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Alternative Disinfection Methods Use to Control of Microbial Quality and Shelf-Life of Raw Eaten Vegetables (Case Study)

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Abstract

Background: *Fresh-cut vegetables are particularly susceptible to microbial growth and, therefore, the use of an effective sanitizer on this product is great importance.*
Methods: *The common vegetable types obtained from a local wholesale market in Isfahan and washed with different common disinfectants such as edible salt, sodium hypochlorite, electrolyzed water (EW), complex disinfectant, calcium hypochlorite and dishwashing liquid. Disinfection time and concentration select according to the Official Protocol in Iran. A total of 126 samples including Mint, Savory, parsley, Garden cress, Common Purslane, Chives, Basil, Radish and mixed vegetables were analyzed for total bacteria, total coliform bacteria and E. coli..*
Results: *Results demonstrated that calcium hypochlorite has the best efficiency for total coliforms in separate vegetable of parsley, Garden cress, Common Purslane, Chives, Radish, with100% efficacy. For total coliforms BGB (Brilliant Green Broth) test, complex disinfectant and calcium hypochlorite have 100% removal for all of the vegetables. E.coli test result showed in four vegetables not E.coli but for another vegetable except Dishwashing Liquid efficiency was 100%. Among all disinfectants, EW has a high efficiency for mixed vegetables. For Basil disinfected with edible salt and mint washing with complex disinfectant, Eimeria parasite and in savory disinfected with calcium hypochlorite Hook worm parasitic was found. Shelf-life was between 1 to 5 days.*
Conclusions: *Our research suggested that EW has a good effect for total coliforms, fecal coliforms, E.Coli and helmet eggs removal, also save the vegetable shelf life.*

Keywords: Disinfection, Raw vegetable, Raw foods, Fecal coliform, Eating, Egg parasite.
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Introduction

During last three decades, Global production and consumption of fresh vegetables have been increasing concurrently. The foodborne outbreaks related to fresh vegetables have been increased by increasing the consumption of fresh vegetables.[1] vegetables contain high levels of micronutrients and fibers, because of that, their consumption is recommended by many organizations (World Health Organization—WHO, Food and Agriculture Organization—FAO, United States Department of Agriculture—USDA and European Food Safety Authority—EFSA) to reduce the risk of cardiovascular diseases and cancer.[2] Statistical studies show that the consumption of fresh vegetables in different countries is not uniform and varies from 1-1.5 to 30 kg per person per year.[3] The increasing of the occurrence of foodborne illness related to the consumption of fruit and vegetables reported by recent studies.[4] The frequency and type of microorganisms found on fresh produce are variable. Listeria monocytogenes, Salmonella, and E. coli are among the pathogens isolated from vegetables.[5] Mesophilic bacteria in raw vegetables after harvest are around 103 –109 CFU/g, that is related to the product and the growing conditions.[6] Foodborne disease outbreaks from fresh produce have increased significantly in recent decades. It was reported that during the 1970s -1990s produce-associated outbreaks increased 5.3% and the median number of illnesses associated with the outbreaks increased by 11% in 2007, there were 37 reported vegetables related outbreaks which were responsible for over than 800 illnesses.[7] 38%, 35% and 11% of reported raw produce outbreaks were related to vegetable row crops, fruits and seeded vegetables, respectively.[8] Possible contaminates source for farm crops include using the raw manure, contaminated irrigation water, dust, animals, poor worker hygiene, dirty equipment and harvest containers.[7] It is well known that one of the most effective ways of producing high quality and safe end products by long shelf- life is using an effective disinfection method for fresh-cut vegetable production.[9] There are numerous chemicals (chlorine, chlorine dioxide, acidified sodium chlorite, and electrolyzed water and physical (modified atmosphere packaging and nanocomposite packaging methods for reducing contaminating pathogens and microflora.[10] For disinfection purpose, Chlorine is the most commonly used disinfectant, but it is used under widely different procedures.[11] Electrolyzed Oxidizing Water (EOW) is an encouraging alternative decontamination technique with a strong bactericidal effect. This technique has been mentioned as an effective disinfection tool in the processed vegetable industry.[12] Casteel et al. evaluated chlorine disinfection for inactivation of hepatitis A virus and coliphage MS2. They found that when produce exposed for 5 – 10 min to 20 mg/L free chlorine solution, the inactivation efficiency for HAV and MS2 achieved 90– 99% and in some cases more than 99%.[11] Effects of Per acetic acid disinfectant on some fresh vegetables investigated by Carrasco et al. and concluded that acid mixtures

(PAA) have better disinfection performance than the aqueous solution of sodium hypochlorite.[13] Likotrafiti et al. reported that vegetable storage in cool temperature enhanced the L. Monocytogenes reduction. 2.6 log CFU/g decrease as a maximum efficiency achieved by decontamination using acetic acid 2% v/v putting vegetables in sodium hypochlorite solution (200 mg/l) for 15 minutes is the most common method for vegetables disinfecting in Brazil.[14] Samadi and et al. reported that reducing 2.3 log CFU/g for total mesophilic bacteria and 1.16 to 2 log MPN/g for coliforms, fecal coliforms and fecal streptococci are achievable by using dishwashing liquid (333 ppm for 10 min) and Benzalkonium chloride (92 ppm for 15 min) for vegetables treatment.[15] Another study established that using a combination of sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2) in the presence of $CuSO_4$ has a synergistic effect for inhibition of the growth of P. digitatum, G. candidum and mycelial.[16] Because of the importance of using appropriate disinfectant for raw vegetable in prevention and control of related diseases outbreak, we designed this study to determine the efficacy of identified common disinfectants and finding the suitable one for improving the microbial quality and shelf-life of raw eaten vegetables.

Materials and Methods

Eight Ready-to-eat types of vegetables (mint, savory, parsley, garden cress, common purslane, chives, basil, radish) were obtained from a local wholesale market in Isfahan, Iran. Common vegetable disinfection method in Iran is based on the protocol of the Iranian Ministry of Health and Medical Education that was used in this study.[3] The vegetables were washed with Sodium Hypochlorite solution (NaOCl, SH, Aldrich Chemical Co., Inc., Milwaukee, Wis.) at 0.2 g/L concentration and 5 min contact time, Calcium Hypochlorite (200 ppm free chlorine with 700 g/kg chlorine) at 0.2 g/ L concentration and 5 min contact time, 3 to 5 drops of dishwashing Liquid (Golrang Group, Tehran, Iran) containing alkyl Benzene Sulfonate, Sodium Lauryl Ether Sulfate, Isothiazolones, and coconate fatty acid diethanol amide was used as the detergent with 5 min contact time, Edible salt (Sepid daneh, Iran), complex disinfectant (Calcium Hypochlorite + edible salt + fruits and vegetables dishwashing Liquid), and EW (water containing 4 grams of edible salt for 30 minutes electrolyzed with titanium electrodes plate and created solutions) were used as disinfectants. Because of the measurement of the disinfectant complex not possible, we can't determine the disinfectant concentration. But based on the references[17] this solution contains chlorine, sodium hypochlorite, hypochlorous acid, hydrogen peroxide and ozone.

The purchased products were placed separately in a sterile plastic bag and immediately hauled to the laboratory. All vegetables were physically inspected and remove any other matter (e.g. soil) before treatment. All of the samples were 63 number, 8 samples before disinfection for any vegetable, 8 samples for any 6 disinfectant, 1 sample for mixed vegetable before disinfection, and 6 samples for mixed vegetable and with two replicate, 126 experiments done.

Because of varied infection level for different vegetable types, each of these vegetable samples were disinfected in two different methods; in the first method each vegetable type was disinfected separately and in the second method the mix of vegetable types was disinfected. In both methods, the average level of Total Coliforms and Parasite Eggs were determined before and after disinfection as fallow 100 g of vegetable was transferred into the distilled water and was blended for 20 min to achieve homogenized mixture. Samples were analyzed using the 9 tubes Most Probable Number (MPN) method. Incubation was done at 30°C for 24 h.[18] For Parasitological analysis, physiological saline solution (0.95% NaCl) was used for washing 100 g of vegetable samples. After 24 h sedimentation the supernatant was discarded and after centrifuging remaining washing water at 2000 rpm for 2 min and discarding supernatant, residual was used for identification of the eggs at 40× magnification.[19]

Results

Table 1, 2 and 3 shown the results of MPN, EC (E. Coli), and BGB test in vegetables before and after disinfection. Table 4 shows the Efficiency of the removal of Total Coliforms, Fecal Coliforms, and E. Coli by the different disinfectants for mixed vegetables.

As shown in Table 1, in the mint, savory, and parsley the MPN was 23 /100gr and between used disinfectant, sodium hypochlorite and calcium hypochlorite had better efficiency. The sample control MPN was 240 /100gr for garden cress and radish, by use of sodium hypochlorite, MPN reached to 0/100gr, also MPN for garden cress and radish after disinfectant by dishwashing liquid obtained 43 and 23/100ml, respectively (minimum efficiency). Sodium hypochlorite had 100 percent efficiency for mint, radish, chives, common purslane, garden cress, and parsley but complexed disinfection by 99 percent (98% for sodium hypochlorite) had high efficiency for mixed vegetables.

Table 2 shows the result of the EC test on the vegetables. In chives, common purslane, garden cress, and parsley not seen any fecal coliform. In basil vegetables, the MPN of fecal coliform in control vegetable from 23 /100gr decreased to 0/100gr by all of the disinfectants. The same results were obtained for a mixed vegetable but dishwashing liquid disinfectant had no effect in reducing the burden of fecal coliform. Dishwashing liquid, calcium hypochlorite, electrolyzed water, and sodium hypochlorite decreased the MPN of E. Coli from 23/100gr to 0/100gr (100% removal) but in complex disinfectant and readable salt, E. Coli was 23/100gr and 9.1/100gr, respectively. In radish and control, dishwashing liquid and EW had the same result (3.6 MPN/100 gr) and another disinfectant decreased the MPN of fecal coliform to 0/100gr. Finally, in this study, all of the disinfectant except of dishwashing liquid have a 100% efficiency for E. coli in mixed vegetable (table 4).

The results of the BGB test in vegetable before and after disinfection has been shown in table 3. Between used disinfectant, edible salt had the lowest efficiency in the reduction of BGB and only in garden cress vegetable, the MPN decreased from 43/100gr to 0 /100gr. Indicated that sodium hypochlorite has a good performance in BGB test and except basil and mixed vegetable, the MPN of all reached to 0/100gr. EW could totally disinfect mixed vegetables and reduce the microbial load in other vegetables. Complex disinfectant and calcium hypochlorite have a 100% Efficiency for mixed vegetable and each of the vegetables separately. After edible salt, lowest efficiency was for dishwashing liquid and only in a savory, BGB reached to 0 from 3.6 /100gr.

In three samples of vegetables, contamination of parasitic observed. In basil disinfected with edible salt, mint washing with complex disinfectant, Eimeria parasite and in savory disinfected with calcium hypochlorite, Hook worm parasitic was found. Reson of present Eimeria and Hook worm could be the use of manure or graze livestock in the region.

Table 5 shows the vegetable shelf-life. According to table 5, Shelf-life was between 1 to 5 days, the maximum and minimum shelf-life obtained to parsley (by complex disinfectant) and basil (by sodium hypochlorite and electrolyzed water), respectively. In mixed vegetables, edible salt and dishwashing liquid have a maximum shelf-life but according to table 1 these disinfectant has the lowest efficiency, also shelf-life for EW was 2 days that was a good shelf-life. Also 1-day shelf-life obtained for sodium hypochlorite, complex disinfectant and calcium hypochlorite disinfectant.

Table1. The results of MPN test in vegetable before and after disinfection

Vegetable / Disinfectant	Mint	Radish	Basil	Chives	Common Purslane	Garden cress	parsley	Savory	Mixed vegetable
Control	23	240	460	460	9.1	240	23	23	460
Edible salt	23	23	460	460	2.3	23	23	9.8	53
Sodium hypochlorite	0	0	23	0	0	0	0	3.6	23
Electrolyzed water	9.1	9.1	3.6	23	0	3	9.1	4.3	9.1
Complex disinfectant	0	3.6	23	0	0	3.6	0	23	3.6
Calcium hypochlorite	0	0	23	0	3.6	3.6	0	3.6	9.1
Dishwashing liquid	3.6	23	240	240	3.6	43	23	23	43

Table 2. The results of the EC test in vegetable before and after disinfection

Vegetable / Disinfect	Mint	Radish	Basil	Chives	Common Purslane	Garden cress	parsley	Savory	Mixed vegetable
Control	23	23	260	460	0	43	23	3.6	460
Edible salt	9.1	3.6	240	460	0	0	23	3.6	53
Sodium hypochlorite	0	0	3.6	0	0	0	0	0	9.1
Electrolyzed water	0	9.1	0	23	0	0	9.8	0	0
Complex disinfectant	0	0	0	0	0	0	0	0	0
Calcium hypochlorite	0	0	0	0	0	0	0	0	0
Dishwashing liquid	3.6	23	240	240	0	43	23	0	43

Table 3. The results of BGB test in vegetable before and after disinfection

Vegetable / Disinfect	Mint	Radish	Basil	Chives	Common Purslane	Garden cress	parsley	Savory	Mixed vegetable
Control	0	3.6	23	0	0	0	0	23	43
Edible salt	0	0	0	0	0	0	0	9.1	0
Sodium hypochlorite	0	0	0	0	0	0	0	0	0
Electrolyzed water	0	3.6	0	0	0	0	0	0	0
Complex disinfectant	0	0	0	0	0	0	0	23	0
Calcium hypochlorite	0	0	0	0	0	0	0	0	0
Dishwashing liquid	0	3.6	0	0	0	0	0	0	43

Table 4. Efficiency of the removal of Total Coliforms, Fecal Coliforms, and E. Coli by the different disinfectants for mixed vegetables

Disinfectants	Conditions		MPN test	BGB test	E.Coli test
			MPN/100g		
	Concentration(ppm)	Contact time(min)			
Edible salt	30000	5	88.5	88.5	100
Sodium hypochlorite	200	5	95.0	98.0	100
Electrolyzed water	200	5	98.0	100	100
Complex disinfectant	200	5	99.2	100	100
Calcium hypochlorite	200	5	98.0	100	100
Dishwashing Liquid	3 to 5 drops	5	90.7	90.7	0.0

Table 5. The vegetable shelf-life

Vegetable / Disinfectant	Mixed vegetable	Radish	Basil	Chives	Common Purslane	Garden cress	parsley	Savory	Mint
Edible salt	3	3	2	4	4	3	5	3	3
Sodium hypochlorite	1	2	1	3	3	4	4	2	4
Electrolyzed water	2	3	1	2	3	3	4	3	3
Complex disinfectant	1	3	2	3	3	3	5	3	3
Calcium hypochlorite	1	3	2	3	3	4	4	2	4
Control	2	3	2	3	4	3	4	3	4
Dishwashing liquid	3	2	2	3	3	4	4	2	4

Discussion

Some factors such as the type of vegetables or fruits and type of the microorganisms that are to be removed can affect the disinfectants efficiency for microorganisms removal.[20] Chlorine is disinfectant that most widely used for produces washing.[21] In a study performed by Nascimento et al. in 2003 in Brazil, initial load of total coliform was evaluated on 10 lettuce samples. Results indicated that the initial load was 3.25 log for CFU/g that after disinfection by sodium hypochlorite decreased to 1.34±0.26 log for CFU/g (57.8% efficiency).[22] Yarahmadi, et al. evaluated the efficiency of Lettuce disinfection according to the official protocol in Iran, the result shown that the removal efficiency of total coliforms after washing with water was 78.1% that increased to 94.85 % and 98.3 % after using detergent and chlorine, respectively.[3] In this study total coliform removal obtained 91% and 98% (table 4) when detergent and calcium hypochlorite used for vegetable washing which is consistent with Yarahmadi, et al. results.[3] In a detailed study with Lettuce and Chlorine (200 ppm), it was observed that cells of E. Coli survived to a minor degree in the surface of the leaves rather than in the inner structures of vegetables such as stomata or damage tissue. Cells can protect themselves from Chlorine action when bacteria penetrated 0 to 10 mm, the viability was between 50.8 and 45.6%. Meanwhile, when the penetration was 30 to 40 mm, viable cells were 68.3%.[23] The fecal contamination degrades bacteriological quality of farm produces, frequently. This contamination can occurred before reaching the products to the consumer results from various unsanitary cultivation and marketing processes. Inadequately treated municipal, animal, or food processing wastes that used for irrigating or fertilizing known as a Primary cause if contain pathogenic organisms . In addition, contamination can also may occur during the growing season or after harvesting because of the careless handling of the crops.[24] Keeratipibul et al. survived the removal of E. coli on tomato fruits after soaking with aqueous chlorine (75 ppm) or per-acetic acid (50 ppm) for 10 min and found the efficiency of this disinfectant were 43% and 35.4%, respectively.[25] Bermúdez-A guirre et al. reported that citric acid was not effective for inactivation of E. coli from selected vegetables.[21] Maria V. Selma et al. studied the efficacy of an electrochemical treatment in water disinfection, using boron-doped diamond electrodes for removal of E. coli O157: H7 in the tap water containing organic matter, and resulted 5 log reduction for E. Coli O157: H7.[26] According to Gomez et al. the MPN of fecal coliforms for lettuce and mexican coriander was 75 and 150 /gr, respectively and reached to 0.43 and 2.1/gr after disinfection by silver base disinfectant. That shows the 99.4% and 98.6% efficiency that confirm the experiment result and efficiency for sodium hypochlorite, electrolyzed water, complex disinfectant, calcium hypochlorite, that were 98, 100, 100 and 100%, respectively.[27] This results are similar to Nadafi et al.[15] research, where they used detergent and calcium hypochlorite for fecal coliform removal and concluded that the efficiency was 98.5% and 100%, respectively. The Gomez et al. recommended stoping irrigation by contaminated water and for irrigation and using more efficient methods such as as diluted chlorine solutions for pathogenic microorganisms removal to deacrising the crops contamnation.[27] In a study conducted by Homayuni et al. in Tehran, reported that 41.3% of 270 studied vegetable samples were had different types of helminths egg contamination.[15] Current reports indicate that in porpuse ofpathogens removal from the surface of lettuce and apples, using lonely surfactants are not significantly more effective than water and only can significantly reduce the population on surface of lettuce and strawberry fruit by 0.2 log CFU/cm 2 and 0.4 log CFU/g, respectively.[28] Avcioğlu et al. reported that raw vegetables washing, can remove all Helminths egg from studied vegtable samples In their study Ascaris lumbricoides and Toxocara spp. were detected in four and two unwashed vegetables, respectively.[29] Using detergent facilitates, not only can separate helminthes egg from the vegetables, also can decrease the water phobia characteristics of the vegetables, and increas the effetiency of disinfection by improving the contact between the pathogens and disinfectant.[28] When the Fresh leafy vegetables stores in inappropriate conditions, will have short shelf-life, Because the Storage temperature and humidity influencing the quality and safety of leafy greens.[30] Gómez-López et al. studied the effect of neutralized EW on increasing the stored shelf life of shredded cabbage under equilibrium modified atmosphere. They recommended that EW is useful for improving the visual acceptability and increase the shelf life at least 5 and 3 days.[31]

Many studies have reported that chlorine is the most common method for fresh produces disinfection, because of its confirmed effetiency in water disinfection, easy to use in an industrial setting, requiring short contact time, high effitiency in chilled water (which is necessary to maintain quality throughout shelf life) and its cost-effectiveness.[32] Gomes et al.[33] reported that chlorine cannot eliminate the internalized microorganisms, another deficiency of chlorine is its impact on environmental and occupational health issues because of its possible by-products left after washing process. acid EOW (AcEOW) and alkaline EOW (allow) are two separated streams to produce EW bya salt diluted solution and currently are passed through the chamber dissociating the solution: AcEOW solution is antimicrobial solution with an action mode similar to chlorine (DNA mutations, disruption of cell proteins and enzymes). AcEOW solution acidity, leads to cell membrane disruption and the action of hypochlorous acid is facilitated, as well as the alkaline solution works as a detergent and has a negative ORP (-800 to -900 mV).[34] EW is considered as an eco-friendly technology becuse of useing convenient and safe raw materials such as water and NaCl.[35] As shows in table 4 and 5 among studied disinfectant the EW not only has a good effect in vegtable decontamination also has a high shelf life for mixed vegetables. The advantages of EW includes inactivatation of several pathogenic types at same time, strong bactericidal effect on pathogens, and neutralizing harmful substances.[2]

Vegetables are indispensable part of the human diet but controlling foodborne outbreak related to vegetables is necessary. The efficacy of disinfectant for removal of survived and total bacteria, total coliform bacteria, and E. coli on common vegetables was investigated in this study. Total coliform was found in all of the raw vegetables. Except common parsley, the other vegetables have a fecal coliform. E.coli just found in radish, basil, savory and mixed vegetables. Chlorine basin compound has a high efficiency for disinfection but because of THM production in Chlorine usage, the EW has preference. EW has high efficacy and shelf life for vegetables.

Between the studied disinfectants for mixed vegetables, the lowest efficiency of edible salt and dishwashing liquid was found for total and fecal coliforms and for E.coli, respectively. Also, the shelf life of vegetables survived and showed that the edible salt and dishwashing liquid have low efficiency but they have a highest shelf life for mixed vegetables. Finally, this research suggests that according to the microbial load and parasites egg removal and good shelf life after disinfection, the DW is a better disinfectant for vegetables disinfection.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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