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Effect of Different Household Decontamination Procedures on Antioxidant Activity and Microbial Load of Vegetables

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Abstract

Aims: Decontamination procedures are different in each country, as the other applications of disinfection, and standards. The aim of this study was to evaluate the effects of household decontaminations and storage time on the antioxidant activity and microbial load of salad vegetables.

Instrument & Methods: This analytic-descriptive study was conducted on 4 types of salad vegetables; cucumber, tomato, lettuce, and sweet basil. After washing, samples with storage time of 0 day were analyzed immediately. Other samples were held in 4°C for 3 and 5 days. Five different washing and decontamination methods were compared; water washing, detergent washing, benzalkonium chloride, sequential washing and Kanz disinfecting method. The Ferric Reducing Ability of Plasma assay was used to measure the antioxidant activity. Aerobic mesophyll bacteria and total coliforms were chosen as microbial load index. ANOVA and Tukey post-hoc tests were used to analyze the data.

Findings: By increasing the storage time, the antioxidant activity of all types of vegetables reduced. There was a significant decrease in antioxidant activity in all types of vegetables using sequential washing method with water, detergent, and benzalkonium chloride and Kanz disinfection method. All washing methods were effective in decontamination for either mesophyll bacteria or total coliforms, except for total coliforms in lettuce. There was no significant difference in microbial load among first 4 methods of washing ($p>0.05$), but a significant difference was observed in Kanz disinfection method ($p<0.05$).

Conclusion: Kanz disinfection is the most effective decontamination method to eliminate microorganisms index, which also reduce the antioxidant activity.

Keywords
Vegetables [https://www.ncbi.nlm.nih.gov/mesh/68014675];
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Introduction

Fruits and vegetables are valuable resources to provide nutritious materials, e.g. antioxidants, vitamins, minerals, etc., for human body. Moreover, mentioned resources could be potential factors to transfer microorganisms, and emerge of different diseases due to ignoring hygiene regularities and using inappropriate and unhealthy washing procedure [1-4]. Both nutritious and microorganisms are under performed processes on vegetables and fruits. These processes are including irrigation, harvest, transportation, storage, decontamination, drying, etc. [5, 6].

One of the most important mentioned processes is decontamination, which is conducted as a process to eliminate pathogen's microbial load, delay spoil in fruits and vegetables, and reduce the risk of related diseases [7]. New procedures of decontamination have been considered since usual methods such as decontaminating with chloride and its derivatives were proven to produce adverse by-product compounds [8]. Although, chemical replacements and physical methods were used to reduce pathogen microorganisms in recent reports, the rate of reduction was not favorable (1 to 2 log10 CFU/gr); also, the applications were in the laboratory scale in many of them [1, 9]. New methods are used to decontaminate nutrition materials, fruits, and vegetables in developed countries, but traditional methods such as decontaminating with chloride derivatives or detergents and then washing with drinking water are still used to decontaminate fruits and vegetables in developing countries [10]. These decontamination procedures are carried out mostly according to recommended regularities and standards, and available disinfectants [11].

It seems that household decontaminations are less considered in terms of the effect on nutritious materials, antioxidant content, and microbial load of fruits and vegetables in various surveys. On the other hand, salad vegetables have been studied more in comparison to other vegetables. These materials have been assessed as separate and as group in the studies [12, 13]. Qiang *et al.* have indicated that the use of all procedures (e.g., chloride, hydrogen peroxide, and ozone) led to significant reduction in antioxidant content; also, using of chloride disinfectant is the most effective procedure for reducing microbial load [4]. Serea *et al.* have reported that the storage of vegetables in the freezing conditions is a reducer factor of antioxidant activity in lettuce [14].

Few comprehensive studies have been conducted in terms of household decontamination's effect on antioxidant content and microbial load. Therefore, the aim of this study was to evaluate the effects of household decontaminations and storage time on the antioxidant activity and microbial load of salad vegetables.

Instrument & Methods

This analytic-descriptive study was conducted on 4 types of salad vegetables; cucumber *(Cucumis sativus),* tomato *(Solanum lycopersicum),* lettuce (*Lactuca sativa),* and sweet basil *(Ocimum basilicum)*. All the samples were purchased from the stores of Tehran, Iran, and were transferred to the laboratory in less than 1 hour. After the washing stage in sampling day, samples with storage time of 0 day were analyzed immediately. Other samples were held in 4°C for 3 and 5 days, and then were analyzed. However, as some studies have reported that microbial load should be assessed immediately after decontamination [3, 7, 8], the effect of time was not surveyed on microbial load.

Five different washing and decontamination methods were compared:

Method 1: Water washing (irrigating for 10min);

Method 2: Detergent washing (2ml of dish soap in 6l of water for 10min of irrigating);

Method 3: Benzalkonium chloride (with concentration of 92ppm and irrigating for 15min);

Method 4: Sequential washing (with water and dish washing liquid, and disinfection by benzalkonium chloride with concentration of 92ppm and irrigating for 15min);

Method 5: Kanz disinfecting (benzalkonium chloride and *Cocamidopropyl betaine,* with concentration of 110ppm and irrigating for 20min).

One group of vegetables was considered as control (contaminated). Vegetables in this group were analyzed without any washing method. The method and concentration used for decontamination were labeled on the purchased can according to recommended method.

To measure the antioxidant activity, first 20g of each vegetable sample was extracted by 60ml of 99% ethanol (Merck; Germany) and were exposed to laboratory temperature in dark condition. Then, the solution was centrifuged for 15min at 10°C. The obtained solution had antioxidant combinations, which were removed and filtered by Whatman leach paper (No. 1440-125) to obtain light extract.

The FRAP assay (Ferric Reducing Ability of Plasma), which was primarily described by Benzie & Strain [15] and then developed by Guo *et al.* [16] was used to measure the antioxidant activity. 0.1ml of extracted solution was diluted with 0.3ml of double-distilled water. Then, 3ml of FRAP indicator was added to the subsequent solution. After 8min of storage, the solution was used to measure the antioxidant content. This process was performed using spectrophotometry (Perkin Elmer; United States) in 593nm. The solution of spectrophotometer reference was used to measure the antioxidant activity of Trolox as control (Fluka, 98%; UK).

To measure the microbial load, after washing vegetables, 25g of each vegetable was put into plastic bags to eliminate contaminations. To maintain pH in relative neutral condition, 225ml of phosphate buffer was added to each plastic bag and fairly shaken for 1min. This process was repeated for every procedure of washing the vegetables. Afterwards, 10mg of each vegetable sample was removed and put in a blender. The obtained solution was diluted to 100ml using distilled water.

Aerobic mesophyll bacteria and total coliforms were chosen as microbial load index. Deep plating of samples on plate count agar (Merck; Germany) was used to evaluate the aerobic mesophyll population. In this method, 1ml of each sample was cultivated and incubated for 48h at 37°C and emerged colonies were counted. The determination of total coliforms was done by a two-step diagnostic and confirmatory method [17, 18].

After entering data to SPSS 21 software, the ANOVA was used to determine the antioxidant activity. Tukey post-hoc test was used to assess the difference between the trial and control groups.

Findings

Sweet basil and lettuce had higher antioxidant activity than tomato and cucumber. By increasing the storage time, the antioxidant activity of all types of vegetables reduced. In addition, there was no significant change in antioxidant activity amongst washing methods by water, detergent, and decontamination with benzalkonium chloride. Conversely, there was a significant decrease in antioxidant activity in all types of vegetables using sequential washing method with water, detergent, and benzalkonium chloride and Kanz disinfection method. The highest decrease was observed in Kanz disinfection method comparing to other decontamination methods and the control ($p<0.05$; Figure 1).

Figure 1) Rate of antioxidant power in vegetables as mmol/l Trolox equivalents per kilogram of fresh weight of antioxidant capacity based on washing method, decontamination procedure used and storage time

Methods	Day Zero	Day 3	Day 5
Sweet basil			
Control	54.08±0.89	43.28±1.05	32.75±1.10
Water washing	54.87±0.95	43.53±1.27	32.85±1.01
Detergent washing	54.34±0.62	42.88±1.52	32.09±2.10
Decontaminant	54.12±0.73	42.81±0.98	32.26±0.61
Sequential washing	44.25±0.59	35.54±0.78	25.15±0.75
Kanz disinfecting	39.18±1.00	30.75±1.34	20.29±0.76
Lettuce			
Control	46.49±0.99	40.26±0.62	31.59±0.91
Water washing	46.24±0.90	40.98±1.21	31.91±1.07
Detergent washing	46.39±0.93	40.89±1.07	31.98±1.92
Decontaminant	46.02±1.33	40.71±0.96	31.99±1.57
Sequential washing	38.60±0.80	32.08±0.88	21.77±1.13
Kanz disinfecting	35.79±0.97	28.53±0.64	18.33±1.24
Tomato			
Control	27.87±1.15	24.48±0.49	19.21±0.67
Water washing	27.73±1.38	24.76±1.01	19.20±1.09
Detergent washing	27.43±1.31	24.09±0.77	19.28±0.74
Decontaminant	27.98±1.24	24.55±0.57	19.34±0.59
Sequential washing	25.61±0.85	22.13±0.78	16.70±0.79
Kanz disinfecting	24.33±0.71	19.33±0.89	13.34±0.76
Cucumber			
Control	20.55±0.71	19.66±0.76	16.44±0.57
Water washing	20.38±0.84	19.78±0.52	16.31±0.61
Detergent washing	20.81±1.26	19.25±0.75	16.37±0.53
Decontaminant	20.53±0.68	19.38±0.51	16.16±0.51
Sequential washing	19.12±0.31	17.58±0.65	13.44±0.68
Kanz disinfecting	17.89±0.25	14.95±0.83	10.91±0.62

Lettuce and sweet basil had more microbial load than cucumber and tomato in both aerobic mesophyll bacteria and total coliforms. All washing methods were effective in decontamination for either mesophyll bacteria or total coliforms, except for total coliforms in lettuce. The rate of microbial load was decreased by developing washing methods (i.e., from washing with water to decontaminating by Kanz disinfecting method). In addition, the pattern increased in different washings by water, detergent, disinfecting with benzalkonium chloride and sequential decontaminating with water, detergent, and benzalkonium chloride. This increase was more significant in Kanz disinfecting method. There was no significant difference in microbial load among first 4 methods of washing (p>0.05), but a significant difference was observed in Kanz disinfection method (p<0.05; Figure 2).

Figure 2) Microbial load including population of total aerobic mesophyll and total coliforms microorganisms in vegetables based on washing methods (log10 CFU/gr)

Methods	Mesophyll bacteria	Total coliforms
Cucumber		
Control	3.70±0.45	3.72±0.71
Water washing	1.72±0.01	2.61±0.69
Detergent washing	1.51±0.19	2.51±0.54
Decontaminant	1.43±0.11	2.48±0.86
Sequential washing	1.38±0.25	2.31±0.63
Kanz disinfecting	0.11±0.00	0.65±0.01
Tomato		
Control	3.81±0.66	3.99±0.59
Water washing	1.86±0.16	2.76±0.73
Detergent washing	1.52±0.09	2.70±0.66
Decontaminant	1.50±0.02	2.52±0.71
Sequential washing	1.45±0.03	2.11±0.34
Kanz disinfecting	0.12±0.01	1.12±0.03
Lettuce		
Control	5.81±0.51	4.63±0.68
Water washing	3.88±0.57	3.45±0.49
Detergent washing	3.61±0.87	3.58±0.43
Decontaminant	3.53±0.76	3.31±0.44
Sequential washing	3.46±0.71	3.63±0.53
Kanz disinfecting	1.59±0.07	1.65±0.15
Sweet basil		
Control	6.88±0.54	5.85±0.81
Water washing	4.89±0.39	4.58±0.62
Detergent washing	4.63±0.69	4.41±0.74
Decontaminant	4.52±0.48	4.22±0.68
Sequential washing	4.48±0.52	3.98±0.48
Kanz disinfecting	2.64±0.56	2.79±0.31

Discussion

The aim of the present study was to evaluate the effects of washing and decontamination methods on antioxidant activity and microbial load of the salad vegetables. The method used to measure antioxidant activity was the Ferric Reducing Ability of Plasma (FRAP) Assay. In terms of microbial load, after washing and decontamination, the populations of mesophyll bacteria and total coliforms were evaluated as remained microbial load index.

Current findings showed an indirect correlation between storage time in fridge with antioxidant activity. Similar results were reported in some previous studies [19, 20]. This is probably resulted from passing the time and the effects of conducted processes. Some studies reported that each growing, process, and ageing of the vegetables might have significant effects on bioactive properties of the vegetables and fruits; also, passing time can have a significant effect on vegetables and fruits [5, 21]. Paradoxically, Kevers *et al.* have reported that FRAP antioxidant activity in vegetables and fruits is steady by passing the storage time. However, the evaluation of antioxidant was performed without disinfectant during the time [22]. Moreover, antioxidant capacity (total phenolic and antioxidant activity) was stable using different disinfectants in a similar study [23]. It should be noted that the applied methods and fruits for evaluation of antioxidant were different in the aforementioned studies. In terms of storage time's effect, there was a significant association between 5-day storage time and antioxidant decrease. Correspondingly, Ahmed *et al.* have reported similar results, as the lowest decrease in antioxidant activity was occurred in the first 3 days for tomato [24].
By comparing trial and control groups (without any washing) in first 3 methods (washing with water, decontaminating with detergent and benzalkonium chloride), no significant association was found between decontamination and antioxidant activity decrease. Conversely, in two other methods (washing with water and decontamination with detergent and benzalkonium chloride and Kanz disinfection method), there was a significant association between decontamination and antioxidant activity decrease. In latter two methods, this is probably due to the increase of irrigation and contacting with disinfectant materials for antioxidant activity, as one of the effective factors in antioxidant activity change was described as type and time of disinfectant and contacting with it [25]. In consistence, no significant effect was observed in a study on separate decontamination procedures with water and chloride (by a short contact time) in comparison with the control group [26].
The highest and lowest antioxidant activity belonged to sweet basil and cucumber,

respectively. There was no report to compare antioxidant activity of sweet basil and cucumber with, to our knowledge, but in some reports cucumber was introduced as vegetable with least antioxidant power [27]. Some studies reported various antioxidant powers for tomato and lettuce, but their antioxidant level is totally more than cucumber [22]. Antioxidant activity and antioxidant level would be different by changing some parameters relating to the growth and storage of fruits and vegetables, e.g. graying, climate condition, season of production, soil condition, and experiment related conditions (extract method) [22, 28, 30].

Kanz was the most effective decontamination method. The decrease in microbial load was observed to be 2 to 2.5 log10 CFU/g for mesophyll bacteria, with low differences among the first 4 methods. Benzalkonium chloride with the concentration of 0.1mg/ml was used in the study of Velázquez *et al.* and decreased the microbial load in tomato to 4.2 log10 CFU/g. This additional performance comparing to the present study is probably due to its higher concentration [31]. Furthermore, Tubajika has reported that this disinfectant in concentrations lower than 100ppm could not significantly decrease the growth of *Physalospora fungus*, but higher concentrations could control the growth of this fungus [32]. Considerable change was observed in decreasing mesophyll bacteria population by means of Kanz disinfecting method. The decrease level was 3.6, 3.7, 4.3, and 4.2 log10 CFU/g for cucumber, tomato, lettuce, and sweet basil, respectively. For total coliforms, the decrease level was ranged from 1.1 to 1.8 in first 4 methods, but in Kanz disinfecting method this value was 3.1, 2.7, 3, and 3 log10 CFU/g for cucumber, tomato, lettuce, and sweet basil, respectively.

For the first 4 methods, it could be noted that benzalkonium chloride and detergent had insignificant effect than potable water, but for sudden decrease in method 5, it could be noted that *Cocamidopropyl betaine* was probably a synergistic factor for benzalkonium chloride. Similarly, Cho *et al.* have reported that chlorine dioxide with chloride can promote the power of chloride to inactive microorganisms [33]. In terms of microbial load for different types of vegetables, the microbial load of lettuce and sweet basil was more than cucumber and tomato in both mesophyll bacteria and total coliforms. This is probably due to the rough shape and uneven surface of lettuce and sweet basil comparing to cucumber and tomato. In accordance with our results, Velázquez *et al.* have reported more microbial load for aerobic mesophyll bacteria in lettuce than tomato and a direct association between microbial load and elimination of that with biofilms and uneven surfaces of leafy vegetables [34]. However, based on the type of vegetable, temperature of washing water, irrigation time, and pH, microbial load decrease can change.

The limitation of the present study was the lack of developing a time-concentration model for optimal decontamination using national standards in Iran; nevertheless, surveying of important chemical and microbial factors in decontamination process of high-consumed vegetables could be a power point of this study. The present study suggests the evaluation of consolidated decontamination methods (e.g., physical-chemical) in antioxidant capacity, also designing optimal time-concentration models for chloride and non-chloride disinfectants.

Conclusion

Kanz disinfection is the most effective decontamination method to eliminate microorganisms index, which also reduce the antioxidant activity.

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References

1- Gil MI, Selma MV, López-Gálvez F, Allende A. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. Int J Food Microbiol. 2009;134(1):37-45.

2- Martin A, Cherubini A, Andres-Lacueva C, Paniagua M, Joseph J. Effects of fruits and vegetables on levels of vitamins E and C in the brain and their association with cognitive performance. J Nutr Health Aging. 2001;6(6):392-404.

3- Grassmann J, Hippeli S, Elstner EF. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol Biochem. 2002;40(6-8):471-8.
4- Qiang Z, Demirkol O, Ercal N, Adams C. Impact of food disinfection on beneficial biothiol contents in vegetables. J Agric Food Chem. 2005;53(25):9830-40.
5- São José JFBd, Andrade NJd, Ramos AM, Vanetti MCD, Stringheta PC, Chaves JBP. Decontamination by ultrasound application in fresh fruits and vegetables. Food Control. 2014;45:36-50.
6- Shahdadi F, Mirzaei H, Garmakhany AD. Study of phenolic compound and antioxidant activity of date fruit as a function of ripening stages and drying process. J Food Sci Technol. 2015;52(3):1814-9.
7- Beltrán D, Selma MV, Marín A, Gil MI. Ozonated water extends the shelf life of fresh-cut lettuce. J Agric Food Chem. 2005;53(14):5654-63.
8- Ölmez H, Kretzschmar U. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. LWT Food Sci Technol. 2009;42(3):686-93.
9- Huang TS, Xu C, Walker K, West P, Zhang S, Weese J. Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. J Food Sci. 2006;71(4):M134-9.
10- Pérez-Gregorio M, González-Barreiro C, Rial-Otero R, Simal-Gándara J. Comparison of sanitizing technologies on the quality appearance and antioxidant levels in onion slices. Food Control. 2011;22(12):2052-8.
11- Gorny JR. Food safety guidelines for the fresh-cut produce industry [Internet]. Alexandria: International Fresh-cut Produce Association (IFPA) [Published 2001, 16 August; Cited 2016, 4 December]. Available from: https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169008.pdf.
12- Sharma PB, Handique PJ, Devi HS. Antioxidant properties, physico-chemical characteristics and proximate composition of five wild fruits of Manipur, India. J Food Sci Technol. 2015;52(2):894-902.
13- Gayán E, García-Gonzalo D, Álvarez I, Condón S. Resistance of Staphylococcus aureus to UV-C light and combined UV-heat treatments at mild temperatures. Int J Food Microbiol. 2014;172(0):30-9.
14- Serea C, Barna O, Manley M, Kidd M. Effect of storage temperature on the ascorbic acid content, total phenolic content and antioxidant activity in lettuce (Lactuca sativa l.). J Anim Plant Sci. 2014;24(4):1173-7.
15- Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem. 1996;239(1):70-6.
16- Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res. 2003;23(12):1719-26.
17- Downes FP, Ito K. Compendium of methods for the microbiological examination of foods. Washington, DC: American Public Health Association; 2000.
18- Eaton AD, Clesceri LS, Rice EW, A. E. Greenberg AE, Franson MH. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association; 2005.
19- Vina SZ, Chaves AR. Antioxidant responses in minimally processed celery during refrigerated storage. Food Chem. 2006;94(1):68-74.
20- Antonia Murcia M, Jiménez AM, Martínez-Tomé M. Vegetables antioxidant losses during industrial processing and refrigerated storage. Food Res Int. 2009;42(8):1046-52.
21- Serrano M, Martinez-Romero D, Guillen F, Castillo S, Valero D. Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Postharvest Biol Technol. 2006;39(1):61-8.
22- Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommes J, Pincemail J. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J Agric Food Chem. 2007;55(21):8596-603.
23- Siddiq M, Sogi D, Dolan K. Antioxidant properties, total phenolics, and quality of fresh-cut 'Tommy Atkins' mangoes as affected by different pre-treatments. LWT Food Sci Technol. 2013;53(1):156-62.
24- Ahmed L, Martin-Diana AB, Rico D, Barry-Ryan C. The antioxidant properties of whey permeate treated fresh-cut tomatoes. Food Chem. 2011;124(4):1451-7.
25- Karaca H, Velioglu YS. Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley. Postharvest Biol Technol. 2014;88:46-53.
26- Kenny O, O'Beirne D. The effects of washing treatment on antioxidant retention in ready-to-use iceberglettuce.IntJFoodSciTechnol.2009;44(6):1146-56.
27- Song W, Derito CM, Liu MK, He X, Dong M, Liu RH. Cellular antioxidant activity of common vegetables. J Agric Food Chem. 2010;58(11):6621-9.
28- Tabart J, Kevers C, Sipel A, Pincemail J, Defraigne J-O, Dommes J. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem. 2007;105(3):1268-75.
29- Howard LR, Clark JR, Brownmiller C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. J Sci Food Agric. 2003;83(12):1238-47.
30- Markus F, Daood H, Kapitany J, Biacs P. Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. J Agric Food Chem. 1999;47(1):100-7.
31- del Carmen Velázquez L, Barbini NB, Escudero ME, Estrada CL, de Guzmán AMS. Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing Escherichia coli O157: H7 and Yersinia enterocolitica on fresh vegetables. Food Control. 2009;20(3):262-8.
32- Tubajika KM. Efficacy of alkyl dimethyl benzyl ammonium chloride on suppression of Physalospora vaccinii in laboratory assays. J Food Prot. 2006;69(10):2460-4.
33- Garcia A, Mount J, Davidson P. Ozone and chlorine treatment of minimally processed lettuce. J Food Sci. 2003;68(9):2747-51.
34- Kondo N, Murata M, Isshiki K. Efficiency of sodium hypochlorite, fumaric acid, and mild heat in killing native microflora and Escherichia coli O157: H7, Salmonella Typhimurium DT104, and Staphylococcus aureus attached to fresh-cut lettuce. J Food Prot. 2006;69(2):323-9.