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The Washing and Disinfection Efficiency of Leek in Reducing Aerobic Mesophilic Microorganisms and *Escherichia Coli* in an Industrial Plant in Shiraz, Southern Iran

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ABSTRACT

Background: In Iran in recent years, the number of vegetable processing factories had an increasing trend. Therefore, quality of freshly cut vegetables has an important role in health that is dependent not only on the microbial flora of vegetables, but also on the hygiene of equipment and in the plant environment. This study investigated the effect of washing and packaging steps on reducing microbial contamination of freshly cut vegetables and has identified critical points during the production processes.

Methods: Leek samples from different stages (raw material, washing tank, chlorine tank, ozone tank, centrifuge, chopping and packaging stage) were taken from the vegetable processing plant and transferred to the laboratory. Samples were evaluated for presence of aerobic mesophilic and *Escherichia coli* microorganisms.

Results: In the leek vegetable plant, *E. coli* was shown to decrease to 0.7 log cfu g^{-1}. However, there was no significant difference between raw vegetables and final products regarding aerobic and *E. coli* microorganisms. Also, aerobic mesophilic bacteria showed a significant increase (p=0.04) between chopping and packing leeks and a significant decrease (p=0.032) between ozonization and centrifugation for *E. coli*.

Conclusion: The processing steps in this plant had little effect on the reduction of aerobic mesophilic microorganisms and *E. coli*. Therefore, it is recommended to use a proper washing system and disinfectants, and also to pay attention to the cleanliness of tools and equipment in contact with vegetables.

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Introduction

Fresh fruits and vegetables are essential nutrients in the human diet that are beneficial to the health (1). Vegetables are rich in vitamins (A, C, B1, B6, B9, and E), minerals, dietary fibers and phytochemicals. A daily diet containing vegetables was shown to improve gastrointestinal health, reduce the risk of heart disease, stroke, and chronic diseases such as diabetes and some forms of cancer (2). The major concern about fruits and vegetables is their microbial contamination. Since there is no general standard approach to ensure the elimination of microbial contamination of ready-to-eat vegetables, these products are potential sources of human pathogens (3), therefore, microbial contamination of semi-processed vegetables can be a concern for the prevalence of foodborne illnesses. Several studies have reported the prevalence of foodborne pathogens (such as Salmonella, *Listeria monocytogenes*, and *Escherichia coli*) in vegetables, especially in semi-processed products. The researchers have focused on the relation of these products and hygiene-index microorganisms (such as aerobic mesophilic microorganisms, psychrotrophic, Pseudomonas, coliforms, and *E. coli*) (4).

The Food plant surfaces, equipment and washing stages can be potential sources of contaminations (3), and also water quality management is of great importance. Good Manufacturing Operations (GMP) principles are important steps in prevention or reduction of spread of pathogens in water and food. The use of antimicrobial agents to reduce cross-contamination during the process is also very important (5). The washing stage is an essential part of the production unit of ready-to-use products factories. Washing fruits or vegetables can be undertaken by spraying or immersing in water (1-10°C) (6). At this stage, any soil and mud residues can be appropriately removed. In the next step, water containing a disinfectant is utilized to reduce the microbial load of the products (7).

Chlorine is the most popular disinfectant used to decline the microbial load of fresh fruits and vegetables. Hypochlorous acid has free chlorine and has the highest bactericidal activity against microorganisms in fresh fruits and vegetables. Ozone is another disinfectant that is widely used in reduction of the microbial load of fresh fruits and vegetables. Unlike chlorine, ozone is very unstable in water and is converted to oxygen in a short time and can oxidize organic elements in the water (8). In recent years, several factories and vegetable processing pilot plants have increased in many countries, including Iran. Therefore, the quality of their products has an important role in health status. So this study was aimed to investigate the effect of different processing methods (washing, disinfection and packaging of fresh and sliced leeks) on reduction of microbial contamination of the products during the processes.

Materials and Methods

Leek samples were taken from different steps of the processing line of the vegetable processing plant (raw material, rinse tank, detergent tank, chlorine tank, ozone tank, centrifugation, chopping and packaging steps), transformed to the laboratory under aseptic conditions and analyzed for aerobic mesophilic microorganisms and *E.coli*. All experiments were performed in triplicates.

Microbiological assessments were carried out using a 10 g sample of leek. The sample was gently mixed with 90 mL of sterile normal saline in a sterile bag and homogenized in a laboratory stomacher (Easy Mix, AES Chemunex, France) for 120 s. For *E. coli* bacteria, the cultured tryptone bile x-glucuronide (TBX) petri dishes were incubated for 1 day at 35°C. Aerobic mesophilic bacteria were recorded using plate count agar (PCA) incubated at 38°C for 2 days.

SPSS statistical software (Version 21, Chicago, IL, USA) was utilized to analyze the data. Descriptive statistics and a T-test were used to analyze the data. Paired-Samples T-Test with a significance level of $p<0.05$ was applied to evaluate the microbial status of vegetables during various processing steps.

Results

The results of aerobic mesophilic microorganisms during the processing steps were shown in Table 1. There was a significant difference in the processing stages, and in the crushing-packaging stage ($p=0.04$). Aerobic mesophilic microorganisms decreased by 0.35, 0.69, 0.23, 0.79 and 0.82 log cfu g^{-1} in water washing, detergent washing, chlorination, ozonation and centrifugation stages, respectively. Also, in the crushing and packing stages, the log cfu g^{-1} increased by 0.51 and 0.34, respectively.

Table 2 shows the results for *E. coli* during the leek processing. There was a significant difference between the processing steps in relation to the ozonization-centrifugation step ($p=0.032$). The *E. coli* count showed a decrease in the stages of washing with water, washing with detergent, chlorination, ozonization and centrifugation as 0.07, 0.69, 0.38, 0.02 and 1.1 log cfu g^{-1}, respectively. A significant reduction was reported in the centrifugation step. In the crushing and packaging stages, the number of *E. coli* increased by 1.09 and 1.78 log cfu g^{-1}, respectively.

Table 3 shows the results in relation to the aerobic mesophilic microorganisms during sampling of water tanks in the plant in the leek vegetables. The effect of detergent and disinfectants was shown to be identical and no significant difference was noticed

Table 1: Results for aerobic mesophilic bacteria during the leek processing steps in the plant.

Stages	Mean difference (CFU/g)	Standard deviation	Significance level	Assurance distance 95%	
				Low limit	High limit
Raw vegetable stage-water washing stage	0.35	1.31	0.68	-2.90	3.61
Washing stage with water-Washing stage with detergent	0.69	0.69	0.22	-1.02	2.40
Detergent washing step-chlorination stage	-0.23	0.56	0.55	-1.63	1.17
Chlorination stage-ozonization stage	-0.79	0.44	0.08	-1.89	0.29
Ozonization-centrifugation stage	0.82	1.43	0.42	-2.74	4.39
Centrifugation stage-chopping stage	-0.50	1.81	0.67	-5	3.99
Chopping stage-packing stage	-0.32	0.12	0.04	-0.64	0.03

Table 2: Results for *E. coli* bacteria during the leek processing steps in the plant.

Stages	Mean difference (CFU/g)	Standard deviation	Significance level	Assurance distance 95%	
				Low limit	High limit
Raw vegetable stage-water washing stage	0.06	2.90	0.97	-7.13	7.27
Washing stage with water-Washing stage with detergent	-0.69	1.29	0.45	-3.89	2.51
Detergent washing step-chlorination stage	-0.38	1.97	0.77	-5.28	4.52
Chlorination stage-ozonization stage	-0.02	0.38	0.92	-0.98	0.94
Ozonization-centrifugation stage	1.10	0.34	0.03	0.23	1.96
Centrifugation stage-chopping stage	-1.08	2.22	0.48	-6.60	4.43
Chopping stage-packing stage	1.77	1.54	0.18	-2.07	5.62

Table 3: Results of aerobic mesophilic bacteria and *E.coli* during sampling of the water tanks in the plant.

Stages	Mean difference (CFU/g)	Standard deviation	Significance level	Assurance distance 95%	
				Low limit	High limit
Washing tank-detergent tank (aerobic mesophilic bacteria)	0.21	0.17	0.17	-0.22	0.64
Detergent tank-chlorine tank (aerobic mesophilic bacteria)	0.69	1.98	0.60	-4.23	5.62
Chlorine tank-ozone tank (aerobic mesophilic bacteria)	0.25	2.42	0.87	-5.77	6.28
Washing tank-detergent tank (*E. coli*)	0.18	0.99	0.78	-2.29	2.66
Detergent tank-chlorine tank (*E. coli*)	0.09	0.92	0.87	-2.20	2.38
Chlorine tank-ozone tank (*E. coli*)	0.71	0.67	0.21	-0.97	2.39

between the water tanks in the plant for the leek. The results for *E. coli* were similar to the findings of aerobic mesophilic microorganisms (Table 3).

In Table 4, the results of comparison for aerobic mesophilic bacteria in raw vegetables with process vegetables showed no significant difference between raw leek and any of the processing steps; demonstrating that washing and processing steps had little effect on reduction of aerobic mesophilic microorganisms.

The results of comparing *E. coli* in raw vegetables and leek processing stages in the plant showed no significant difference between *E. coli* in raw vegetables-centrifuged vegetables, raw vegetables-chopping vegetables and raw vegetables-packaging vegetables (Table 5).

Discussion

In this study, leek was collected from ready-to-eat vegetables in the processing plant during various processing stages from vegetables entrance to the plant to the packaging stage. The researchers have shown that in the crude vegetable plant, aerobic and *E. coli* microorganisms were 6.40 and 1 log cfu g^{-1} in the leeks, respectively. In the study undertaken by Aycicek *et al.*, *E. coli* contained 3.8 log cfu g^{-1} in the raw product of parsley and dill (9-11). Also, in Cardamone's study, aerobic mesophilic

Table 4: Results for comparison of aerobic mesophilic bacteria in raw vegetables and the final product.

Stages	Mean difference (CFU/g)	Standard deviation	Significance level	Assurance distance 95%	
				Low limit	High limit
Raw vegetable stage-centrifuge stage	0.84	0.91	0.25	-1.43	3.12
Raw vegetable stage-chopping stage	0.33	1.18	0.67	-2.61	3.28
Raw vegetable stage-packaging stage	0.00	1.14	0.99	-2.84	2.82

Table 5: Results for comparison of *E. coli* in raw vegetables and the final product.

Stages	Mean difference (CFU/g)	Standard deviation	Significance level	Assurance distance 95%	
				Low limit	High limit
Raw vegetable stage-centrifuge stage	0.07	1.41	0.93	-3.43	3.57
Raw vegetable stage-chopping stage	-1.01	3.51	0.66	-9.73	7.71
Raw vegetable stage-packaging stage	0.76	1.96	0.56	-4.11	5.64

microorganisms in fresh vegetables at the point of sale were reported 2 to 7 log cfu g^{-1} (12). According to a study by Zare-Jeddi *et al.* regarding the microbial status of raw vegetables, aerobic mesophilic microorganisms were between 4 and 8 log cfu g^{-1}, coliforms were between 0.6 and 6 log cfu g^{-1}, and *Listeria monocytogenes*, Salmonella and *E. coli* O157:H7 were rarely found (2).

Among the factors affecting the microbial quality of raw vegetables in different countries, the observance and non-observance of GAP (Good Agricultural Practices) principles, planting conditions, water quality used in agriculture, type of fertilizer used, transportation conditions and supply of vegetables were reported (13). Also, the temperature of the vegetables entering the plant was very important. It must enter the production process immediately; otherwise, it would be kept at a temperature of 5°C. In this plant, there was no storage for pre-cooling of raw vegetables, and this can increase the microbial load of the raw vegetables before entrance to the production process. According to Abadias *et al.*, findings, vegetables were shown not to be free of microbial agents and the processing of freshly cut products such as transportation, washing, slicing, storage and distribution were also potential sources of contamination in these products and increased the microbial load of vegetables freshly cut (1). In the present study, the washout phase with water decreased aerobic mesophilic microorganisms by 0.35 log cfu g^{-1} and *E. coli* by 0.07 log cfu g^{-1}.

In the study of Yarahmadi *et al.*, aerobic mesophilic microorganisms in water washing stage decreased to 0.67 log cfu g^{-1} (13). Also, according to Adams *et al.* and the study of Frank and Seo, washing vegetables with water reduced only 1 logarithmic cycle of the microbial load of vegetables (14, 15). Washing fruits and vegetables in water and rinsing them were shown to help elimination of microorganisms (8). However, in a studied plant, this process was not very effective in reducing the microbial load of leek. According to Gil *et al.*, the water used in the process was shown to be a potential source of cross-contamination with fecal index microorganisms and human intestinal pathogens (16). In a study by Holvoet *et al.*, It was found that when lettuce contaminated with *E. coli* was in the wash tank, *E. coli* was transmitted from lettuce to water. Therefore, the use of disinfectants in the washing system in ready-to-use vegetable processing plants is a necessity (17). We showed in the washing process of leek with detergent in the plant, aerobic mesophilic microorganisms decreased by 0.69 log cfu g^{-1} and *E. coli* increased by 0.69 log cfu g^{-1}.

Barak *et al.* used Bacdown hand soap in vegetable processing, which reduced 1 logarithmic cycle of *Salmonella enterica* (18). In Yarahmadi's study, washing with detergent reduced fecal coliforms by 1.9 log cfu g^{-1} (13). *E. coli* and aerobic mesophilic bacteria increased in leek after chlorination to 0.23 and 0.38 log cfu g^{-1}, respectively. Also in a study by Nascimento *et al.*, a concentration of 200 ppm sodium hypochlorite in lettuce during 10 minutes treatment reduced aerobic mesophilic bacteria, mold and yeast by 2.5-3 log cfu g^{-1}, as well as less than 2 log cfu g^{-1} for coliforms (19). In a study by Niemira *et al.*, sodium hypochlorite at 300 ppm and 600 ppm decreased *E. coli* O157:H7 in lettuce and spinach by 0.5 log cfu g^{-1} (20). Chlorine compounds are widely used in the food industry for disinfection and to decline bacterial load, while the optimal pH is considered 6.5-7; and at 4°C has the greatest effect and the chlorine concentration for a product of 50-200 ppm is 1-2 minutes (3).

Chlorination is a critical point in a plant. If the proper concentration and time are not utilized, it can have little effect on reducing the microbial load of vegetables. Ozonization in leek can increase the aerobic mesophilic microorganisms by 0.79 log cfu g^{-1} and increase the *E. coli* by 0.02 log cfu g^{-1}. In a study by Yuk *et al.*, 5 ppm ozone for 5 minutes in lettuce decreased the *E. coli* O157:H7 by 1.09, while was ineffective for *L. monocytogenes* (21). Ozone decomposes rapidly at room temperature and is converted to oxygen (3). Unfortunately in this plant, ozonization was done at room temperature and in conditions where workers were exposed to it, and this induced tissue injuries to the respiratory system on one hand, and on the other hand caused a bad smell and an eye irritation. So the workers turned off the ozone generator, and as a result, the appropriate dose was not injected into the vegetables emphasizing the critical role of ozonization in the plant (3).

In the centrifugation stage of leek, aerobic mesophilic microorganisms decreased by 0.82 log cfu g^{-1} and *E. coli* by 1.1 log cfu g^{-1}, which was significant for *E. coli* population (p=0.032). In a study by Castro-Ibanez *et al.*, Salmonella was found in two centrifuged water samples (3). Unfortunately, the washing and disinfection of the baskets inside the centrifuges were not scientifically and correctly carried out, and this can affect the microbial load of the product. Therefore, this stage was considered a critical point too. In the crushing stage, aerobic mesophilic and *E. coli* microorganisms increased by 0.51 and 1.09 log cfu g^{-1}, respectively. Several researchers have reported that cutting tools, peelers and centrifuges can be contaminated and cause secondary contamination in products during the production and distribution lines (2); because at this stage, the processing of vegetables is not well conducted and an increase in the microbial load of vegetables can occur, denoting to the critical role of this stage. In a Bahreini *et al.*'s study, aerobic mesophilic microorganisms in vegetables and lettuce and lactic acid bacteria in lettuce increased during the crushing, drying and packaging stages (22).

Conclusion

The processing steps in this plant did not have much effect on reduction of *E. coli* and aerobic mesophilic bacteria that was higher than the standard limit of Iran for freshly chopped and packaged mixed vegetables. Therefore, it is recommended to use a proper washing system, to utilize suitable disinfectants, to observe workers' personal hygiene, and to clean the equipment used for processing of vegetables.

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Conflict of Interest

None declared.

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