

## Microbial Contamination of Fresh Vegetable and Salad Samples Consumed in Tehran, Iran

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### Abstract

**Background:** The criteria for the quality control of Iranian fresh produce are the total microbial count, Enterococcal count and the detection of *Escherichia coli*. The aim of this study was to assess microbial contamination of fresh vegetable and salad samples consumed in Tehran, Iran.

**Methods:** From May to October 2013, freshly vegetable (n=100) and salads (n=100) samples were randomly collected in three area of Tehran including Islamshahr (n=35), south of Tehran (n=30) and Shahr-e-Rey (n=35). The samples were tested microbiologically according to institute of standards and industrial research of Iran (ISIRI) for determination of total microbial, Enterococcal and *E. coli* count. The results were analyzed by SPSS software v. 16.0.

**Results:** Overall, 71% of salad and 82% of vegetable samples had contamination rate more than acceptable limits in the case of at least one of the three microbial parameters. All of spinaches, wheat sprouts, mung bean sprouts, mixed leafy vegetables, Kuku vegetables, and green peppers were exceeded acceptable limits at least in case of one parameter. From 71 ineligible salad samples, 24 samples (68.6%) were for Islamshahr, 26 samples (74.3%) for south of Tehran and 21 ones (70%) for Shar-e-Rey, with no significant difference ( $p>0.05$ ).

**Conclusion:** This study showed poor microbiological quality of salad and fresh vegetables used in Tehran. Measures to reduce the contamination might be advised such as a proper handling and washing before consumption of these products as well as public education and awareness.

### Introduction

Health benefits of fresh products, e.g. vegetables with high fiber and vitamin content make them more popular for the people who care about proper diet. The consumption

rate of such products has been increased in recent years. These foodstuffs could be vehicles for some kinds of pathogens if they are used as raw, therefore, illnesses and outbreaks maybe resulted from their consumption

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(Beuchat, 2002; Lewis Ivey et al., 2012; Olaimat and Holley, 2012). The outbreak of *Escherichia coli* O157:H7 due to consumption of contaminated spinach resulted in 200 patients and 3 cases of death (Abadias et al., 2008). The presence of *E. coli* can represent the existence of fecal pathogens like *Salmonella* and *Shigella*. Thus, it can be a good indicator of food sanitation. Also, some *E. coli* strains are pathogenic for human (Enabulele and Uraih, 2009; Ishii et al., 2006; Tadesse et al., 2012; Weintraub, 2007). *Enterococcus* spp. are another bacteria which live in the gastrointestinal tract of human and warm blooded animals, so it is a fecal indicator for quality control of foods. Furthermore, some species possess pathogenic characteristics for human. *E. faecalis* and *E. faecium* are more common species involved both in clinical infections and food contaminations (Abriouel et al., 2008; Fisher and Phillips, 2009; Franz et al., 1999; Gomes et al., 2008) which their presence in vegetables has previously been reported (Johnston et al., 2005). Another parameter used to evaluate the quality of foods is total microbial count, which reflects the efficiency of hygienic measures and shows any kind of contamination in foodstuffs (Aycicek et al., 2006). Three mentioned microbial parameters have been considered in Iran national standards to control vegetables microbial quality (ISIRI, 2007c).

So, this study was carried out to estimate total microbial count, enterococcal count and detection of *E. coli* in fresh vegetable and salad samples supplied in Tehran, Iran.

## Materials and methods

### Sampling

In this descriptive study from May to October 2013, a total of 100 fresh vegetable and 100 salad samples were randomly collected in selective restaurants and delicatessens in three area of Tehran, including Islamshahr (n=35), south of Tehran (n=30) and Shahr-e-Rey (n=35). Ten kinds of fresh vegetables consist of chopped spinach, green onion, ready to eat (RTE) mixed leafy vegetables, chopped Kuku (a Persian dish) vegetable, green pepper, mushroom, broccoli, wheat sprout, mung bean sprout and basil were selected. The basil vegetable was obtained from local Kebab retails. Salad samples were included 88 manually prepared samples and 21 industrially packaged ones.

### Microbial analysis

The total microbial count, enterococcal count and detection of *E. coli* were based on Iran national standards (ISIRI, 2004; ISIRI, 2006b; ISIRI, 2007d). Dilutions

were prepared by sterile normal saline. Total microbial count and enterococcal count were carried out by pour plate culturing of proper dilution in plate count agar and kenner fecal agar (Scharlau), respectively. Incubation period was 48-72 h at 30 °C for plate count agar and 24-48 h at 37 °C for enterococcal count. For *E. coli* detection, 10 cc of 0.1 dilutions was added to double strength lauryl sulfate broth (Merck) with durham tubes and incubated at 37 °C for 24-48 h. In the case of turbidity and gas production, 1 cc of lauryl sulfate broth was added to *Escherichia coli* broth (EC broth, Merck) with durham tubes. After incubation at 37 °C for 24-48 h, if turbidity and gas production developed, 1 cc of EC broth was added to pepton water (Merck) and also streaked on macconkey agar (Merck). Pepton water was incubated in water bath at 44 °C and macconkey agar was incubated at 37 °C both for 24-48 h followed by some biochemical tests including sugar fermentation, motility, gas and SH<sub>2</sub> production. According to Iran national standard (ISIRI, 2007c), limitation levels were considered as 10<sup>6</sup> CFU/g for total microbial count, 10<sup>2</sup> CFU/g for enterococcal count and lack of *E. coli* in 1 g of samples.

### Statistical analysis

The results were analyzed by SPSS (USA, Il, Chicago, SPSS Inc) software package v. 16.0. Significant level was considered as  $p < 0.05$ .

## Results

Overall, 71% of salad and 82% of vegetable samples had contamination rate of more than acceptable limits, regarding at least one of the three microbial parameters.

Table 1 displays frequency distribution of vegetable samples with unacceptable level of contamination for each microbial parameter. All of spinaches, wheat sprouts, mung bean sprouts, mixed leafy vegetables, Kuku vegetables, and green peppers were exceeded acceptable limits at least in case of one parameter. This exceeding rate for broccolis, green onions, basil and mushrooms were 30%, 40%, 60% and 90%, respectively.

Out of 71 ineligible salad samples, 24 (68.6%) were obtained from Islamshahr, 26 (74.3%) from south of Tehran and 21 (70%) from Shar-e-Rey, indicating no significant difference ( $p > 0.05$ ) between contamination rate in three sampling areas (Table 2).

Ten of 12 (83.3%) industrially packaged salads were not acceptable to use because they had contamination rate more than allowed. From 88 manually salads, 61 (69.3%) samples were out of standards. Fig. 2 shows the data regarding to two kinds of salads with contamination rate of more than acceptable level.

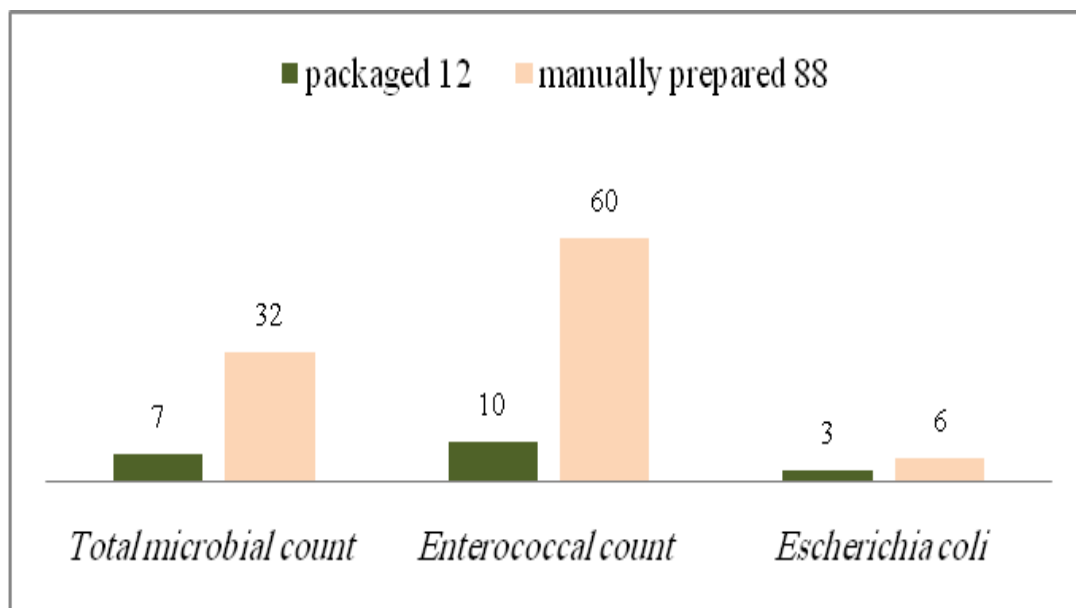
**Table 1:** Frequency distribution (%) of vegetable samples with unacceptable level of contamination for each microbial parameter

Sample type	Sample size	Total microbial count	Enterococcal count	<i>E. coli</i>
Spinach	10	100	100	10
Broccoli	10	0	30	0
Green onion	10	10	40	0
Wheat sprout	10	100	100	20
Mung bean sprout	10	90	100	30
Basil	10	30	60	0
RTE mixed leafy vegetables	10	100	100	20
Kuku vegetable	10	100*	100*	30
Green pepper	10	60	100	0
Mushroom	10	80	50	10

\* These parameters have not been considered for Kuku vegetable in Iran guidelines

**Table 2:** Frequency distribution (%) of salad samples with unacceptable level of contamination for each microbial parameter

Sampling area	Sample size	Total microbial count	Enterococcal count	<i>E. coli</i>
Islamshahr	35	40	68.6	2.8
South of Tehran	30	40	71.4	11.4
Shahr-e-Rey	35	36.7	70	13.3



**Fig. 2:** Number of packaged and manually prepared salads with unacceptable level of contamination for each microbial parameter

## Discussion

In this survey, among vegetable samples, the lowest contamination rate was for broccolis followed by green onions. Basils had less contamination rate, compared to other ready to use packaged leafy vegetables. Industrially packaged salads were also more contaminated than manually salads. This may indicate that the washing treatments performed in factories is no more effective than traditional washing in restaurants, or insufficient hygiene control in industrial packaging. This is probably due to lack of use or inefficient use of disinfectants in the industries.

Avazpour et al. (2013) reported that 69% of salad samples served in restaurants of Ilam, Iran, were contaminated to *E. coli*. In an investigation done in India on several kinds of sprout, the aerobic plate count was 7.6 to 8.9 log CFU/g which is similar to present study. However, it was shown that 13% of mung bean sprouts had more than  $10^6$  CFU/g *E. coli* contamination that was less than our results (Saroj et al., 2006). Average rate of aerobic plate count of sprouts in Spain was 7.3 log CFU/g which is similar to our results (Abadias, 2008). In a pre-harvest survey performed in USA, *E. coli* was not detected in non-organic broccolis and green peppers, as our equivalent samples (Mukherjee et al., 2004). Results of total bacterial count of spinaches in Japan were 4.5-4.9 log CFU/g which was much lower than contamination level of our spinaches (Izumi et al., 2004). According to another research in USA, enterococcal count of corianders, dills, parsleys and spinaches were 1.9, 3.6, 2.5 and 2.1 log CFU/g, respectively (Johnston et al., 2005). It is known that aerobic plate count during germination stage are increased, because of optimal condition for bacterial growth. Moisture, high temperature and nutrients released from seeds are some reasons for proliferation of pathogenic bacteria such as *E. coli* during germination (Ibenyassine et al., 2007; Rajkowski et al., 2003). On the other hand, microbial ecosystems of various products are affected by storage, processing, packaging, distribution and marketing conditions. Many other parameters such as soil, feces, irrigation water, animals, fungicides, fertilizers, dust, containers, handlings, etc. can be potential sources of contamination (Beuchat, 2002).

Due to high contamination rate of examined vegetables and salad samples, more education of individuals involved in production, microbial assessment of water sources and fertilizers, extensive researches in the field of food contamination in our country and improvement of rapid methods for the identification of such contaminants are recommended. Furthermore, revision of some Iranian national standards used to control vegetables and salad

quality seems necessary due to some existing defects, as lack of microbial parameters in some published ones (ISIRI, 2003; ISIRI, 2006a; ISIRI, 2007a; ISIRI, 2007b; ISIRI, 2008). Restricted number of brands available in the market was the limitation of this research, so statistics may vary by development of new production units.

## Conclusion

This study showed poor microbiological quality of salads and fresh vegetables consumed in Tehran. Measures to reduce the contamination might be advised like proper handling and washing before consumption of these products.

## Conflicts of interest

All the authors indicated that there is no conflict of interest in this research.

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## References

- Abadias M., Usall J., Anguera M., Solsona C., Vinas I. (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*. 123: 121-129.
- Abriouel H., Omar N.B., Molinos A.C., Lopez R.L., Grande M.J., Viedma P.M., Ortega E., Cañamero M.M., Galvez A. (2008). Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. *International Journal of Food Microbiology*. 123: 38-49.
- Avazpour M., Rostami Nejad M., Seifipour F., Abdi J. (2013). Assessment of the microbiological safety of salad vegetables from different Restaurants in Ilam. *Journal of Paramedical Sciences*. 4: 111-115.
- Aycicek H., Oguz U., Karci K. (2006). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health*. 209: 197-201.
- Beuchat L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection*. 4: 413-423.

- Enabulele S.A., Uraih N. (2009). Enterohaemorrhagic *Escherichia coli* O157: H7 prevalence in meat and vegetables sold in Benin City, Nigeria. *African Journal of Microbiology Research*. 3: 276-279.
- Fisher K., Phillips C. (2009). The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology*. 155: 1749-1757.
- Franz C.M., Holzapfel W.H., Stiles M.E. (1999). Enterococci at the crossroads of food safety? *International Journal of Food Microbiology*. 47: 1-24.
- Gomes B.C., Esteves C.T., Palazzo I.C.V., Darini A.L.C., Felis G.E., Sechi L.A., Franco B.D., De Martinis E.C. (2008). Prevalence and characterization of *Enterococcus* spp. isolated from Brazilian foods. *Food Microbiology*. 25: 668-675.
- Ibenyassine K., Mhand R.A., Karamoko Y., Anajjar B., Chouibani M., Ennaji M. (2007). Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. *Journal of Environmental Health*. 69: 47-51.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2003). Chives-Specification and test method. National Standard No. 7932. URL: <http://www.isiri.org/portal/files/std/7932.doc>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2004). Microbiology of food and animal feeding stuffs-Detection and enumeration of presumptive *Escherichia coli*-Most probable number technique. National Standard No. 2946. URL: <http://www.isiri.org/portal/files/std/2946.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2006a) Edible mushrooms-Specifications. National Standard No. 1627. URL: <http://www.isiri.org/portal/files/std/1627.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2006b). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms-Colony count technique at 30 °C. National Standard No. 5272. URL: <http://www.isiri.org/portal/files/std/5272.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2007a). Sprout of wheat-Specifications. National Standard No. 5833. URL: <http://www.isiri.org/portal/files/std/5833.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2007b). Sprout of vetch-Specifications. National Standard No. 11245. URL: <http://www.isiri.org/portal/files/std/11245.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2007c). Packed fresh vegetable to ready for use-Specifications and test methods. National Standard No. 10082. URL: <http://www.isiri.org/portal/files/std/10082.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2007d). Microbiology of foods and food stuffs-Detection and enumeration of *Enterococcus* in food. National Standard No. 2198. URL: <http://www.isiri.org/portal/files/std/2198.pdf>. Accessed 3 May 2015.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2008). Fresh spinach-specifications and test methods. National Standard No. 387. URL: <http://www.isiri.org/Portal/File/ShowFile.aspx?ID=91b7e482-1971-40b3-b8c8-61a567109f83>. Accessed 3 May 2015.
- Ishii S., Ksoll W.B., Hicks R.E., Sadowsky M.J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior Watersheds. *Applied and Environmental Microbiology*. 72: 612-621.
- Izumi H., Nagano M., Ozaki Y. (2004). Microbial evaluation of fresh marketed vegetables. *Memoirs of the School of Biology-Oriented Science and Technology of Kinki University*. 13: 15-22.
- Johnston L.M., Jaykus L.A., Moll D., Martinez M.C., Anciso J., Mora B., Moe C.L. (2005). A field study of the microbiological quality of fresh produce. *Journal of Food Protection*. 68: 1840-1847.
- Lewis Ivey M.L., LeJeune J.T., Miller S.A. (2012). Vegetable producers' perceptions of food safety hazards in the Midwestern USA. *Food Control*. 26: 453-465.
- Mukherjee A., Speh D., Dyck E., Diez-Gonzalez F. (2004). Preharvest evaluation of *Coliforms*, *Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *Journal of Food Protection*. 67: 894-900.
- Olaimat A.N., Holley R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*. 32: 1-19.
- Rajkowski K.T., Boyd G., Thayer D.W. (2003). Irradiation D-values for *Escherichia coli* O157:H7 and *Salmonella* sp. on inoculated broccoli seeds and effects of irradiation on broccoli sprout keeping quality and seed viability. *Journal of Food Protection*. 66: 760-766.
- Saroj S.D., Shashidhar R., Dhokane V., Hajare S., Sharma A., Bandekar J.R. (2006). Microbiological evaluation of sprouts marketed in Mumbai, India, and its suburbs. *Journal of Food Protection*. 69: 2515-2518.
- Tadesse D.A., Zhao S., Tong E., Ayers S., Singh A., Bartholomew M.J., McDermott P.F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases*. 18: 741-749.
- Weintraub A. (2007). Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *Journal of Medical Microbiology*. 56: 4-8.