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Chlorine and ozone applications used for fruit and vegetable disinfection in tourism accommodation facilities

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ABSTRACT

Samples of vegetable and fruit were taken from the kitchens of tourism accommodation facilities and microbiological analyses were made. The samples were analysed after being disinfected with 100 ppm chlorine for 2-10 minutes and with 2 ppm ozone for 2-10 minutes. In the examined vegetables and fruit samples, *Escherichia coli*, *E. coli* O157, *Salmonella* spp., *Listeria monocytogenes* were examined in terms of microorganisms. Before disinfection, *E. coli* was detected in all 30 samples: *E. coli* O157 in 8 samples, *Salmonella* spp. in 4 samples, and *L. monocytogenes* in 3 samples. As a result of disinfection applications with 100 ppm chlorine for 2 minutes, *E. coli* decreased by more than 45%. *Salmonella* spp. was detected in 1 sample and *E. coli* O157 was detected in 2 samples. After disinfection applications with 2 ppm ozone for 2 minutes, *E. coli* decreased by more than 60%. A decrease of more than 70% was observed in *E. coli.* after disinfection applications with 100 ppm chlorine for 5 minutes. As a result of disinfection applications with 2 ppm ozone for 5 minutes, *E. coli* decreased by more than 85%. No bacteria were found in the samples after disinfection with 100 ppm chlorine and 2 ppm ozone for 10 minutes. It was observed that there was a decrease in the microorganism loads as a result of the chlorination and ozonation processes of the vegetable and fruit samples.

1. Introduction

A healthy life and nutrition are among peoples most basic needs. The main problem in food products in developing countries is the inability to disinfect foods properly and effectively. In order to protect food and ensure its hygiene and sanitation, it is necessary to protect raw materials and water, to prevent cross-contamination of cooked and raw foods, to hold the storage conditions at the appropriate temperature, to cook the food at the appropriate time and temperature, to protect the food from the pollution of humans, animals and parasites (Gökçe 2011).

In recent years, in line with the increasing demands for raw fruit and vegetable products, consumers' orientation towards quality, healthy and nutritious products has increased. Therefore, disinfection processes for these food products are of great importance. Chlorine, ozone, organic acid, hydrogen peroxide, calcium oxide, and thyme water are disinfectant agents used in places of mass consumption.

Today, chlorinated compounds continue to be widely used in the food industry for the disinfection processes of fruit and vegetable in tourism accommodation facilities, especially because they are economical.

Ozone is accepted as a usable antimicrobial substance in waters by the US Food and Drug Administration (FDA) and as a substance that has generally been accepted as safe a (GRAS) status in foods. As a result of the research, it has been revealed that ozone, which can be used in gas or liquid form, and reduces the presence of disease-causing microorganisms, can quickly decompose and leave the environment when applied to food products (Gücükoğlu and Küplülü 2005).

Vegetable and fruit samples were analyzed after being subjected to chlorination and ozonation processes. Vegetable and fruit samples were examined in terms of microorganisms corresponding to washed, chopped and packaged raw vegetables and frozen or dried vegetables separately or mixed in the Turkish Food Codex Microbiological Criteria Regulation Annex-1 section and the ready-to-eat chopped fruits and vegetables groups in Annex-2 (Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği 2011).

In the light of the information obtained from the findings in the study, it was necessary to reach the source of the problems. The disinfection processes of fruits and vegetables in tourism accommodation facilities were examined and the advantages and disadvantages of disinfection agents were revealed. It is thought that the research will benefit ozonation and chlorination applications in enterprises such as places of mass consumption. The findings obtained from the study will be a prediction for the disinfection activities in vegetables and fruits to continue more effectively and well, and to eliminate the threats posed by the product groups that can be consumed raw and present them to the consumer in a healthy way.

Disinfection processes have an important place in order to prevent physical, chemical and microbiological deterioration that may occur in the chain from the production to consumption of vegetables and fruits (Aytemiş 2021).

According to studies (Erkoç 2019), diseases caused by pathogenic microorganisms, due to the consumption of vegetables and fruits, have increased in recent years. It has been observed that the increase in foodborne diseases are caused by conditions such as cross-contaminations in food products and food contact materials, sewage water used in vegetable and fruit irrigation, the land where the products are planted, and lack of personnel training.

Chlorine is one of the most preferred disinfectant agents due to its low cost and ease of use. Hypochlorite and liquid chlorine are the most commonly used chlorine forms to extend the shelf life of vegetables and fruits (Gölgeçen 2014). However, the disadvantages, such as the fact that bacteria are not sufficient to destroy spore forms of bacteria and viruses, that they can produce harmful products and that they have a high risk of harming the environment have led to a tendency towards different types of disinfection (Sevilgen 2009).

In a study conducted with chopped lettuce, which was disinfected with chlorinated water containing 100 mg l^{-1} concentration at 4°C and 47°C it was reported that lettuce washing at 47°C was more effective in the inhibition of microorganisms. As a result, the effect of chlorine activity on microorganism inactivation, depending on temperature, was observed (Delaquis et al. 1999).

In another study, iceberg and fresh broccoli vegetables were kept in a solution containing *Escherichia coli* for 1 minute and then for 2-5 minutes in a chlorine solution (50 ppm-100 ppm chlorinated water studies). As a result, it has been reported that chlorinated water causes a decrease in *E. coli* (Behrsing et al. 2000).

One of the areas where ozone applications are used the most is food product groups (Tümay 2019). Fresh vegetables and fruits are foods that are offered to the consumer without any processing. The risk of contamination is high due to mechanical damage during and after harvest. Therefore, it is important to increase the shelf life of chopped vegetables and fruits and to protect their nutritional values and sensory properties (Savaş et al. 2014).

Ozone is used as a disinfectant that can be easily adapted to processes, eliminating disease-causing microorganisms (pathogens) in food products, preventing quality losses, prolonging the shelf life of products, removing pesticide residues and mycotoxins (Tetik et al. 2006; Karaca 2010).

It is stated that very low application concentrations of ozone give very positive results in microorganism inactivation during cold chain storage in vegetables and fruits. Ozone is applied as a gas during transport or storage in the food chain. At the same time, it acts as a strong fumigation and sanitizer, protecting food products against pests, reducing the number of microorganisms on the surfaces of the products (Xu 1999).

This research was carried out with the aim of microbiological examination of disinfection processes with chlorine and ozone of vegetables and fruits offered for the consumption of the guests in the kitchens of tourism accommodation facilities.

2. Material and Method

2.1. Materials

In this study, samples of cress, lettuce, parsley, mint, arugula, dill, tomato, apple, plum, and carrot, which were offered to the consumption of guests in June, July and August of 2020 from some tourism accommodation facilities in Antalya, were used. Random selections were made for the analysis of food samples.

2.2. Methods

2.2.1. Preparation of the samples

The products were purified from coarse dust, dirt and insects by using only tap water in the kitchen. At least 700 g of these samples were taken and at least 100 grams of them were analyzed without any disinfection process.

2.2.2. Chlorination and ozonization applications

Afterwards, 300 grams of the samples were divided into 3 parts, and disinfection was carried out with 2 minutes-5 minutes-10 minutes of chlorination at 100 ppm chlorine concentration with chlorination (Diversey, South Carolina, USA). Rinsing was performed again in order to prevent chlorine from leaving residue. The remaining average of 300 grams of the samples was divided into 3 and disinfection was carried out with 2 minutes-5 minutes-10 minutes ozonation at a concentration of 2 ppm with ozonizer (Prozon, Antalya, Türkiye). No rinsing was done as ozonation does not leave any residue.

The samples were delivered to the laboratory under aseptic conditions, in the cold chain, for analysis. They were analyzed by classical methods in terms of *E. coli*, *E. coli* O157, *Salmonella* spp., and *Listeria monocytogenes.* According to the product groups of "washed, chopped and packaged raw vegetables and frozen or dried vegetables separately or mixed" in the Turkish Food Codex Microbiological Criteria Regulation Annex-1 section and "the ready-to-eat chopped fruits and vegetables" groups in Annex-2, and the validation stages were carried out (Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği 2011).

2.2.3. Escherichia coli Analysis

Ten g of the sample was homogenized with 90 ml of Maximum Recovery Diluent (Biolife 4016912). It was inoculated into 1 ml empty sterile petri dish. Approximately 15 ml of Tryptone Bile Glucuronide Agar (Himedia M1591) previously cooled in a water bath at 44-47°C was poured into each petri dish and mixed. After incubation at 44°C for 18-24 hours, blue-green colonies were counted as *E. coli* (TSE 2012).

2.2.4. Escherichia coli O157 analysis

A 25g sample was homogenized with 225 ml of Modified Tryptone-Soy *Broth* (MTSB) with Novobiocin (Merck 1092050500) under aseptic conditions. As a result of immunomagnetic separation, streaking was performed on Cefixime Tellurite Sorbitol Macconkey Agar (Biolife 4016692) and Harlequin SMAC-BCIG (Labm HAL 006) medium. It was incubated for 18-24 hours at 37°C. Colonies with clear and usually colorless yellowish-brown zones were taken for indole verification and incubated at 37°C for 18-24 hours. Petri dishes with positive indole confirmation were confirmed with the latex confirmation test (TSE 2003).

2.2.5. Analysis of Listeria monocytogenes

A 25g sample was incubated in 225 ml Half Fraser Broth (Biolife 4014952) medium at 30°C for 25±1 hour. The culture was taken with a loop from the pre-enrichment medium incubated for 25±1 hours at 30±1°C, streaked on Listeria According to Ottaviani and Agosti Agar (Biolife 4016052) and Oxford Agar (Biolife 4016002) media.

The 0.1 ml taken from the pre-enrichment medium into 10 ml second enrichment medium was transferred to a tube containing Fraser Broth (Biolife 4014952). Fraser Broth was incubated at 37°C for 24±2 hours. Streaks on ALOA and Oxford Agar media were incubated for 24±2 hours at 37°C. The culture was taken with a loop from Fraser Broth, the second pre-enrichment medium, which was incubated for 24±2 hours at 37°C, and streaked again on ALOA and Oxford Agar mediums. It was incubated in ALOA medium for 48±2 hours at 37°C and in Oxford Agar at 37°C for 24 hours. Colonies of *L. monocytogenes* on the ALOA medium were colonies with a blue-green opaque zone. On the Oxford Agar, black-brown colonies with 2-3 mm diameter blackish sunken center were *L. monocytogenes* colonies (TSE 2017a). Commercially available Microgen Listeria-ID kits were used for the confirmation test (Kemiteks Kimya 2021a).

2.2.6. Salmonella spp. analysis

A 25g sample was homogenized with 225 ml Buffered Peptone Water (Biolife 4012782) under aseptic conditions. Enrichment was done by incubating at 34-38°C for 18 hours. 0.1 ml of the pre-enriched sample was inoculated into 10 ml of Rapaport Vassiliadis Medium (Biolife 4019812) and 1 ml into tubes containing 10 ml of Muller-Kauffman Tetrathionate Novobiocin Broth (Biolife 4017452). A second enrichment was made by incubating RVS broth at 41.5°C for 24 hours and MKTTn broth at 37°C for 24 hours. At the end of the incubation, streaks were made from RVS and MKTTn onto solid media Brillant Green Phenol Red Agar (Biolife 4012552) and XLD agar (Biolife 4022082) with loops. It was incubated for 24±3 hours at 37±1°C. Typical colonies, pink-red, rarely colorless, on the BGA medium after incubation formed a red zone around them. In the XLD medium, on the other hand, they formed pink colonies with black centers (TSE 2017b). A commercially available biochemical Microgen GN A-ID panel was used for validation (Kemiteks Chemistry 2021b).

3. Result and Discussion

From the tourism accommodation facilities in Antalya province, in June, July and August of 2020, cress, lettuce, parsley, mint, arugula, dill, tomato, apple, plum, carrot, vegetable and fruit samples, including the appropriate microorganisms according to the Turkish Food Codex Microbiological Criteria Regulation Annex-1 and Annex-2, were taken into analysis before and after the disinfection process and examined (Tables 1 and 2).

Table 1 shows detected microorganisms according to samples before and after the disinfection process and Table 2 shows the number of samples detected before and after the disinfection process.

In the analyzes performed before the disinfection applications of vegetable and fruit samples, *E. coli* bacteria were detected in all 30 samples, *E. coli* O157 in 8 samples, *Salmonella* spp. in 4 samples, and *L. monocytogenes* in 3 samples. *E. coli* is known as a fecal-derived bacterium that lives in the large intestine of mammals. *E. coli* O157 is the serotype of *E. coli*, and it is a toxin-forming bacterium that lives in the human intestines like *E. coli*.

The reason why it is found in fruit and vegetable samples is that these samples, which are consumed raw, are cross-contaminated by washing with unclean washing water before reaching the consumer. Before these foods are washed, they provide a suitable environment for the reproduction of microorganisms by contacting different unhygienic products in

Table 1. Detected microorganisms according to samples before and after the disinfection process

Vegetable and fruit	**Without disinfection**	**Chlorine application (100 ppm)**			**Ozone application (2 ppm)**		
Time	-	2 min	5 min	10 min	2 min	5 min	10 min
Cress	1	0	0	0	0	0	0
Lettuce	1, 2, 4	1	0	0	0	0	0
Parsley	1, 2	0	0	0	0	0	0
Mint	1	1	0	0	0	0	0
Rocket	1, 2, 3	1, 2	0	0	1	0	0
Dill	1, 2	1	0	0	1	0	0
Tomatoes	1	0	0	0	0	0	0
Apple	1	0	0	0	0	0	0
Plum	1	0	0	0	0	0	0
Carrot	1	0	0	0	0	0	0

0: not detected, 1: *E. coli*, 2: *E. coli* O157, 3: *Salmonella* spp., 4: *L. monocytogenes.*

Table 2. Number of samples detected microorganisms according to before and after the disinfection process

Vegetable and fruit	**Without disinfection**	**Chlorine application (100 ppm)**			**Ozone application (2 ppm)**		
Time	-	2 min	5 min	10 min	2 min	5 min	10 min
E. coli	30	16	7	0	11	4	0
E. coli O157	8	2	0	0	0	0	0
Salmonella spp.	4	2	0	0	0	0	0
L. monocytogenes	3	0	0	0	0	0	0

the storage and storage areas. Waste water, soil, air, animal feed, insects, birds, mice, rodents are known as factors that are effective in the spread of *Salmonella* spp.. *L. monocytogenes* can be found in many areas such as slaughterhouse waste, water, sewage water, animal-human feces, mastitis or healthy milk. *L. monocytogenes* contaminates green fodder and soil from infected animals, and it is known that the bacteria re-infects milk and meat animals fed with them. This causes the bacteria to remain alive in nature and form a contamination cycle. The viability of bacteria also varies according to the type of food, storage conditions and storage areas.

As a result of disinfection applications with 100 ppm chlorine (2 minutes), *E. coli* in 16 of 30 samples, *Salmonella spp*.in 1, *E. coli* O157 in 2 microorganisms were detected. *L. monocytogenes* were not found in any of the samples. As a result of disinfection applications with 2 ppm ozone (2 minutes), *E. coli* were detected in 11 of 30 samples. *E. coli* O157, *Salmonella* spp., and *L. monocytogenes* were not detected in any sample disinfected with ozone. Due to the low effect of chlorine on pathogenic bacteria, no pathogenic bacteria were observed in ozone-treated samples. In the study conducted on 30 samples, it was observed that as a result of chlorination disinfection applications, *E. coli* remained in contact with food more than through the ozone application. This is thought to be due to the fact that ozone disinfection processes are more effective than chlorination applications. The detection of *E. coli* O157 and *Salmonella* spp. microorganisms after chlorine application for 2 minutes is due to the lack of time for inactivation of these bacteria. In addition, it shows how important it is that the product groups and the microorganism load they contain are more or less in disinfection.

In the study, *E. coli* bacteria were detected in 7 of 30 samples as a result of disinfection applications with 100 ppm chlorine (5 minutes). *E. coli* O157, *Salmonella* spp., *L. monocytogenes* were not found in any of the samples. As a result of disinfection applications with 2 ppm ozone (5 minutes), *E. coli* were detected in 4 of 30 samples. *E. coli* O157, *Salmonella* spp., *L. monocytogenes* were not detected in any of the products. As a result of disinfection applications with 100 ppm chlorine (10 minutes) and 2 ppm ozone (10 minutes), *E. coli, E. coli* O157, *Salmonella* spp., *L. monocytogenes* were not found in any of the 30 samples. In disinfection processes with chlorine and ozone, when the concentration is constant and the washing times are increased, it has been determined that the vital activities of pathogenic microorganisms are stopped by preventing the proliferation of bacteria. This shows that as the disinfection processes take longer, microorganisms that are harmful to human health are destroyed from the environment.

The high oxidation effect of ozone has a greater effect on the destruction of bacteria than chlorine. It has been observed that ozone is more effective than chlorine in the inhibition of microorganisms in the same period.

In the application of 2 minutes chlorine disinfection of lettuce containing *E. coli* bacteria, a decrease of more than 45% of the bacteria, a decrease of more than 70% in the application of 5 minutes, and 100% elimination in the application of 10 minutes were detected. Aruscavage et al. (2006) in their study, it was determined that there was a 2.5 log cfu g^{-1} decrease in the microorganism as a result of the treatment of lettuce samples containing 10^5 cfu g^{-1} *E. coli* with 200 ppm chlorine. Since *E. coli* is a fecal-derived bacterium, its incidence is high in fruits and vegetables. When the studies are compared, it is seen that chlorine reduces the *E. coli* microorganism by creating an antimicrobial effect.

In the study, the *E. coli* O157 microorganism was detected in 8 of 30 fruit and vegetable samples before the disinfection process. It was detected in 2 of the samples after 2 minutes of chlorination was applied to the products containing this bacterium. The *E. coli* O157 microorganism was not found in 5-10 minute chlorine applications. Nou and Luo (2010) disinfected the lettuces in chlorinated water at a concentration of 70 ppm in their research. They reduced the *E. coli* O157 load, which was 6.3 log cfu g^{-1} at the beginning, to 1 log cfu g^{-1} in the first wash for 60 seconds. In the second wash for 30 seconds, they reported a decrease of 0.6 log cfu g^{-1} in the load. As a result of disinfection processes in food products, it has been determined that the amount of time and concentration of chlorination are important in the gradual decrease of microorganism loads.

In the study, after 2 ppm ozonation treatment, *E. coli* decreased by over 45% after 2 minutes of treatment, over 85% after 5 minutes of treatment, and 100% after 10 minutes of treatment. *E. coli* O157, *Salmonella* spp., *L. monocytogenes* could not be detected in the environment with ozone applications for 2-5-10 minutes. In his study on green leafy vegetables in which he applied disinfection with ozone for 5-10-15 minutes at 2-5-10 ppm concentrations, Tümay (2019) reported the highest reductions of *E. coli, S. aureus, L. monocytogenes, B. cereus* and *S. typhimurium* after ozonation as 0.65 log cfu g^{-1}, 0.32 log cfu g^{-1}, 0.46 log cfu g^{-1}, 0.47 log cfu g^{-1}, 0.15 log cfu g^{-1}, respectively. In his study, Karaca (2010) reported that *E coli* showed a decrease of 1.25-2.09 log cfu g^{-1} and *L. innocua* showed a decrease of 1.54-2.17 log cfu g^{-1} in 5 minutes ozone application in lettuce, spinach and parsley. In the same study, lettuce, spinach and parsley samples were washed with water and chlorine to eliminate *E. coli* and *L. innocua*. He reported that ozone with chlorine achieved better results than washing with pure water. When the studies were compared, it was seen that the microorganism loads decreased depending on the time and concentration after the ozonation process in all of the fruit and vegetable samples.

4. Conclusion

Disinfection processes with chlorination are economical and have low investment costs. However, it causes corrosion as it creates the risk of leaving residues. Disinfection with ozonation is known as an environmentally friendly disinfectant that does not leave any residue, but with a high cost (Karaca 2010).

Features such as eliminating bacteria and viruses, oxidation, environmental sensitivity, color removal, investment cost are more advantageous in ozonation than chlorine. Chlorination can cause more damage to the respiratory and skin in humans than ozonation. However, it is known that ozone is more difficult to apply than chlorine. After the chlorination process, the food must be re-watered so that the chlorine does not leave any residue. This leads to quality loss (Sevilgen 2009). In ozone application, there is no need for such a process. In tourism accommodation facilities, disinfection with chlorine is generally preferred in fruit and vegetable samples due to its costs. As a result, in this study conducted in the kitchens of tourism accommodation facilities, it was concluded that ozonation eliminates microorganisms more effectively than chlorine applications, and that ozonation is a healthier disinfection process in terms of human health. Disinfection processes of fruits and vegetables consumed raw in places of mass consumption such as tourism accommodation facilities should be carried out in an appropriate and reliable manner in a way that does not pose a risk to human health and does not cause food poisoning.

This study is a contribution to other research done on the effectiveness of microorganism inactivation by comparing ozone and chlorine applications to be used for disinfection in the kitchens of tourism accommodation facilities.

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