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The study of the effect Ozonated water on E. Coli. O157 on the tomatoes surface

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Abstract
The effectiveness of ozonated water in lowering *Escherichia coli O157:H7* contamination on tomato surfaces is assessed in this study. Despite their nutritious value, tomatoes can become contaminated by pathogens during handling and processing.For different exposure durations (3, 6, 9, 12, and 15 minutes), tomatoes were inoculated with *E. coli O157:H7* and treated with ozonated water at a concentration of 0.5 mg/L. The viable plate count method was used to determine the number of bacteria.The results showed that *E. coli O157:H7* populations significantly decreased over time, with the largest drop at 15 minutes after treatment. Ozone's short halflife (around 20 minutes) and instability in water further restrict its longterm antibacterial effectiveness.Physical barriers on the tomato surface limit the effectiveness of ozonated water, even though it was found to be beneficial in lowering surface contamination when compared to untreated controls.According to these findings, ozonated water is a promising chemicalfree substitute for decontaminating produce; however, in order to improve microbial safety and stop regrowth during storage, delivery methods should be optimized and combined with other preservation strategies (such as cold storage or edible coatings).Ozone's potential as a nonthermal food safety intervention in the preparation of fresh produce is supported by this study.

Key words: Ozone , Food safety, Tomatoes, Ozonated water, *E. coli O157*.

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دراسة تأثير الماء المُعالج بالأوزون على بكتيريا الإشريكية القولونية O157 على سطح الطماطم

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الملخص

تُقيم هذه الدراسة فعالية الماء المُعالج بالأوزون في خفض تلوث أسطح الطماطم ببكتيريا الإشريكية القولونية O157:H7. على الرغم من قيمتها الغذائية، إلا أن الطماطم قد تُصاب بالتلوث بمسببات الأمراض أثناء التعامل معها ومعالجتها. لفترات تعرض مختلفة (3، 6، 9، 12، و15 دقيقة)، تم تطعيم الطماطم ببكتيريا الإشريكية القولونية O157:H7، وعولجت بالماء المُؤَوزون بتركيز 0.5 ملغم/لتر. واستُخدمت طريقة العد الطبقي الحيوي لتحديد عدد البكتيريا. أظهرت النتائج انخفاضًا ملحوظًا في أعداد بكتيريا الإشريكية القولونية O157:H7 مع مرور الوقت، وبلغ أكبر انخفاض بعد 15 دقيقة من المعالجة. إن عمر النصف القصير للأوزون (حوالي 20 دقيقة) وعدم استقراره في الماء يحدان من فعاليته المضادة للبكتيريا على المدى الطويل. وتحد الحواجز الفيزيائية على سطح الطماطم من فعالية الماء المؤزن، على الرغم من أنه وجد أنه مفيد في تقليل تلوث السطح عند مقارنته بالضوابط غير المعالجة. ووفقًا لهذه النتائج، فإن الماء المعالج بالأوزون هو بديل واحد خالٍ من المواد الكيميائية لتطهير المنتجات؛ ومع ذلك، من أجل تحسين السلامة الميكروبية ووقف إعادة النمو أثناء التخزين، يجب تحسين طرق التسليم ودمجها مع استراتيجيات الحفظ الأخرى (مثل التخزين البارد أو الطلاءات الصالحة للأكل). وتدعم هذه الدراسة إمكانات الأوزون كتدخل غير حراري لسلامة الأغذية في تحضير المنتجات الطازجة.

الكلمات المفتاحية: الأوزون، سلامة الغذاء، الطماطم، الماء المعالج بالأوزون، الإشريكية القولونية.O157

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Introduction

In early August of 2000, the Electric Power Research Institute (the EPRI) filed a Food Additive Petition (FAP) with the U.S. Food and Drug Administration (FDA) requesting formal FDA approval for ozone to be used as an antimicrobial agent coming in direct contact with various foods and food products.

Consumption of fresh produce has been suggested to increase public health because fresh vegetables and fruits are thought to be an important part of a healthy diet and are a source of much-needed vitamins, minerals and fiber. Typically, most fruits, vegetables, and ready-to-eat (RTE) foods sold in markets rarely carry pathogenic bacteria. However, there is always the possibility of contamination with food borne pathogens, such as enterohemorrhagic Escherichia coli O157:H7, However, there is always the possibility of contamination with food borne pathogens, such as enterohemorrhagic *Escherichia coli O157:H7*, during any stage of product handling from production at the farms to the point of sale (Hanning et al., 2009; Matthews 2006).

Since tomato has a short shelf life, its extension plays a vital role in both exports and domestic markets (Esua,Chin, & Yusof, 2018; Gharezi, Joshi, & Sadeghian, 2012).

The development of novel technologies to produce safe and high quality foods is urgently needed in terms of food security and food safety, Owing to its low cost and anti-bactericidal impact (Rodgers, Cash, Siddiq, & Ryser, 2004). treatment of heart disease and some types of cancer. Also, it helps in lowering the risk of prostate cancer and improved cardiovascular health markers, such as blood pressure, and LDL cholesterol levels (Godos et al., 2023). For this importance of tomato Because of their abundance of key minerals and antioxidants, tomatoes are an essential part of a balanced diet. It has potent antioxidant qualities that may help in, this study aimed to assess the potential of ozonated water in protecting tomatoes crop as well as consuming people from harmful effect of *E. coli O157:H7* bacterium.

Materials and methods

Area of the study:

This study done in Al-Marj City; 120 km eastern of Benghazi (northeast of Libya). Al-Marj City is an agricultural region contains an agronomic, vegetable (includes tomato and fruit fields .

Ozone measurement:

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For preparation of aqueous ozone concentration which was proceeded by continuously measuring to absorbance at (260, 259 nm) using a UV spectrophotometer DR-2800. Then the ozone calculation was determined as follows, 0.5 mg/L, dissolved O3 mg/ml = Δ A.100/f b v .

Where :

ΔA – the difference in optical absorbance between sample and blank solution.

b – the path length of the cuvette in cm (10 cm) .

V – the volume of sample added into the ampoule, normally, 90 ml .

f – the experimentally obtained factor (0.42).

Experimental design set up:

In this way will describe the design of the tomatoes treatment technique using ozone gas. In addition to materials equipment **system steps** operation, ozonation samples treatment and determination of procedures will be described as in Figure (1).

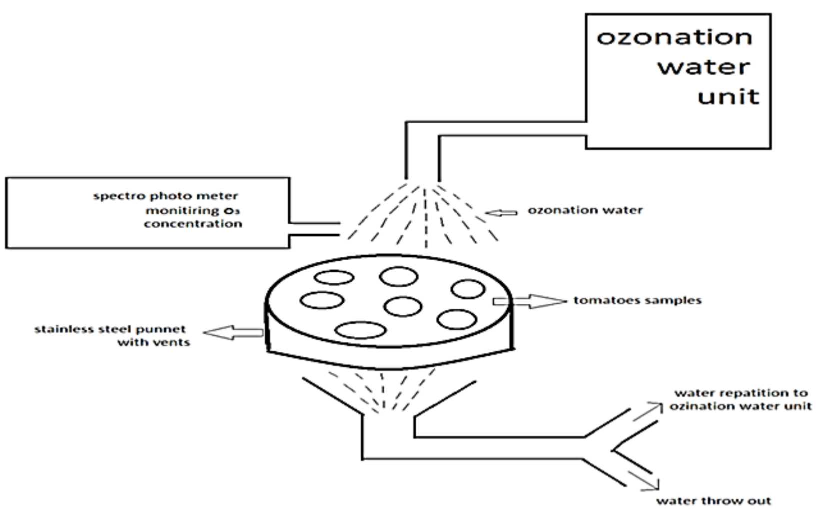

Figure (1): Experimental design set up.

Preparation and Inoculation of Tomatoes

Tomatoes were purchased from a local a market in Almraj, whole sound tomatoes were washed with 0.5% aqueous solution of a

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detergent, rinsed in tap water and wiped dry. Each tomatoes was dipped in the diluted bacterial suspension (to 22h to 25h at 80C .(In a 700-ml beaker, stirred gently for 1 min drained, and placed on a sterile tray. Inoculated tomatoes were incubated for 2.5h at 22 to 25C prior to the sanitization treatments.

Seeding of samples with E.coli O157:H7

Escherichia coli O157:H7 was obtained from the Analysis Laboratory of Microbiology in Almarej Moutwsta Laoratory - cultures, were maintained on slants of trypticase soy agar at 40C with occasional transfers the bacterium was propagated by making 2 successive transfers in 10 ml Trypticase soy broth the inoculated broth was incubated at 370C for 20 to 22h before using the inoculums in these experiments, this bacterium was diagnosed by Phoenix-TM100 This process were from 20-25 May, 2025, a portion (35 ml) of the resulting culture was transferred in to 100 ml TSB. The mixture was incubated at 370C for 18 to 19h with agitation, count at the end of the was incubation period was ~109 CFU/ml This culture was appropriately diluted, using 0.85% NaCl solution and used for inoculation of tomatoes. (Achen and Yousef, 2001).

The steps of ozonation system operation

Tomatoes were prepared and organized in stainless steel pots (4 fruits / pot) samples were treated by ozonided water 0.5 mg/L O3 for Exposure time (3, 6, 9, 12 and 15 mins).

Bacterial analysis:

Counts controls (inoculated unwashed or water-washed tomatoes) and inoculated, ozone After treatment. Tomatoes were placed in 50 ml of Dey-Engley Neutralizing and pummeled for 1 min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water and spirally plated on tryptic soy agar supplemented with 50 µg/mL of nalidixic acid with an Auto plate 4000. Plates were incubated at 37 0C for 24h and then enumerated using the Q-count reductions of bacteria were calculated on basis gram of fruit basis. Random colonies of *E. coli O157:H7* were confirmed serologically using RIM *E. coli O157:H7* latex test For *E. coli O157:H7* 1 mL of 0.1% peptone water was transferred to 9 mL of TSBN and incubated at 37 0C for 24h. After incubation 1 mL of the TSBN was transferred to 9 ml either TT Broth Base Hajna or MacConkey For *E. coli O157:H7*, respectively. TT Broth Base

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Hajna was incubated at 4 0C for 48 h. A loop full of enrichment. Solution was then streaked onto xylose lysine deoxycholate agar and incubated for 24h at 37C. Colonies were again confirmed A-1 latex agglutination test. Mac- Conkey broth tubes were incubated for 24h at 37C and then a loop full of solution will be streaked onto MacConkey agar plates that were incubated for 24h at 37C. Colonies were then confirmed as *E. coli O157:H7* using a RIM *E. coli O157:H7* latex test.

Bacterial count

According to (Robert, 2006), the viable plate count method were used to enumerate the viable bacteria, and the results were recorded as colony forming units per ml (cfu/ ml).

Results:

Using an ozone concentration of 0.5 mg/L, the study assessed how well ozonated water reduced populations of *E.coli O157:H7* on the surface of tomatoes at several exposure duration (3, 6, 9, 12, and 15 minutes). When compared to control samples (inoculated tomatoes that were either unwashed or washed with tap water), the resulta demonstrated a notable decrease in *E. coli O157:H7* counts on tomato surfaces following treatment with ozonated water. Longer exposure periods resulted in a greater decrease in the bacterial populations, suggesting that the effects of ozone treatment are time dependent. The greatest decrease in the bacterial load was noted at the maximum exposure duration (15 minutes), indicating the potential of ozonated water as a surface decontaminant. Even so, the effectiveness of ozone layers was restricted in some parts of the tomato surface, especially in the difficulty to reach places like surface cracks or stem scars, where the bacterial cells might be physically shielded from ozone contact. This restriction is explained by the fact that ozone in water is extremely unstable and has a short half life (about 20 minutes in distilled water), which lowers its long term antibacterial action.

Furthermore, some bacterial cells may be able to survive and even develop during storage since ozone cannot reach inaccessible surface areas. In conclusion, *E. coli O157:H7* on tomato surfaces was successfully decreased by ozonated water at a concentration of 0.5 mg/L; longer contact durations produced better outcomes. The virus was not entirely eradicated, particularly in protected surface regions, which emphasizes the necessity for better ozone delivery techniques to increase its accessibility and overall decontamination

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effectiveness. Figure (2) provides an illustration of these results by demonstrating the impact of exposure duration and ozone concentration (0.5 mg/L) on bacterial decrease.

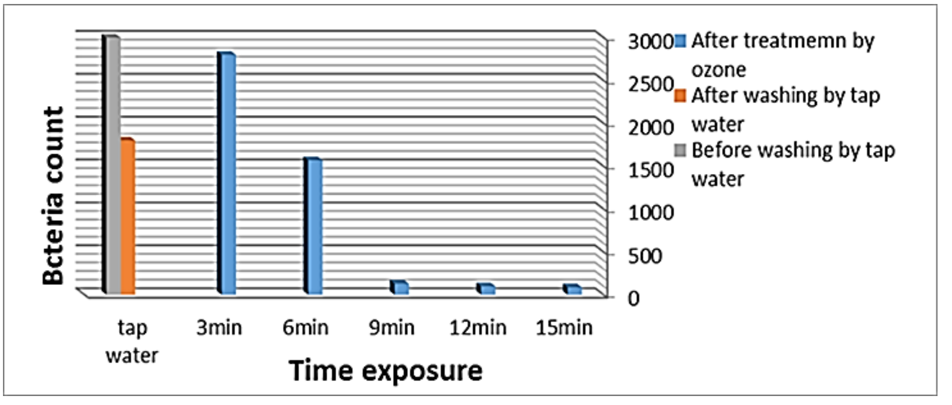

Figure (2): Ozone concentration 0.5 mg/L VS time exposure.

Discussion

With larger decreases seen at longer exposure times (3 to 15 minutes) and an ozone content of 0.5 mg/L, the current study shows that ozonated water is effective in lowering *E. coli O157:H7* populations on the surface of tomatoes. These results are in line with earlier research showing that ozone ruptures microbial cell membranes as a potent oxidizing agent, resulting in cell lysis and death (Kim et al., 1999). As a non thermal way of decontaminating fresh food, ozonated water's antibacterial potential is confirmed by the observed decrease in bacterial load.

The greatest decrease in *E. coli O157:H7* in this investigation was attained after 15 minutes of exposure, indicating that ozone treatment is effective over a longer period. This is consistent with the result of Luo et al., (2021), who found that ozone's bactericidal action on E.coli and other pathogens on tomato surface is greatly increased by extended contact duration.

E. coli O157:H7 was not entirely eradicated, though, especially in places like surface cracks and stem scars that would not have easy access to ozone. This finding bolsters the findings of Bialka and Jack (2004), who observed that the diverse surface topography of tomatoes may protect pathogens from direct ozone exposure, hence diminishing the effectiveness of therapy. In comparison to other research, the ozone concentration employed in this one (0.5 mg/L)

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is in the low to moderate range. Selma et al. (2008), found that doses of 10 – 4.0 mg/ml were more successful in lowering Salmonella and E.coli on lettuce and tomatoes. Lower concentrations are more appropriate for uses, though, as greater concentrations could cause oxidative damage to the produce or lingering ozone issues. Since continual synthesis and application are necessary for the best outcomes, the instability of ozone in water highlighted in this study, with a half-life of roughly 20 minutes, further restricts its effectiveness (Purohit et al., 2020). It's interesting to note that, although ozonated water greatly decreased the microbial burden when compared to tap water washing, initial investigations revealed bacterial regrowth during storage.According to Rajendran and Balasubramaniam (2006), sublethal ozone exposure may cause bacterial stress responses, which could result in recovery and regrowth in the presence of good storage conditions. Thus, ozone treatment may improve shelf life and microbiological safety when combined with other preservation methods like cold storage or edible coatings. Ozone microbubbles and electrostatic spraying are two recent developments in ozone delivery systems that have demonstrated promise in enhancing ozone penetration and distribution on produce surfaces (Gao et al., 2022;Zhang et al., 2023). (Gao et al., 2022). To sum up, ozonated water is a viable substitute for conventional chemical sanitizers when it comes to cleaning fresh fruit, such as tomatoes.

Recommendation

Even though it efficiently lowers E. coli O157:H7, more optimization is required due to its limits in reaching protected surface areas and the possibility for microbial regrowth. To guarantee both microbiological safety and product quality, future research should concentrate on improving ozone delivery techniques and investigating combination treatments.

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