Original Article

Evaluating the Efficiency of Lettuce Disinfection According to the Official Protocol in Iran

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Abstract

Background: The objective of this study was to evaluate the efficacy of Sanitization of Lettuce according to the protocols set forth by Iranian Ministry of Health and Medical Education for reducing populations of total coliform, fecal coliform, and helminth eggs present on lettuce.

Methods: In the present study, we determined the load of total coliform, fecal coliform, and parasites of lettuce. The lettuce was sanitized by protocol of Iranian Ministry of Health and Medical Education. The protocol consists of 3 levels to disinfect the fruits and vegetables. The procedure was as follows: first washing stage. The leaves of leafy vegetables washed with tap water, second stage, separation of helminth eggs by 3 to 5 droplets of detergent per liter for 5 min; third stage, disinfection of vegetables by calcium hypochlorite solution (with 200 mg/l free chlorine) for 5 min; and finally the disinfected vegetables were washed with tap water.

Results: The average initial levels of total coliform and fecal coliform in the samples were 3.36 $\log_{10}$ cfu/g and 2.31 $\log_{10}$ cfu/g, respectively. Helminth eggs were not detected in any of the samples tested. The efficiency of total coliform and fecal coliform removal were 78.1% (0.75 $\log_{10}$cfu/g) and 79.6% (0.67 $\log_{10}$cfu/g), respectively, after washing. This increased up to 94.8(1.44 $\log_{10}$cfu/g) and 98.5% (1.90 $\log_{10}$cfu/g) after the use of detergent. Chlorine disinfection rose these amounts up to 98.3% (2.18 $\log_{10}$cfu/g) and 100% (2.31 $\log_{10}$cfu/g), respectively.

Conclusion: By applying the protocol large parts of microorganisms existing on lettuce have indeed been removed.

Keywords: Raw vegetable, Disinfection, Fecal coliform, Total coliform

Introduction

Fruits and vegetables are known to be rich in nutrients and considered as an important part of people's daily diet (1). Although raw-eaten fruits and vegetables have a primary role in the diet, they can cause food-borne diseases if they are contaminated (2-4). *Listeria monocytogenes*, *Salmonella* (5), and *Escherichia coli* are among the pathogens that have been observed on vege-

tables (4,6), Research indicates that food-born diseases have significantly increased during the recent years, due mainly to the consumption of raw-eaten fruits and vegetables (2,6). Studies have shown the occurrence of 22 outbreaks in the United States between 1995 and 2006, half of which has been attributed to the consumption of contaminated vegetables such as spinage. Ac-

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cording to these studies, tomato, lettuce, sprouts, and spinage are the most important causes of food-born diseases in the United States (7). The American Food and Drug Administration have reported that there are serious concerns about the consumption of vegetables, especially leafy vegetables such as lettuce (8). Vegetables can be contaminated in various ways; for example, at the time of harvest, transport, distribution, sale, and even during the food preparation processes at home or restaurants (9). The main causes of the contamination of the vegetables are application of human or animal fertilizers and the use of wastewater effluent for irrigation purposes (10). It has been estimated, according to the studies conducted in 2006, that raw wastewater, unfinished, or finished wastewater are used in more than 20 million acres of the agricultural lands around the world (11). Preventing the contamination of fruits and vegetables should be set as an important objective during production, transport, and delivery. However, some pathogens are natural flora of the soil and may exist on vegetables even at the time of harvest (3).Rinsing the vegetables with clean water is a simple, but effective way in the disinfection of vegetables with low pathogens load. However, the application of an effective disinfectant is essential in case of high microbial load (12). Rinsing vegetables with clean water decreases the microbial load only by 1 $\log_{10}$ CFU/g (4,13). In a study conducted by Singh et al., it was found that one and several times of water rinse can reduce the load of *Escherichia coli* on lettuce by 0.22 $\log_{10}$ CFU/g and 1 $\log_{10}$ CFU/g, respectively (14). Several studies have been conducted around the world to assess the efficacy of different disinfectant for vegetables disinfection (15-17). However, no studies have yet been conducted in Iran in this filed. Some of the most commonly used disinfectants are calcium hypochlorite, sodium hypochlorite (NaClO), and electrolyzed water with pH of 4-5 (18). A variety of disinfection methods are in use around the world depending mainly on the contamination load and the processes used for the production of the vegetables. Chlorinated water is used by some countries for disinfection of the vegetables after of harvest, transport, and processing (19). The most commonly used method for the disinfection of vegetables in Brazil is the application of sodium hypochlorite solution with a concentration of 200 mg/l for 15 min (20). Result of the study conducted by Garcia-Gomez et al., in Mexico indicated that approximately 30% of the studied population use a disinfectant containing colloidal silver for disinfection of vegetables; 28% use water rinse, 25% apply sodium hypochlorite, and the rest of the population use a variety of other disinfectants such as salt, detergent, salt combined with lemon, and finally iodine combined with detergent and lemon (21). In some countries, such as US, the vegetables are only rinsed with clean water and the use of any kind of disinfectant is not suggested (22). In Iran, in the recent years, the prevalence of food-born diseases, such as gastrointestinal complications, has increased due mainly to the consumption of contaminated vegetables. According to the study of Rahbar et al. in 2006, the consumption of contaminated vegetables irrigated by wastewater effluents was found to be the main cause of cholera (23). In another study conducted in Markazi and Golestan provinces, improper disinfection of vegetables was found to be the major cause of cholera in those provinces (24). Results of another study conducted in Karaj indicated the same cause for the prevalence of cholera in this city (25).

Iranian Ministry of Health and Medical Education has developed a method for the disinfection of raw-eaten fruits and vegetables. The procedure is as follows: first washing stage, the leaves of leafy vegetables were separated and washed with tap water. Thus, mud and dust of these vegetables were removed; second stage, separation of helminth eggs by 3 to 5 droplets of detergent per liter for 5 min, third stage, disinfection of vegetables by calcium hypochlorite solution (with 200 mg/l free chlorine) for 5 min; and finally the disinfected vegetables were washed with tap water (26). The main objective of the

present study was to assess the efficacy of the disinfection method proposed by the Iranian Ministry of Health and Medical Education for decreasing the contamination load on lettuce.

Materials & Methods

Fresh lettuce was purchased from a wholesale market of fruits and vegetables in Tehran, Iran. Having transferred to the laboratory, lettuce was kept in 7 ˚C and all of the experiments were performed within the same day. 200gr samples of lettuce leaves were provided. From this sample, 50 g of lettuce was transferred to a Ziploc bag containing 250 ml 0/1% peptone water with a pH of 7.2 (PW, Merck). The bags containing lettuce leaves and PW were orbitally agitated by a shaker at 150 rpm for 2 min. Dilution series were prepared from the solution and the population of total coliforms and fecal coliforms were determined by MPN test (27, 28). In order to determine the efficacy of water rinse of lettuce in decreasing the microbial load, 50 gr of the lettuce was put in a sterile bag containing peptone water. Afterwards, dilution series were prepared and the population of total coliforms and fecal coliforms were determined by MPN test (27, 28).

The efficacy of detergent in decreasing the microbial levels present on lettuce surfaces showed that on the remaining 100 gr of sample from the previous level submerged in 1 liter of water that contained approximately 3 drops of detergent for approximately 5 minutes. Then, the lettuce sample was washed with water, and 50 grams of which was separated. Again, it was proved that total coliforms and fecal coliforms exist on the surface of the lettuce. Finally, the remaining 50-gr-lettuce sample was immersed in one liter of calcium hypochlorite solution (200 ppm free chlorine with 700 g/kg available chlorine) for approximately 5 minutes at the end of the contact time. Then, treatment solution was drained off and the treated lettuce was rinsed. Total coliforms and fecal coliforms were then determined

by MPN test. calcium hypochlorite solution was prepared by adding calcium hypochlorite (700 g/kg available chlorine) to deionized water. The concentrations of free chlorine were measured using a Hach DR: 2000 meter. Finally, results were reported as CFU/g (27,29,30).

The second step of this study was to assess the efficacy of the disinfection method proposed by the Iranian Ministry of Health and Medical Education in terms of decreasing helminth eggs from lettuce surfaces. In the present study, flotation method was used to determine the helminth eggs load of the lettuce. For this purpose the vegetable samples from wholesalers were brought, they were transported to the laboratory for analysis in nylon bags.

Portion of vegetables was weighted (250 g) and the sample was placed into a beaker, containing a 1.5-l detergent solution. The samples were then completely mixed and kept for approximately 30 min. Afterwards, the lettuce samples were gathered from the beaker and discarded. The water used for washing purposes was filtrated in order to remove all the remaining dirt, filth, and small debris that may have remained in the sample. Then, the container containing the wash water was stored for approximately two hours until helminth eggs layers, and sediments were precipitated. Then, the resulting eggs was transferred to a beaker. This process was repeated for several times in order to ensure the full precipitation. After that, 50 mL of the bottom liquid was transferred to centrifugation tubes and centrifuged for for 3 min at 2000g The sediment was examined at 10× magnification under a light microscope for helminth egg content. The eggs were identified at 40× magnification. (31,32). The experiments were repeated for three times in order to determine their precision and the accuracy.

Results

Results of the present study regarding the initial microbial and parasitological loads of the lettuce as well as the efficacy of the method proposed by the Iranian Ministry of Health and Medical Education are presented in Tables. According to table 1, the initial loads of total coliforms and fecal coliforms on the lettuce were 3.36 $\log_{10}$ CFU/g and 2.31 $\log_{10}$ CFU/g , respectively. The initial load of total coliform was higher than that of fecal coliform. However, no helminth eggs were observed in any of the samples. Table 2 presents the loads of total coliforms and fecal coliforms after the disinfection according to the proposed method. The loads of total coliforms and fecal coliforms after disinfection by this method were 2.18 $\log_{10}$ CFU/g, and 2.31 $\log_{10}$ CFU/g, respectively.

The removal efficiency of total coliforms and fecal coliforms by the method proposed by the Iranian Ministry of Health and Medical Education is presented in Table 3. As given in this table, the removal efficiency of the method is as follows: 78.1% (0.75 $\log_{10}$ CFU/g) and 79.6% (0.67 $\log_{10}$ CFU/g) for total coliforms and fecal coliforms at the first stage of the method (washing stage); 94.8% (1.44 $\log_{10}$ CFU/g) and 98.5% (1.9 $\log_{10}$ CFU/g) for total coliforms and fecal coliforms at the second stage (use of detergent); 98.3%(2.18 $\log_{10}$ CFU/g) and 100% (2.34 $\log_{10}$ CFU/g) for total coliforms and fecal coliforms at the final stage(disinfection). Significant differences were observed between the efficacies of the method in different stages (P=0.007, P=0.015) implying the effectivness of the method.

Table 1: Initial contamination of lettuce samples purchased from a wholesale market of fruit and vegetable in Tehran, Iran 1n 2010

Sample	(Total coliform) CFU/g $\log_{10}$	(Fecal coliform) CFU/g $\log_{10}$	Parasite
1	3.34	2.40	0
2	3.40	2.83	0
3	3.20	2.14	0
4	3.38	1.89	0
5	3.50	2.30	0
Average	3.36	2.31	0

Table 2: Reduction of total coliforms and fecal coliform populations on lettuce by the method of disinfection that proposed by the Iranian Ministry of Health and Medical Education

Treatment	Total coliform ($\log_{10}$ CFU/g)		Fecal coliform ($\log_{10}$ CFU/g)		Parasite
	Population recovered (mean + SD)	Reduction	Population recovered (mean + SD)	Reduction	
Control	3.36± 0.10		2.31±0.34		0
Water	2.61±0.2	0.75	1.63±0.17	0.67	0
Detergent	1.89 ±0.47	1.44	0.41 ± 0.58	1.90	0
calcium hypochlorite (200 ppm,5min)	1.88±0.10	2.18	0	2.31	0

1- Means and standard deviations represent five samples per experiment
2- Values with different letters for total coliform are significantly different (*P*=0.007)
3- Values with different letters for fecal coliform are significantly different (*P*=0.015)

Table 3: Efficiency of the removal of total coliforms and fecal coliforms by the method of disinfection that proposed by the Iranian Ministry of Health and Medical Education

Treatment	Total coliform (%)	Fecal coliform (%)
Water	78.1	79.6
Detergent	94.85	98.5
Calcium hypochlorite (200ppm/L,5min)	98.3	100

Discussion

No studies have yet been conducted in Iran in order to determine the microbial load of vegetables, though a number of studies have been carried at in different countries. It is noteworthy that the initial microbial load of vegetables is different in different countries, due mainly to the different condition of harvest, transport, distribution, and finally delivery to the costumer. In a study performed by Nascimento et al., in 2003 in Brazil, initial loads of total coliforms and *Escherichia coli* were evaluated on 10 lettuce samples. Results indicated that the initial loads were 3.25 $\log_{10}$ CFU/g and 1.64 $\log_{10}$ CFU/g, respectively, which is in agreement with those presented by the present study (20). Studies indicate that the population of microorganisms on vegetables can vary significantly (33, 35). The microbial load of vegetables is not always representative of the use of wastewater effluents or human fertilizers, because some of the microorganisms are considered as the natural microflora of the soil and exist on the vegetables at the time of harvest. In addition, presence of fecal coliforms is not always indicator of fecal contamination since this bacterium is the natural microflora of the plants (34-36).

However, the presence of *Escherichia coli* is undoubtedly representative of irrigation by wastewater effluents or use of human fertilizers (37). No helminth eggs was observed in any of the samples, indicating the lettuce was not irrigated by raw waste water. In a study conducted by Homayuni et al. in 2007 in Tehran, 270 vegetable samples were studied and it was found that 41.3% of the samples had different types of helminth eggs contamination (38). In another study conducted by Davami in 2000, 120 samples of raw-eaten vegetables were evaluated; in this case, 68.3% of the samples had a variety of helminth eggs contamination (39)**.** Similar study in Bushehr indicated the contamination of 12.5% of the samples (40). These finding are not consistent with those observed by the present study. This can be attributed to our smaller sample size, different types of methodologies applied, and the currently more advanced methods of production of vegetables compared to the traditional methods used in the past. At the first stage (water wash of lettuce) of the present study, total coliforms and fecal coliforms decreased by 0.75 $\log_{10}$ CFU/g and 0.67 $\log_{10}$ CFU/g, respectively. Results of the study of Nascimento indicated decreases in aerobic bacteria, yeasts, fungi, and total coliforms of 0.78, 0.87 and 0.82, respectively. In another study carried out by Frank et al. in 1999, it was found that water wash can decrease the microbial load of vegetables by 1 $\log_{10}$ CFU/g (41). In the study of Barak et al, in US, a 1 $\log_{10}$ CFU/g decrease was observed in the population of *Salmonella* induced on cantaloupe by water rinse. The second stage (use of detergent) decreased total coliforms and fecal coliforms by 1.44 $\log_{10}$ CFU/g and 1.9 $\log_{10}$ CFU/g, respectively.

Results of the study of Barak et al. indicated a 1 $\log_{10}$ CFU/g decrease in *Salmonella* population by detergent. Detergent facilitates the separation of helminth egg from the vegetables; in addition,

it decreases the water phobia characteristics of the vegetables, increasing the effectiveness of disinfection due to improved contact between the disinfectant and the pathogens (42). Total coliforms and fecal coliforms decreased by 2.18 $\log_{10}$ CFU/g and 2.31 $\log_{10}$ CFU/g, respectively, at the third stage (use of disinfectant). Results of the study of Zhang et al. indicates 1.3-1.7 $\log_{10}$ CFU/g and 0.9-1.2 $\log_{10}$ CFU/g decreases in the population of *Salmonella* on the lettuce and cabbage using chlorine (with a concentration 200 mg/l) as the disinfectant (43). A concentration of 150 mg/l chlorine decreased the population of *Salmonella* by 1 $\log_{10}$ CFU/g. Overall, the method proposed by the Iranian Ministry of Health and Medical Education.and Medical Education.decreased the total coliforms and fecal coliforms by 98.3% (2.18 $\log_{10}$ CFU/g) and 100% (2.31 $\log_{10}$ CFU/g), respectively. Results of Barak et al. indicated a 3 $\log_{10}$ CFU/g decrease in the population of *Salmonella* induced on cantaloupe by using a combination of water wash, detergent, and chlorine (150 mg/l) (44). The study conducted in Brazil indicates that the common method in that country (vegetable disinfection by 200 mg/l chlorine for 15 min) can decrease total aerobic bacteria by 2.63 $\log_{10}$ CFU/g, yeasts and fungi by 2.78 $\log_{10}$ CFU/g, and total coliforms by 1.96 $\log_{10}$ CFU/g (20). In another study performed in Mexico it was found that the disinfectant containing colloidal silver can decrease the population of total aerobic bacteria and fecal coliforms on lettuce by 52% and 99.4 %,, respectively. Corresponding values for coriander are 28.6% and 98.6 %, respectively. However, this disinfectant did not remove *Salmonella* typhi existing on the vegetables. It was suggested in the study that chlorinated water can be used as an effective alternative for the mentioned disinfectant (21). Removal efficiency of disinfectants depend primarily on the type of product, type of the fruits and vegetables surface, and the type of the microorganism that is to be removed, resulting in a broad range of results (12).

According to the results from the present study, it can be concluded that although the disinfection method proposed by the Iranian Ministry of Health and Medical Educationcan effectively remove the organisms present on vegetables, chlorine can react with the organic matters of fruits and vegetables and produce toxic compounds such as THMs (14,45,46). Instead of disinfection by chemical compounds, applying appropriate control measures at the time of harvest, transport, distribution, and delivery according to the HACCP can surely decrease the contamination load of fruits and vegetables and decline the prevalence of food-born diseases linked to them. For this purpose, all people involved in the production and sale of such products should be well trained. It is also noteworthy that further studies have to be conducted to evaluate the effectiveness of different methods of disinfection.

Ethical considerations

Acknowledgements

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