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Assessment of the efficiency of ozone application for post-harvest microbial disinfection of some fruits and vegetables

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Abstract

The treatment of ozone is widely used for post-harvest fruit and vegetable processing because of its high activity and safety. As opposed to the gaseous form, the aqueous form is more active. The technique and commodities are major factors in determining the ozone efficiency ratio. Fresh products are a source of pathogen organisms such as *Fecal coliform* (FC) that are considered a threat to quality and health. This study applied to evaluate the quality and safety of some fruits such (as apples and oranges) with some fruit vegetables like (tomatoes, bell peppers, eggplant, cucumber, and squash), as well as some vegetables such as (lettuce, and arugula) by detection ratio of FC microorganisms. These classes of products were exposed to (3 mg/L) aqueous ozone for 5 minutes. Overall, these concentrations improved entire products. Ozon application lowered the overall activity of contaminants about 25 times from 2792.26 to 105.96 RLU/cm^2 after processing. Most of the commodities as eggplant, lettuce, apples, and orange were disinfected and other products significantly decreased. The system is capable of inactivating about 6935.3 relative light units (RLU/cm^2) of FC in an ideal situation.

Keywords: aqueous ozone, post-harvest, fruit and vegetable, fecal coliform, Relative Light Unit (RLU).

Introduction

Currently, fruits and vegetables comprise a sizable portion of the food industry and play a significant role in daily diets [1]. They are undoubtedly necessary for a nutritious and well-balanced diet because of their high nutritional content. However, because of their short shelf life, they have been linked to a significant number of outbreaks of foodborne illnesses [2]. Since most fruits and vegetables are eaten raw or with very little processing (such as in salads that are ready to eat), microbiological safety becomes crucial to reducing the dangers to consumers [3,4]. It is necessary to recognize that surfaces need to be disinfected while preparing fruits and vegetables. According to Mendoza *et al.* (2010) and Perera (2020), microbial deterioration causes around 30% of fresh items to be lost after harvest [5,6].

Numerous therapeutic modalities have been investigated, yielding diverse and noteworthy outcomes. Consequently, there is a great deal of interest in substitute sanitizing agents that are safe yet still effective. Ozone has a strong oxidant capacity and works against a far , more comprehensive range of germs than other disinfectants. Therefore, using it as an alternative might be one of these. In addition to having antibacterial properties, ozone may eliminate chemical residues and pesticides, as well as change non-biodegradable organic molecules into ones that can break down naturally [7,8,9]. Ozone is a potent disinfectant that could satisfy producers' demands, regulatory bodies' approval, and customer acceptability. In the United States, ozone was classified as Generally Recognized as Safe (GRAS) in 1997 for direct food contact and in 1995 for the disinfection of bottled water.

Additionally, the US Food and Drug Administration (FDA) authorized ozone in both gas and aqueous phases as an antibacterial agent for direct food contact. Furthermore, ANSES provided technological assistance for the cleaning of prepared salads using ozone in water [10]. Ozone may be administered in two different ways throughout the vegetable handling process: gaseous and aqueous [11]. Aqueous ozone is introduced just after the vegetable harvest or during the washing process. In the latter instance, the product can be sprayed, rinsed, or dipped in water that has dissolved ozone to wash it [12,13].

Ozone has been shown to have antibacterial effects on a variety of species, including spores, vegetative cells, and both Gram-positive and Gram-negative bacteria [14]. Through catalytic oxidation, ozone breaks down the structure of proteins and oligosaccharides in bacteria, viruses, protozoa, and insects. Ozone oxidizes bacterial cell wall components because of its great propensity for oxidation. Numerous components of bacteria, including proteins, unsaturated lipids, respiratory enzymes in cell membranes, peptidoglycans, enzymes, and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coatings and viral capsids, have all been demonstrated to be attacked by ozone. Ozone has been shown to kill bacteria by lysis mechanism by attacking unsaturated membrane lipid double bonds, sulfhydryl groups of membrane-bound enzymes, glycoproteins, and glycolipids [15,16].

Gram-positive bacteria are characterized by thicker peptidoglycan and more hydrophilic walls, whereas coliform bacteria are characterized by thin peptidoglycan lamella coated by an outer membrane composed of lipoproteins and polysaccharides. The gradual oxidation and resulting destruction of the cell envelope's unsaturated lipids is how ozone inactivates vegetative bacteria. A significant portion of the membrane barrier is broken, which causes the cell to become disrupted, allowing cellular contents to flow out and bacteria to lyse. While this is insufficient to cause the rapid death of the cell, ozone can enter the bacteria and oxidize some of its vital components, such as proteins, nucleic acids, and enzymes [13,14,15].

According to the ministry of Planning and the ministry of Agriculture and water resource in 2019 [16], 25-33% of crop losses in KRI was due to insufficient proper storage mechanisms. They mentioned that 70% and 83% of local products was stored in traditional methods for vegetables and fruit, respectively. In facts 90% of the farmers need to store their crops for specific periods before selling them. There is no attempt to disinfect their products before storage. Additionally, disease bacteria, particularly Fecal Coliform (FC) and *E. Coli*, can be considered as source of contamination in fruit and vegetables. Therefore, it is crucial to maintain or increase the microbiological quality of commodities by disinfection. The study set out to assess the effectiveness of ozone application in aqueous phases for post-harvest sanitization and the mitigation of fecal coliform in order to increase further shelf life and safety at the same time that poses a concern to some fresh fruits and vegetables.

Material and Methods

This study was carried out in the microbiology lab of "Hamsa Company for quality control of fruit and vegetables" in Hwana stores/ Sulaimani.

Sample collection

Nine types of top-quality fruit (orange and apple), fruit-vegetable (Tomato, bell peppers, cucumber, eggplant and squash), and vegetables (lettuce and arugula (*Eruca sativa*)) with five replications were selected and collected from "Tamata fresh" company for sanitizing fruit and vegetables. Tamata fresh company import their commodities form local greenhouses at "Bazyan" from farmers which normally 'don't use sewage water for irrigation. Vegetable and fruit-vegetables were harvested at midnight and transferred to industry as soon as possible in order to disinfection process. Fruits sources are from store-keepers who store fruits in cold rooms.

Ozone processing

Disinfection process is based on using aqueous ozonation. The subsequent system briefly consists of: the elimination of low-quality products, cleaning, primary two low turbidity pools with 5 NTU (Nephelometric Turbidity Unit) for prewashing, shower washing, continuous bubbling ozone pool (3 mg/L for 5 minutes), centrifuge drying and automated packaging. Ozone concentration was continuously monitored from the tank and the chamber, respectively, through an ultraviolet absorption ozone analyzer.

Microbial analysis

Untreated and treated samoles transferred to and analyzed in the Microbiology lab of Hamsa Company. Fecal coliform tests were applied using the swab method using MicroSnap instruments (AOAC certified, 2013: license no. 071302). It was applied using a coliform/ *E. coli* enrichment swab covering 100 cm^2 of surface per sample and then incubated at 45 °C for 7 hours. Then 0.1 ml of enrichment was transferred to coliform detection kit and incubated for 10 min. Finally, it was measured and recorded using the Ensure Touch instrument. Results are presented in relative light unit (RLU/cm^2) [17] and then inactivation efficiency percentage was measured.

Statistical analysis

Recorded data statistically analyzed using XLSTAT software (2019.2.2.59614), Factorial-CRD for three replicates and mean comparison was conducted by Duncan test at level ($P \leq 0.05$).

Results and Discussion

At the farms to the consumption, microbial contamination of fruit and vegetables can happen at different times during field growth, harvesting, post-harvest handling and transportation, storage, processing, and marketing for human consumption [18,19].

Recorded data shows significant differences in level of contamination of the commodities ($P \leq 0.05$). The average of FC (RLU/cm^2) contamination for dependent fruit and vegetables before ozonation is shown in (Figure 1). With respect to the variation of sample class, leafy vegetables, fruit vegetables, and fruits were ranked from highest to lowest level of contamination by FC respectively. Arugula and squash loaded the highest level of FC with 6935.3 and 6612.0 RLU/cm^{2,} respectively. In contrast, apples and oranges recorded the lowest level of detection, with only 29.0 and 705.3 RLU/cm^2, respectively. There many factors that effect on microbial load in fruit and vegetables but mainly it related to nature and position of products. Nature of fruits differs from vegetables. Vegetables has higher pH than fruits that makes more suitable environment to growth microbes, therefore vegetables are more susceptible to bacterial deterioration [15,20]. Besides, leafy- and fruit-vegetable attached to soil and therefore they are more susceptible to contamination by bacteria when compared to fruits that are suspended in air and are away from direct contact to soil and it contents.

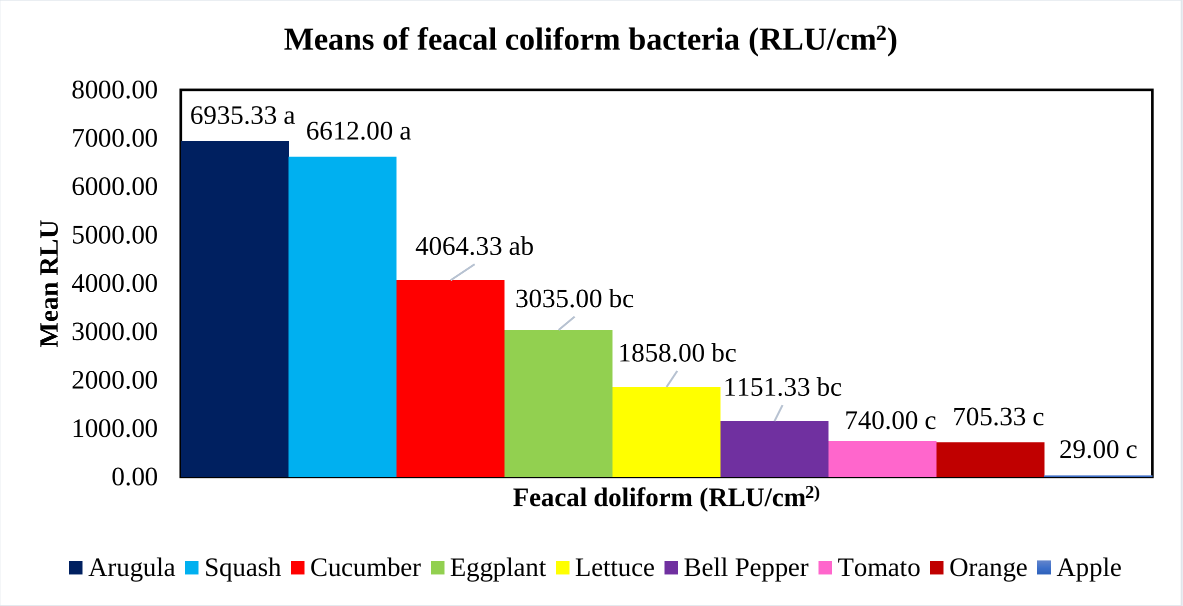

Figure (1): Mean of feacal coliform of some untreated fruits and vegetables.

The primary source of FC contamination refers to field. Major sources in fields come from polluted water (sewage water) and manures (organic fertilizer) during planting and irrigation process [21]. Therefore, its logical that FC less contaminates fruits whereas major spoilage of fruits are birds and during harvesting and handling process. In a study to determine level coliform contamination of some crops, it resulted that the consequence of crops to contaminated by coliform bacteria are leafy vegetables, fruit, and fruits [22].

In the past, ozone treatment in the fruit and vegetable processing sector has been used to gaseously treat or wash all fruits and vegetables with ozone-containing water before or during storage in order to decontaminate their surfaces and increase shelflife [23]. The majority of bacteria found on the surface of plants are usually Gram-negative. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 10^4 to 10^8 per gram [24].

The total mean impact of ozone on the reduction of FC (RLU/cm^2) is shown in (Figure 2). Results showed significant differences ($P \leq 0.05$) of FC activity among the entire samples during the study. Ozone significantly affected the inactivation of bacteria and lowered level to the acceptable range. Total average of FC in raw samples (before treatment) was 2792.26 RLU/cm^2 which decreased to 105.96 RLU/cm^2 after processing. Total efficiency of system to inactivation FC was 96.2%. The ability of ozone to disinfection differs by applied methods, type of food and type of bacteria. Aqueous ozone was shown to have strong inhibitory effects against aerobic bacteria, coliforms, and yeasts during storage [25].

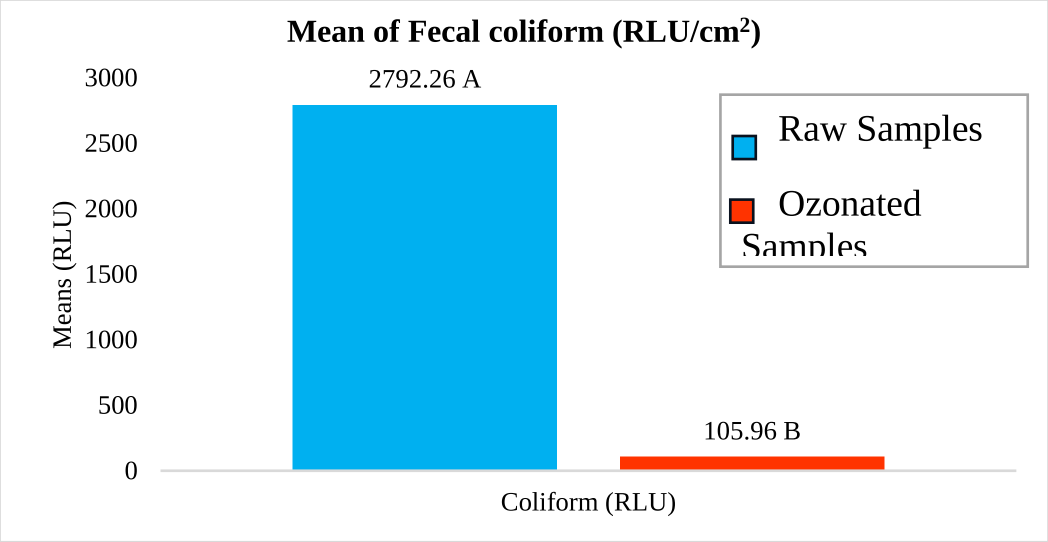

Figure (2): Mean of total fecal coliform bacteria (RLU/cm^2) of raw and ozonated (3 mg/L for 5 min.) commodities.

In aqueous conditions, ozone typically reduces viable cell counts by 5-7 $\log_{10}$ when organic matter is not present [26]. Mustapha *et al.* [27] claimed that if a washing procedure could result in a 2-log decrease or more significant, its efficacy would be noteworthy. Washing lettuce leaves in 10 and 20 mg/L ozonated water for three to five minutes at 4 or 8 °C resulted in a 3 log CFU/g reduction in coliforms [28].

Based on the components of its cell wall, the kind of microbe greatly influences its resistance to ozone therapy. Ozone has more ability to destroy gram-negative bacteria as a coliform group compared to gram-positive groups. The reason is related to variation level of peptidoglycan and lipid chain in cell of bacteria. Ozone can penetrate lipid chains more than peptidoglycan, which is dominant in gram-negative bacteria. Therefore, compared to gram-positive bacteria, coliform bacteria exhibit less resistance to ozone exposure [15,16,29]. Patil *et al*. [30] provided evidence that the bacterial membrane, which includes both lipid and protein components, was the primary target of ozone toxicity in *E. Coli* cells.

In an ideal environment, bacteria can survive and cause problems even if their exposure to ozone is brief and does not damage their cell walls. However, extended exposure to ozone damaged intracellular proteins and DNA, resulting in a reduction in the survival of *E. Coli* cells [29,31].

As this ozonation system mentioned previously, if time and concentration are regarded, these processing techniques meet the requirement of the ideal ozonation system that is explained by Gil *et al*. [32]. They claimed that a shower is a necessary prewashing step in an efficient ozone-washing system to get rid of debris and cell exudates from the cut surfaces.

Submerging the product in a washing tank containing ozone as a cleaning agent comes next [32]. Improved ozone dispersion and nearly consistent ozone concentration during processing are achieved with continuous ozone treatment, leading to higher processing efficiency. Ozone gets more concentrated in the liquid layer that develops at the ozone gas/water contact when it is bubbled into water, as detailed by Karaca and Velioglu [33]. As a result, when the infected lettuce came in contact with ozone bubbles, more microbial inactivation of *E. coli* was obtained (1.97 log CFU/g reduction) after 2 min, as opposed to dipping into the bulk liquid (1.17 log CFU/g reduction) [34]. According to Alexopoulos *et al*. [35], bubble-washing lettuce reduced the number of coliforms by 2.2/2.47 log CFU/g when 0.5 mg/L ozone was applied continuously in distilled water at 15–17 °C for 5–30 minutes.

Studies have also looked at using ozonated water delivered via fine and ultra-fine bubbles with intense mechanical action to wash apples and fresh vegetables. The mean size of particles of tiny bubbles was shown to decrease with a rise in the intake of ozone dosage. This resulted in a much greater interfacial area per unit volume, which in turn increased ozone diffusion over the target material [36,37]. According to Achen and Yousef [38], *E. coli* levels were reduced by 3.7 log and 2.6 log, respectively, as an outcome of bubbling and dipping.

Furthermore, bubble cleaning is helpful in difficult-to-treat areas that ozone finds difficult to penetrate. According to research, the apple's stem-calyx area fell by 0.6 CFU/g after bubble washing, but the dip approach only reduced the CFU/g by 0.5 logs. This was explained by the injected bacteria adhering to the rough surfaces of the stem-calyx area or by the microorganisms in this location being resistant to the sanitizer's activity [39].

In addition, studies have shown that, in comparison to greater levels of ozone, the dynamic bubbling approach using a modest dose of ozone has the same disinfecting effect. In their investigation of 3, 5, and 10 mg/ L, Koseki and Isobe [40] found that when treatment reached levels more than 3 mg/ L, there was no discernible difference in the decrease of microbial load and the log of bacteria was 3 mg/ L. Selma *et al*. [41] showed comparable outcomes at the same period.

Ozone process had a significant impact on sanitation products ($P \leq 0.05$). Average differences between microbial load before and after ozonation of FC (RLU/cm^2) for depended variable are shown in (Figure 3) that can be mentiond as evaluation of efficiency of system. Most variation of living cells was recorded for arugula and less was for apple with 3752.33 and 14.5 (RLU/cm^2) respectively. The initial microbiological load and the contaminating microorganisms on the items are additional parameters that need to be taken into account. The higher the microbial load, the potential decrease in O_3 efficacy. This is because the gas destroys microflora that competes with it, but it also increases the number of aerobic microbes since ozone is constantly breaking down into O_2 [42]. The surface and the properties of the microorganisms (type, contamination load, and degree of adhesion) have a major impact on the inactivation of food microorganisms by ozone [42].

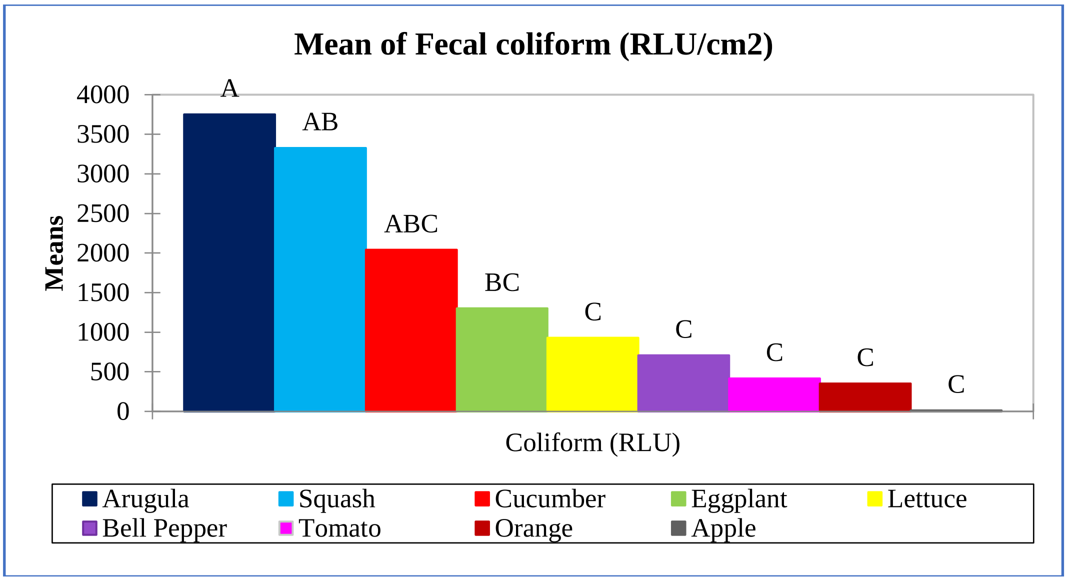

Figure (3): Mean differences of fecal coliform before and after ozonation (3 mg/L for 5 min.) for some fruits and vegetables.

There was a sharp reduction in bacterial loads in all commodities (Table 1). FC of raw treatments ranged from 6935.3 RLU/cm^2 for arugula as maximum value into 29.0 RLU/cm^2 for apple as minimum value. After the ozonation process, these values lowered to 569.33 and 0.0 RLU/cm^2 respectively.

In most products (lettuce, eggplant, orange, and apple), their loads released to non-detectable levels and treated samples were totally disinfected. Surfaces played vital role in the process. In general, all studied treatments contained smooth surfaces that helped to ease of deattach of microbes and representing them to bombarding ozone.

The efficiency of ozone could inactivate 6935.3 RLU/cm^2 load of FC (for arugula as mentioned maximum mean value) by studied process with 3 mg/ L for 5 min. Regarding to mentioned level, during study more load of bacterias (more than 16000 RLU/cm^2) was found for some treatments before ozonaion that they wholly disinfected by ozone. They ignored bio-statistically to it was considered as 'outlaws' data.

Regarding to arugula and squash that, they had the highest level of FC load, but ozone efficiency to disinfection was different in about 8%. The FC loss of arugula was 91.8% while it was 99.3% for squash. Untreated arugula and squash contained 6935.3 and 6612.0 RLU/cm^2, respectively, which decreased to 569.3 and 45.7 RLU/cm^2 after ozonation. This clearly demonstrated that the inactivation kinetics of ozone is different with fruit and vegetable, as well as in the same class of commodity. This may refer to surface and shape nature of products as some products have smooth surface and some have irregular and pose surfaces [44].

Table (1):Inactivation ratio and efficiency of fecal coliform by ozone (3 mg/L for 5 min.)

Type	Fecal Coliform (RLU/cm^2)		Inactivation Efficiency (%)
	Raw	Processed (Ozone)	
Arugula	6935.3 ^{a}	569.3 ^{c}	91.8
Squash	6612.0 ^{a}	45.7 ^{c}	99.3
Cucumber	4064.3 ^{ab}	15.7 ^{c}	99.6
Eggplant	3035.0 ^{bc}	ND* ^{c}	100
Lettuce	1858.0 ^{bc}	ND ^{c}	100
Bell Pepper	1151.3 ^{bc}	265.7 ^{c}	76.9
Tomato	740.0 ^{c}	92.7 ^{c}	87.5
Orange	705.3 ^{c}	ND ^{c}	100
Apple	29.0 ^{c}	ND ^{c}	100

*Not detected (ND)

The surface of squash is waxy and smoother than arugula. Either true for lettuce and arugula as they are in same class (vegetable). These attributes encourage good load of microbes easily detach from smooth surfaces during washing steps (pool and shower washing) before applying ozone in comparison with irregular ones. Das *et al.* [45] resulted in washing fruit and vegetables with tape and filtered water capable of removing 0.85 to 2.05 and 2.38 into 3.36 log bacteria from the surface respectivelly.

Study confirmed when infected whole and sliced apples were washed with tap water simultaneously, the populations of *E. coli* were reduced by around 1 log. Therefore, for items with smooth and unbroken surfaces, including apples, tomatoes, and green peppers, the application of aqueous ozone showed remarkable outcomes with a low ozone demand [46,35]. These items make it simple for the sanitizer to get in close touch with the germs. Microbes to be able to separate from plant tissue with ease.

Microbial inactivation becomes more difficult when the surface is more complex in terms of porosity and roughness, as on arugula, for example, [10,47,48]. According to Tzortzakis and Chrysargyris [15], microorganisms attached to surface irregularities are more protected from ozone than those that are readily exposed. For instance, lettuce and endives are protected by folds and layers that may reduce the impact of ozone for microbial control because it could be more difficult for ozone to reach the microbes. With Salmonella, Kroupitski *et al.*[49] also noted the same outcome. The bulk of cells were found in the cut-edge areas, preferring the injured tissue, and these cells had adhered to the cuticle of the undamaged leaf surface [10].

Regardless of the length of exposure (0.5 to 15 minutes), washing with 3 and 5 mg/L ozonated water decreased the *E. coli* O157:H7 counts in both iceberg and romaine-infected lettuce by around 1 log unit. In order to compare them with unwashed samples, freshly cut spinach leaves were submerged in ozonated water (5 ppm) for 3 minutes at room temperature [50]. Exposure to O_3 significantly reduced the populations of *E. coli* O157:H7 in inoculation leaves by 1.22 log units as compared to the untreated control (12).

Among the same category of fruit vegetables, limited inactivation was found. For instance, cucumber had relatively the same smoothness as tomato and bell pepper, but ozone activity was different regarding to load of FC. Untreated cucumber loaded more bacteria with ratio of 3 and 5 fold over bell pepper and tomatoes, respectively, while the ozonation process eliminated almost entire FC on cucumber and only 87.5 and 76.9% for tomatoes and bell pepper, respectively. Besides the irregularity of surface, the reason may be explained by the arbitrary action of ozone towards bacteria related to attachment degree and age of colonies as a result of limited penetration. When germs stick to fruits or vegetables, the ozone's oxidizing qualities lose some of its effectiveness. It was discovered that the microbes' adhesion to the food product decreased their viability by 1 $\log_{10}$ in comparison to the control scenario [26]. Moreover, new research by Wani *et al*. [51] indicates that ozone resistance may result from both the kind of microbe and colony age, with older colonies becoming more resistant to ozone and perhaps having a stronger attachment to the product. Older colonies (7, 10, and 12 days old) of Pseudomonas spp. cells showed greater resistance to gaseous ozone than did cells. Cells showed greater resistance to gaseous ozone than younger colonies (2 and 4 days old).

Size and shape of treatments also has major role in elimination and lowering loads of bacteria by ozone. Small size commodities are heavy to disinfect by ozone. This is true when size character choose as comparion for treatments. For example, leafy arugula has samller size than squash, but its activation was less than squash (91.8 and 99.3% disinfection respectively). Similar results was found bt Inatsu *et al*. [52], which they reported that after water ozone disinfection of lettuce and spinach, total coliform vaiable cells are 3.8 and 5.2 log CFU/g respectively. Aqueous ozone is not particularly effective in cleaning cherry tomatoes, as reported by Mustapha *et al*. [27], who found a minimal reduction in mesophilic bacteria, yeasts, and molds (< 1 log CFU/g). This may related to heavy exposure of entire surface to ozone particles especially when irregularity and hidden points are present on the surface. Rotation and flipping of arugula and squashe may be another reason for insufficient inactivation. Squashes are easily rotate in dynamic pools than flat leafy vegetables. Venta *et al*. [53] insisted to ensure that the tomatoes' whole surface is exposed to the dissolved ozone. They should be rotated inside the water. The effectiveness of this movement is also dependent on the rinsing system. The industrial facilities should take these factors into account.

Data clearly showed that shape has important role on bacterial population. As well as it can impact on efficiency of ozone. Long shape treatments as squash, cucumber and eggplants totally cleared from bacterial load. In contrast, round shape fruit vegetable as tomato and bellpeppers contained much remained bacteria (87.5 and 76.9% respectively). Significant differences have been observed in the weight/surface area (g/cm^2) among several product varieties, including tomatoes (spheres) and lettuce (two-sided planes) [54]. According to these authors, a decontamination procedure intended to, for instance, produce a 3-log decrease in CFU/g of tomato or lettuce would provide, in turn, around 0.114- and 18-log decreases in CFU/cm^2 [32]. This may be refers to represented area of treatments to bombarded ozone. Also it may be related to temperature of water during processing. In several investigations, ozone application at higher temperatures resulted in greater efficiency. For instance, in tomatoes, ozone application at 50 °C (4.14 log reduction) considerably reduced *S. enterica* compared to 4 °C (2.54 log reduction) [48].

Ozone, which is recognized as an alternative sanitizer, has taken the place of all conventional sanitizing agents in the surface cleaning of fresh horticultural produce. The effectiveness of aqueous ozone to reduce viable bacterial cells of FC surface-attached bacteria on nine fruits and vegetables tested. We concluded from these results that the use of aqueous ozone can provide limited effectiveness for the increasing shelf life and sanitizing of fruit and vegetable surfaces and prevent cross-contamination via washing. It is possible to use lower ozone dosage in aqueous form to inactivate of fruit and vegetables, especially those with regular shape and surface.

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