Epidemiology and Infection 123: 469-474.

Rahman, F. and Noor, R. 2012. Prevalence of pathogenic bacteria in common salad vegetables of Dhaka metropilis. Bangladesh Journal of Botany 41(2): 159-162.

Sahilah, A.M., Tuan Suraya, T. S., Noraida., I., Ahmad Azuhairi, A., Chai, L. C. and Son, R. 2010. Detection of virulence genes and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis among raw vegetables isolates of *Campylobacter jejuni*. International Food Research Journal 17: 681-690.

Soriano, J.M., Rico, H., Molto, J.C. and Ma, J. 2001. Incidence of microbial flora in lettuce, meat and Spanish potato omelette from restaurant. Food Microbiology 18: 159-163.

Tambekar, D. H. and Mundhada, R. H. 2006. Bacteriological quality of salad vegetables sold in Amravati city, Indian Journal of Biological Science 6: 28–30.

Tauxe, R., Kruse, H., Hadberg, C., Potter, M., Madden, J. and Wachsmuth, K. 1997. Microbial hazards and emerging issues associated with produce. A preliminary report to the National Advisory Committee on Microbiologic Criteria for Food. Journal of Food Protection 60: 1400-1408.

United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference, Release 24, 2011, accessed on January 06, 2014. *http://ndb.nal.usda.gov/.*

World Health Organization (WHO), 2002. Food and Agriculture Organization of the United Nations. Risk assessments of *Salmonella* in eggs and broiler chickens. Microbiological Risk Assessment Series 2.

World Health Organization (WHO). 2008. WHO Initiative to Estimate the Global Burden of Foodborne Diseases: First formal meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG) in 2007. World Health Organization, Geneva, Switzerland.

Zhao, T., Clavero, M.R.S., Doyle, M.P. and Beuchat, L.R. 1997. Health relevance of the presence of fecal coliforms in iced tea and in leaf tea. Journal of Food Protection 60: 215-218.