Ozone Treatment as a Sustainable Alternative for Suppressing Blue Mold in Mandarins and Extending Shelf Life

Lemić, Darija; Galešić, Marija Andrijana; Bjeliš, Mario; Virić Gašparić, Helena

agriculture

Article

Ozone Treatment as a Sustainable Alternative for Suppressing Blue Mold in Mandarins and Extending Shelf Life

Darija Lemic [1,2,*], Marija Andrijana Galešić [2], Mario Bjeliš [3] and Helena Viric Gasparic [1,2]

[1] Department of Agricultural Zoology, Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia; hviric@agr.hr
[2] Green Environmental Research Ltd., 10020 Zagreb, Croatia; marija.a.galesic@gmail.com
[3] Department of Marine Studies, University of Split, 21000 Split, Croatia; mbjelis@unist.hr
* Correspondence: dlemic@agr.hr

Abstract: Citrus fruits, particularly mandarins, are highly valued globally for their nutritional benefits and versatile culinary uses. However, the challenge of post-harvest decay, primarily due to blue mold (*Penicillium italicum*) infections, results in significant food losses and necessitates effective preservation strategies. Traditional methods often rely on fungicides, raising concerns about chemical residues and environmental impact. This study investigates the efficacy of ozone as an alternative approach to controlling blue mold in mandarins. Various gaseous ozone treatments were tested, including single, double, and triple treatments, with durations ranging from 10 to 60 min and concentrations from 3.3 to 20 ppm. Additionally, ozonated water treatments were evaluated with concentrations of 2, 4, and 6 ppm. To simulate a realistic infestation scenario, mandarins were artificially infected with *P. italicum* spores before undergoing both gaseous ozone and ozonated water treatments. The storage conditions for the mandarins were meticulously controlled, maintaining a humidity level of 50–60% and a temperature range of 10–12 °C. Each fruit was analyzed, and the presence of *P. italicum* infection was determined two and three weeks after the ozonation. Results indicated that ozone treatments significantly reduced mold growth, with gaseous ozone demonstrating efficacy rates up to 97.5% and ozonated water treatments achieving preservation rates between 95% and 97%. These results underscore ozone's potential as a safe, efficient, and sustainable alternative to conventional fungicides, offering promising solutions for extending the shelf life of mandarins. Further research is recommended to optimize ozone treatment parameters, assess long-term effects on fruit quality and nutritional content, and refine application techniques to harness ozone's potential in citrus fruit preservation fully. This approach not only addresses food security challenges but also aligns with global efforts to reduce chemical inputs in agriculture and promote environmentally sustainable practices.

Keywords: mandarins; *Penicillium italicum*; gaseous ozone; ozonated water

check for updates

Citation: Lemic, D.; Galešić, M.A.; Bjeliš, M.; Viric Gasparic, H. Ozone Treatment as a Sustainable Alternative for Suppressing Blue Mold in Mandarins and Extending Shelf Life. *Agriculture* **2024**, *14*, 1196. https://doi.org/10.3390/agriculture14071196

Academic Editor: Perla A. Gómez

Received: 26 May 2024
Revised: 17 July 2024
Accepted: 19 July 2024
Published: 20 July 2024

1. Introduction

Citrus is the most widely cultivated fruit tree species in the world [1]. They are grown in more than 140 countries, with the majority being produced in the northern hemisphere in tropical and subtropical regions [2–4]. Their importance lies in their diverse uses, as they are used either as fresh fruit or as fruit juices [5]. Mandarins are the second most widely cultivated fruit in the world [6]. From 2010 to 2015, global mandarin production increased by more than 30% from 22 to 29 million tonnes [7]. In addition to the pulp, mandarin peel is beneficial for human consumption, containing polyphenols like hesperidin, nobiletin, and tangeretin, known for their anti-inflammatory properties. Consequently, mandarin peel is used in producing peel paste, an additive in both the food industry [8] and pharmaceuticals and cosmetics production [9].

Consumer demand for safe, organically grown food, particularly for products consumed with the skin, is rising. The shelf life of fresh produce, including citrus fruits, is influenced by the quality at harvest and storage conditions [10,11]. Globally, 30 to 50% of produced food is not consumed, with citrus fruits experiencing a 10–30% loss due to rot, which can escalate to 50% under unfavorable conditions [12,13].

Rot from blue mold (*Penicillium italicum*) and green mold (*Penicillium digitatum*) is the primary postharvest infection in citrus fruits [14,15]. These molds account for 80% of all postharvest decay in citrus fruits in Mediterranean climates [16]. Mold spores activate on citrus surfaces when the peel is damaged by insects, branches, or improper handling during harvest [17–19].

Mandarins spoil more easily than other fruits, and their storage should ideally not exceed 2 to 4 weeks; hence, quick marketing is important. Under ambient conditions or in high humidity, mandarins rapidly lose moisture and deteriorate [20,21]. Packaging choices, such as non-perforated plastic films, also significantly impact freshness by increasing relative humidity and spoilage [22]. To extend mandarins' longevity and maintain their sensory properties, measures such as waxing, chemical treatments, or washing with antimicrobial solutions are employed to prevent microbial contamination [23,24].

One widely used method for controlling mold growth and maintaining the freshness of fruit is the application of pesticides. Imazalil is the most commonly used protectant for citrus fruits [13,25–29]. While effective, imazalil tends to accumulate in the fruit's skin, which poses concerns since mandarin peels are used in food. Several washing techniques have been developed to remove phytochemicals from fruits; however, none have been significantly successful in removing imazalil [13].

Due to concerns about residue levels, resistance, and negative impacts on human health, alternative methods are needed to protect citrus fruits from mold infections [30–32]. Ozone, a triatomic oxygen molecule with strong antimicrobial properties and a high oxidation potential, is one such promising alternative. It decomposes into oxygen molecules either spontaneously or upon contact with oxidative surfaces [33]. Recognized as Generally Recognized as Safe (GRAS) by the US FDA since 1997, and approved as a disinfectant in food production [34,35], ozone can oxidize substances in both water and air. This is particularly relevant for air-transmitted mold spores [30,36–39]. Research shows that ozone treatments effectively delay the onset of diseases caused by molds by inhibiting spore germination by 99.5% and reducing their viability, which helps prevent the development of conidia on infected fruit [34,38–40].

The use of ozone shows promising potential for reducing microbiological contamination in the processing of different types of fruits [41,42]. Studies have demonstrated that ozone treatment can effectively reduce the presence of mold and bacteria on citrus surfaces [42]. Additionally, ozone has been found to degrade pesticide residues on fruits, making it a dual-purpose treatment that enhances both safety and freshness [43,44]. To exploit its potential fully, it is necessary to familiarize oneself with the possible advantages and disadvantages of ozone use for future experiments in citrus production and shelf-life extension [31]

The aim of this study was to determine the antifungal potential of ozone used as a gas or dissolved in water as an alternative control method for stored mandarins (in two different packaging methods) for the curative suppression of blue mold *P. italicum.*

2. Materials and Methods

This study was conducted at the storage facility in Opuzen, Croatia, in the period from October to December 2021. The volume of the storage facility was 12 m^2. All experiments were conducted via two different (standard) packaging methods (cardboard boxes and polystyrene boxes). (Expanded) Polystyrene, known as EPS (not to be confused with Styrofoam), is a lightweight foam product that is 100% recyclable as it is made up of 98% air and 2% recyclable plastic.

2.1. Fruits in the Experiment

In the study, we used the dominant mandarin variety cultivated in the Neretva River valley of Croatia "Zorica rana", which is a local mutation of the Kawano Wase group of Satsuma mandarins. Fruits were purchased from a local producer directly from the orchard and treated on the same day of the harvest. The fruit was not waxed and not treated with pesticides, neither in the orchard not in storage before the experiment.

One day before conducting the experiment, all mandarin fruits were artificially infected with a mixture of blue mold *P. italicum* in water (spore concentration: 10,000 CFU/mL).

Two variants of the experiment were treated with imazalil according to the standard treatments of mandarins received in industrial warehouses/refrigerators commonly used in the Neretva valley. Imazalil (NEOZIL 50 EC) agent was applied in a concentration of 0.1% (100 mL agent/100 L water) by soaking the fruits with the "drencher" system. The fungicidal variant 1 was treated once with imazalil, while the fungicidal variant 2 was treated twice with imazalil at intervals of half an hour. The control variant was untreated.

2.2. Gaseous Ozone Treatments

An ozonator of the OZ-10 series, a 130 W ozone generator, was used for ozonization. Ozonation was conducted in separate chambers, and no additional ozonation was performed in the storage facility during the monitoring period. This device uses an electrical charge to convert O_2 from the air into O_3 (ozone). The ozone output of 20 mg/m^3 with a reactivity of max. 30 ppm/h was used. The ozone output could not be changed, and the time of exposure to ozone also defined the amount of ozone used as a test variable.

Considering the test parameters, we started the study to determine the ozone production per hour and then determine the ozone concentration in parts per million (ppm) in the storage chamber. Table 1 shows the amount of gaseous ozone used in the variants in this study.

Table 1. Applied amount of ozone in variants.

Variants in the Experiment (Exposure to Ozone in Minutes)	Applied Concentration of Ozone in the Air (ppm)
10	3,3
30	10
60	20

Ozonization was carried out in three groups. The first group consists of the 10-, 30-, and 60-min variants, which were ozonized only once. The second group consists of the 10-, 30-, and 60-min variants, which were ozonized on two consecutive days with an interval of 24 h between treatments. The third group consisted of the 10-, 30-, and 60-min variants, which were ozonized on three consecutive days with an interval of 24 h between treatments.

A total of 12 variants took part in the trial (two fungicide-treated, three ozone-treated in three ozonation groups, and the control variant). Each variant was planted in four replicates. Each replicate contained 10 mandarin fruits. A total of 40 fruits were treated per variant, so that a total of 480 mandarin fruits were tested in this study. During the trial, the fruits (per replicate) were stacked in cardboard boxes to minimize the possibility of contact between the fruits and to prevent the spread of infection through contact. This decision was made to control the treatment conditions precisely and to facilitate uniform exposure to ozone for each piece of fruit.

The same procedure was repeated with the same experimental setup and the same number of fruits. The fruits were stacked in polystyrene boxes to minimize the possibility of contact between the fruits and to prevent the spread of infection by contact.

2.3. Ozonated Water Treatments

PURITAS EOG PLUS ozone was equipped with "ozone generators" of the "immersion" type and was used for the production of ozonized water based on electrolysis. The generator was set up according to the manufacturer's instructions. The ozone concentration and the duration of the water treatment were varied according to the experimental design. The ozone photometer (Palintest Lumiso Ozone Handheld Photometer) was used to measure the ozone concentration in the ozonated water up to 3 mg/L using the DPD method. Ozone reacts with diethyl-p-phenylene diamine (DPD) in buffered solution in the presence of potassium iodide to produce a pink coloration. The intensity of the color is proportional to the ozone concentration. The concentrations of ozonated water for this experiment were 2, 4, and 6 ppm. It was applied via immersion in ozonated water to ensure uniform coverage of the fruit surface.

Ozonization was carried out in three groups. The first group consists of the variants with 2, 4, and 6 ppm, which were immersed only once in ozonized water (25–30 s). The second group consists of the variants with 2, 4, and 6 ppm, which were immersed in ozonized water on two consecutive days, with an interval of 24 h between the treatments. The third group consists of the variants with 2, 4, and 6 ppm, which were immersed in ozonized water on three consecutive days at intervals of 24 h.

A total of 12 variants took part in the experiment (two treated with fungicide, three treated with ozonated water in three ozonation groups, and the control variant). Each variant was planted in four replicates. Each replicate contained 10 mandarin fruits. A total of 40 fruits were treated per variant, so that a total of 480 mandarin fruits were tested in this study.

During the trial, the fruits (per replicate) were stacked in cardboard boxes to minimize the possibility of contact between the fruits and to prevent the spread of infection through contact. The controlled storage temperature was 10–12 °C, and the humidity was 50–60% throughout the study period.

The same procedure was repeated with the same experimental setup and the same number of fruits. The fruits were stacked in polystyrene boxes to minimize the possibility of contact between the fruits and to prevent the spread of infection by contact.

2.4. Readings and Data Analysis

The measurements were carried out twice (14 and 21 days after the start of the experiment). Each fruit was analyzed, and the presence of *P. italicum* infection was determined. The percentage of healthy fruit per variant and storage method was subjected to analysis of variance (ANOVA). When deemed suitable, the data were log-transformed (logx + 1). Following the attainment of significant results in the testing process ($p < 0.05$), a Tukey post hoc test was employed to identify specific mean variant values that exhibited statistically significant differences.

3. Results

3.1. Efficacy of Gaseous Ozone Treatments

In mandarins subjected to gaseous ozone treatment and stored in polystyrene boxes (Table 2), initial observations two weeks after treatment showed a minimum efficacy of gaseous ozone in the preservation of mandarins of 82% for a single application of gaseous ozone for 60 min and the untreated control variant (81%). In contrast, the success rates for the other ozonized treatment variants were between 87% and 99%. After three weeks, the control variant (without treatment) had the lowest maintenance rate, with only 35% healthy fruit, while the double fungicide treatment achieved a maintenance rate of 99%. However, no significant differences were found between the fungicide treatments (single and double) and the ozonized variants (double and triple treatments) with an exposure time of 30 and 60 min. The least effective treatment, with an average preservation rate of 62%, was observed with the single application of gaseous ozone over 10 min. The untreated control variant was heavily infected with mold and had only 35% healthy fruit.

Table 2. Healthy mandarin fruits stored in polystyrene (%±SE) after treatments with gaseous ozone.

			Healthy Mandarin Fruits (%)	
Variant	**No. of Treatments**	**Duration of Ozonation (min)**	**Reading 1**	**Reading 2**
Fungicide (Imazalil)	1	-	100 ± 0 a *	95.0 ± 2.9 ab
	2	-	99.4 ± 4.6 ab	98.8 ± 0.6 a
Gaseous ozone	1	10	89.4 ± 4.6 bc	62.5 ± 4.8 c
	1	30	87.8 ± 2 bc	85.0 ± 2.9 b
	1	60	82.5 ± 3 c	81.5 ± 2.5 ab
	2	10	99.4 ± 4.6 ab	90.0 ± 4.1 b
	2	30	99.4 ± 4.6 ab	95.0 ± 2.9 ab
	2	60	97.4 ± 5.3 ab	97.5 ± 2.5 ab
	3	10	98.7 ± 6.6 ab	95.0 ± 2.9 ab
	3	30	99.4 ± 4.6 ab	92.5 ± 2.5 ab
	3	60	99.4 ± 5.6 ab	95.0 ± 2.9 ab
No treatment	-	-	81.3 ± 4.6 c	35.0 ± 9.6 d
HSD p = 0.05			11.94	14.43

* Means followed by same letter or symbol do not significantly differ. Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

No significant differences in mold infestation were observed in mandarins stored in cartons two weeks after treatment with gaseous ozone (Table 3). In all variants, the preservation of the fruit was between 94 (no ozone treatment) and 100%. After three weeks, the lowest percentage of healthy fruit (35%) was observed in the control variant. Very high fruit preservation was observed with both fungicide variants and all ozonated variants with double and triple treatment (95–98%). Significantly lower preservation was observed with the single ozone treatment ranging between 75 and 87%.

Table 3. Healthy mandarin fruits stored in cardboard (%±SE) after treatments with gaseous ozone.

			Healthy Mandarin Fruits (%)	
Variant	**No. of Treatments**	**Duration of Ozonation (min)**	**Reading 1**	**Reading 2**
Fungicide (Imazalil)	1	-	100 ± 0 ns	95.0 ± 2.9 a*
	2	-	99.4 ± 4.6 ns	98.0 ± 0.5 a
Ozone	1	10	100 ± 0 ns	87.5 ± 2.5 ab
	1	30	100 ± 0 ns	87.5 ± 4.8 ab
	1	60	99.4 ± 4.6 ns	75.0 ± 2.9 b
	2	10	99 ± 4.6 ns	95.0 ± 2.9 a
	2	30	97.4 ± 5.3 ns	92.5 ± 2.5 a
	2	60	100 ± 0 ns	95.0 ± 2.9 a
	3	10	99.4 ± 4.6 ns	92.5 ± 2.5 a
	3	30	99.4 ± 4.6 ns	97.5 ± 2.5 a
	3	60	100 ± 0 ns	97.5 ± 2.5 a
No treatment	-	-	94.3 ± 4.6 ns	35.1 ± 9.6 c
HSD p = 0.05			-	13.96

* Means followed by same letter or symbol do not significantly differ. Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

3.2. Efficacy of Ozonated Water Treatments

Mandarins stored in polystyrene boxes and treated with ozonized water showed significant differences in preservation two weeks after treatment (Table 4). The lowest treatment efficacy of 80% was observed with a single application of 2 ppm ozonated water. The highest preservation rate (100%) was observed with the double treatment with fungicide and the triple treatment with ozonated water of 6 ppm. The other variants of the experiment showed consistent efficacy, with no significant differences in prolonging the storage time of mandarin fruit, which ranged from 85 to 95%. After three weeks, the significantly lowest treatment efficiency of 33% was observed in the control variant without treatment. The best preservation was achieved with the double fungicide treatment (100%), followed by the double and triple ozone treatments at all concentrations (80–87%). The lowest percentage of healthy fruit was observed in the untreated variant (35%) and all single ozone treatments (60–65%).

Table 4. Healthy mandarin fruits stored in polystyrene (%±SE) after treatments with ozonated water.

			Healthy Mandarin Fruits (%)	
Variant	**No. of Treatments**	**Ozon Concentration (ppm)**	**Reading 1**	**Reading 2**
Fungicide (Imazalil)	1	-	97.5 ± 2.5 ab *	95 ± 2.9 ab
	2	-	100 ± 0 a	100 ± 0 a
Ozone	1	2	80 ± 4.1 c	60 ± 4.1 d
	1	4	85 ± 2.9 abc	65 ± 2.9 cd
	1	6	82.5 ± 2.5 bc	62.5 ± 4.8 d
	2	2	95 ± 2.9 abc	82.5 ± 2.5 b
	2	4	95 ± 2.9 abc	87.5 ± 2.5 ab
	2	6	92.5 ± 4.8 abc	85 ± 2.9 ab
	3	2	90 ± 4.1 abc	85 ± 6.5 ab
	3	4	92.5 ± 2.5 abc	82.5 ± 4.8 b
	3	6	100 ± 0 a	80 ± 0 bc
No treatment	-	-	92.5 ± 2.5 abce	33 ± 9.6 e
HSD $p = 0.05$			15.39	16.79

* Means followed by same letter or symbol do not significantly differ. Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

Two weeks after treatment, the average percentage of healthy fruit in mandarins stored in cartons was between 90 and 100%, but with no significant differences between the variants (Table 5). The lowest preservation rate of 80% was observed both in the control variant and in the individual treatments with 2 ppm. Three weeks after ozonation, the lowest percentage of healthy fruit (77%) was observed in the control variant, followed by the single ozonated variant at 2 and 4 ppm (80–82%). The highest fruit preservation rates between 87 and 97% were observed in the double and triple treatments (at all concentrations), with no significant differences between the fungicide variants (single and double).

Table 5. Healthy mandarin fruits stored in cardboard (%±SE) after treatments with ozonated water.

			Healthy Mandarin Fruits (%)	
Variant	**No. of Treatments**	**Ozon Concentration (ppm)**	**Reading 1**	**Reading 2**
Fungicide (imazalil)	1	-	100 ± 0 ns	100 ± 0 a*
	2	-	100 ± 0 ns	100 ± 0 a
Ozone	1	2	92.5 ± 4.8 ns	82.5 ± 2.5 b
	1	4	95 ± 5 ns	80 ± 4.1 b
	1	6	92.5 ± 4.8 ns	92.5 ± 4.8 ab
	2	2	97.5 ± 2.5 ns	95 ± 5 a

Table 5. *Cont.*

			Healthy Mandarin Fruits (%)	
Variant	**No. of Treatments**	**Ozon Concentration (ppm)**	**Reading 1**	**Reading 2**
Ozone	2	4	97.5 ± 2.5 ns	97.5 ± 2.5 a
	2	6	95 ± 2.9 ns	90 ± 4.1 ab
	3	2	95 ± 2.9 ns	87.5 ± 4.8 ab
	3	4	95 ± 5 ns	87.5 ± 2.5 ab
	3	6	100 ± 0 ns	90 ± 0.4 ab
No treatment	-	-	90 ± 4.1 ns	77.5 ± 2.5 c
HSD p = 0.05			-	14.1

* Means followed by same letter or symbol do not significantly differ. Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

4. Discussion

In our study, we wanted to demonstrate the effectiveness of ozone in extending the shelf life of the dominant mandarin variety in Croatia and, at the same time, to investigate its potential as a substitute for chemical agents, particularly fungicides. The initial decision to focus our study on a single variety of mandarins was driven by the need to establish a controlled, uniform experimental baseline from which to gauge the effect of ozone accurately. This approach minimized biological variability, allowing for clearer interpretation of the treatment effects. This approach aims to reduce the environmental impact and minimize residues in the treated fruit. To thoroughly test the effectiveness of ozone, we artificially infected mandarin fruits with the blue mold *Penicillium italicum*, simulating a serious real-life scenario. By optimizing the conditions conducive to mold growth, such as storage at 10–12 °C and moderate humidity, we tried to exploit the potential of ozone treatment fully. Regarding the relative humidity settings of 50–60%, our intent was to mirror conditions that are commonly encountered in storage environments, rather than ideal laboratory conditions. This was to ensure that our findings are applicable to real-world settings, where such optimal conditions may not always be attainable.

Our results emphasize two critical points: first, treatments with gaseous ozone and ozonated water effectively extend the shelf life of mandarins; second, these treatments can either partially or completely replace conventional fungicide applications. In the experiments, mandarins were stored in both cardboard boxes and polystyrene and treated with gaseous ozone and ozone dissolved in water. In particular, the double treatment with 60 min of gaseous ozone proved to be the most effective for the mandarins stored in polystyrene, achieving a remarkable 97% efficacy. Conversely, triple treatments of 30 and 60 min were optimal for mandarins in cardboard, resulting in the same efficacy rate. Treatments with ozone dissolved in water gave slightly different results. Dual treatments with 2 and 4 ppm ozone were most effective for mandarins in cardboard, resulting in 95% and 97% preservation, respectively. For mandarins stored in polystyrene and treated with ozonated water, the most effective treatment was a double application of 4 ppm ozonated water, which achieved 87% efficacy. Overall, the results suggest that while both types of packaging benefit significantly from ozone treatments, cardboard packaging has a slightly higher preservation efficacy when treated with ozonated water compared to polystyrene. Cardboard can absorb and regulate moisture better than polystyrene, which helps to maintain an optimum moisture level around the fruit. Treatment with gaseous ozone was very effective for both types of packaging, although the number of applications required for optimum results varied slightly. These results emphasize the versatility of ozone as a treatment method that can be adapted to different packaging scenarios to effectively extend the shelf life of mandarins.

Our results are consistent with previous studies that have confirmed the role of ozone in extending the shelf life of citrus fruit by preventing mold growth. For example, Garcia-Martin et al. [45] demonstrated the inhibitory effect of ozone on mold growth in various citrus fruits, while Palou et al. [31] found that ozone inhibits mold growth and prolongs fruit freshness. Although our experimental treatments did not completely eliminate mold growth, they significantly reduced it compared to the untreated control.

Furthermore, our studies confirmed the effectiveness of ozone in reducing mold spores on the fruit surface, which is consistent with the findings of Smilanick [46] and Di Renzo et al. [14]. It is noteworthy that ozone treatments not only prevent the development of pathogens but also maintain the quality characteristics of the fruit. In contrast to fungicides, ozone treatments leave no residues in the fruit, which eliminates concerns about chemical residues in the peel of mandarins, a staple food [34,35]. Ozone treatments on mandarins can potentially impact both their nutritional quality and sensory properties. While our primary focus was on evaluating the immediate efficacy of ozone treatment, we recognize the importance of investigating its residual effects and impact on sensory qualities. This aspect is critical for assessing the long-term viability and consumer acceptance of ozone treatment in commercial scenarios. Ozone exposure has the capacity to oxidize and degrade antioxidants, vitamins, and other bioactive compounds, thereby reducing the overall nutritional value of the fruit. Additionally, prolonged or high concentrations of ozone may alter the taste, aroma, and texture of mandarins, potentially leading to undesirable changes in flavor profiles that could affect consumer acceptance. The potential for phytotoxic effects from ozone exposure was continuously monitored throughout our experiments. Although no immediate detrimental impacts were observed under the treatment conditions utilized, further studies to explore long-term effects and ensure there are no subtle impacts on fruit quality that could affect marketability and consumer satisfaction are needed [47]. To mitigate these concerns, careful management of ozone treatment protocols, including monitoring ozone levels, optimizing application techniques, ensuring appropriate packaging and storage conditions, and adhering to regulatory standards, are necessary. Continuous monitoring of ozone levels is needed to keep concentrations within safe limits, which can be achieved using ozone monitoring systems that provide real-time data. Optimizing application techniques involves adjusting the delivery system for uniform distribution of ozone across all fruits. This may include modifying flow rates and exposure times based on the storage area's volume and the density of the packed fruits, as well as using oscillating fans or specialized duct systems to ensure even distribution, particularly in large storage facilities. Maintaining proper packaging and storage conditions is vital for the effectiveness of ozone treatments. Using permeable packaging materials such as micro-perforated plastic or breathable cardboard allows for better ozone circulation. Controlling storage conditions, specifically maintaining humidity and temperature, is important for preserving fruit quality and enhancing treatment effectiveness.

In the face of regulatory pressure to reduce the use of pesticides, ozone is proving to be a promising alternative for crop protection. The mandate of the European Green Deal to reduce the use of pesticides by 50% by 2030 emphasizes the urgency of sustainable alternatives. The importance of the alternative use of ozone for combat *P. italicum* in mandarins treatment is even more evident due to the recent research and confirmation of the development of resistance of the fungus *P. italicum* to the fungicide imazalil in the area of the Neretva River valley [48].

Ozone, which is recognised by the EFSA [49] for its broad spectrum of activity, is a viable ecological method for crop protection. Our study primarily focused on the efficacy of ozone treatments in maintaining the health of mandarins during storage by preventing the development of visible fungal infections. However, we recognize that the absence of disease during storage does not fully confirm the inactivation of all potential pathogens on the fruit surface. The pathogens may remain inactivated rather than being completely inactivated. This presents a risk that the fruit could develop mold during the sale and shelf-life periods.

To comprehensively address this concern, future studies should include in vitro assessments of antifungal and antimicrobial efficiency. With these additional tests, we can provide a more thorough evaluation of ozone's potential to ensure the long-term safety and quality of the fruit.

5. Conclusions

The potential use of ozone to extend the shelf life of mandarins is a promising strategy by which to reduce post-harvest losses and ensure food safety. The strong antimicrobial properties of ozone provide an effective means of combating microbial contamination, thereby delaying spoilage and maintaining fruit freshness. Its ability to inhibit the growth of various pathogens makes it a versatile tool in fruit preservation. In addition, ozone treatments have been shown to reduce the incidence of common post-harvest diseases such as blue mold. This approach not only extends the storage time of the fruit but also preserves its quality characteristics. In addition, ozone treatment leaves no harmful residues, so there is no need to worry about chemical residues in the fruit skin, which is often consumed together with the flesh. With increasing consumer demand for safe and organic produce, ozone is proving to be a sustainable alternative to traditional chemical treatments and offers a safe and environmentally friendly solution for extending the shelf life of mandarins.

Author Contributions: Conceptualization, D.L., H.V.G., and M.B.; methodology, D.L.; software, D.L.; validation, M.B., H.V.G., and M.A.G.; formal analysis, D.L.; investigation, D.L., M.B., and H.V.G.; resources, D.L. and H.V.G.; data curation, M.B. and M.A.G.; writing—original draft preparation, D.L. and M.A.G.; writing—review and editing, D.L.; visualization, D.L. and M.A.G.; supervision, D.L.; project administration, H.V.G.; funding acquisition, D.L. and H.V.G. All authors have read and agreed to the published version of the manuscript.

Funding: Project development of an advanced ecological solution for pest control in agriculture—Go Green Ozonator (GGO3) (NPOO.C1.1.2.R2-I3.02)—funded by Eu funds through National Recovery and Resilience Plan (NPOO).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful to the Zagreb Innovation Centre (ZICER) for their support in this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Aleza, P.; Cuenca, J.; Juarez, J.; Pina, J.A.; Navarro, L. 'Garbi' Mandarin: A New Late-maturing Triploid Hybrid. *HortScience* **2010**, *45*, 139–141. [CrossRef]
2. Liu, Y.; Heying, E.; Tanumihardjo, S.A. History, Global Distribution, and Nutritional Importance of Citrus Fruits. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 530–545. [CrossRef]
3. Agusti, M.; Mesejo, C.; Reig, C.; Martinez-Fuentes, A. Citrus Production. *Hortic. Plants People Places* **2014**, *1*, 159–195. [CrossRef]
4. Cheng, Y.; Lin, Y.; Cao, H.; Li, Z. Citrus Postharvest Green Mold: Recent Advances in Fungal Pathogenicity and Fruit Resistance. *Microorganisms* **2020**, *8*, 449. [CrossRef]
5. Talibi, I.; Boubaker, H.; Boudyach, E.H.; Ait Ben Aoumar, A. Alternative methods for the control of postharvest citrus diseases. *J. Appl. Microbiol.* **2014**, *117*, 1–17. [CrossRef]
6. Usman, M.; Fatima, B. Mandarin (*Citrus reticulata* Blanco) Breeding. *Adv. Plant Breed. Strateg. Fruits* **2018**, *3*, 465–533.
7. Goldenberg, L.; Yaniv, Y.; Porat, R.; Carmi, N. Mandarin fruit quality: A review. *J. Sci. Food Agric.* **2017**, *98*, 18–26. [CrossRef] [PubMed]
8. Tsurunaga, Y.; Takahashi, T.; Nagata, Y. Production of persimmon and mandarin peel pastes and their uses in food. *Food Sci. Nutr.* **2021**, *9*, 1712–1719. [CrossRef] [PubMed]
9. Shorbagi, M.; Fayek, N.M.; Shao, P.; Farag, M. Citrus reticulata Blanco (the common mandarin) fruit: An update review of its bioactive, extraction types, food quality, therapeutic merits, and bio-waste valorization practices to maximize its economic value. *Food Biosci.* **2022**, *47*, 101699. [CrossRef]
10. Glowacz, M.; Colgan, R.; Rees, D. The use of ozone to extend the shelf-life and maintain quality of fresh produce. *J. Sci. Food Agric.* **2014**, *95*, 662–671. [CrossRef]

11. Shah, N.N.A.K.; Sulaiman, A.; Sidek, N.S.M.; Supian, N.A.M. Quality assessment of ozone-treated citrus fruit juices. *Int. Food Res. J.* **2019**, *26*, 1405–1415.
12. Zainuri, H.; Hasim, A. Post-harvest losses of citrus fruits and perceptions of farmer sin marketing decisions. *E3S Web Conf.* **2021**, *306*, 2–6. [CrossRef]
13. Bahtta, U.K. Alternative Management Approaches of Citrus Diseases Caused by *Penicillium digitatum* (Green Mold) and *Penicillium italicum* (Blue Mold). *Front. Plant Sci.* **2022**, *12*, 833328. [CrossRef]
14. Di Renzo, G.C.; Altieri, G.; D'Erchia, L.; Lanza, G.; Strano, M.C. Effects of gaseous ozone exposure on cold stored orange fruit. *Acta Hortic.* **2005**, *682*, 1605–1610. [CrossRef]
15. Ashebre, K.M. Pre-Harvest and Post-Harvest Factors Affecting Citrus Fruit and Post-Harvest Treatments. *J. Biol. Agric. Healthc.* **2015**, *5*, 19–29.
16. Nunes, C.; Usall, J.; Manso, T.; Torres, R.; OImo, M.; Garcia, J.M. Effect of High Temperature Treatments on growth of *Penicillium* spp. and their Development on 'Valencia' Oranges. *Food Sci. Technol. Int.* **2007**, *13*, 63–68. [CrossRef]
17. Droby, S.; Eick, A.; Macarisin, D.; Cohen, L.; Rafael, G.; Stange, R.; McColum, G.; Dudai, N.; Nasser, A.; Wisniewski, M.; et al. Role of citrus volatiles in host recognition, germination and growth of Penicillium digitatum and Penicillium italicum. *Postharvest Biol. Technol.* **2008**, *49*, 386–396. [CrossRef]
18. Papoutsis, K.; Mathioudakis, M.M.; Hasperue, J.H.; Ziogas, V. Non-chemical treatments for preventing the postharvest fungal citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). *Trends Food Sci. Technol.* **2019**, *86*, 479–491. [CrossRef]
19. Dabargainya, B.; Acharya, B.; Acharya, P. Effect of different packaging materials on post-harvest life of mandarin (*Citrus reticulata* Blanco). *Rev. Food Agric.* **2022**, *3*, 87–91. [CrossRef]
20. D'Aquino, S.; Angioni, M.; Schirru, S.; Agabbio, M. Quality and Physiological Changes of Film Packaged 'Malvasio' Mandarins during Long Term Storage. *LWT Food Sci. Technol.* **2001**, *34*, 206–214. [CrossRef]
21. Lopez-Gomez, A.; Ros-Chumillas, M.; Buendia-Moreno, L.; Navaroo-Segura, L.; Martinez-Hernandez, G.B. Active Cardboard Box with Smart Internal Lining Based on Encapsulated Essential Oils for Enhancing the Shelf Life of Fresh Mandarins. *Foods* **2020**, *9*, 590. [CrossRef]
22. Baswal, A.K.; Dhaliwal, H.S.; Singh, Z.; Mahajan, B.V.C. Influence of Types of Modified Atmospheric Packaging (MAP) Films on Cold-Storage Life and Fruit Quality of 'Kinnow' Mandarin (*Citrus nobilis* Lour X *C. deliciosa* Tenora). *Int. J. Fruit Sci.* **2020**, *20*, 1552–1569. [CrossRef]
23. Mahajan, B.V.C.; Dhatt, A.S.; Satish, K.; Manohar, L. Effect of pre-storage treatments and packaging on the storage behaviour and quality of *Kinnow mandarin*. *J. Food Sci. Technol.* **2006**, *43*, 589–593.
24. Kang, J.H.; Choi, H.Y.; Park, H.H.; Min, S.C. Effects of washing and packaging combined treatments on the quality of satsuma mandarins during storage. *LWT Food Sci. Technol.* **2020**, *121*, 108982. [CrossRef]
25. Smilanick, J.L.; Michael, I.F.; Mansour, M.F.; Mackey, B.E.; Margosan, D.A.; Flores, D.; Weist, C.F. Improved Control of Green Mold of Citrus with Imazalil in Warm Water Compared with Its Use in Wax. *Plant Dis.* **1997**, *81*, 1299–1304. [CrossRef] [PubMed]
26. Moscoso-Ramirez, P.A.; Montesinos-Herrero, C.; Palou, L. Control of citrus postharvest penicillium molds with sodium ethylparaben. *Crop Prot.* **2013**, *46*, 44–51. [CrossRef]
27. Sukorini, H.; Sangchote, S.; Khewkhom, N. Control of postharvest green mold of citrus fruits with yeast, medicinal plants, and their combination. *Postharvest Biol. Technol.* **2013**, *79*, 24–31. [CrossRef]
28. Erasmus, A.; Lennox, C.L.; Korsten, L.; Lesar, K.; Fourie, P.H. Imazalil resistance in *Penicillium digitatum* and *P*. italicum causing citrus postharvest green and blue mold: Impact and options. *Postharvest Biol. Technol.* **2015**, *107*, 66–76. [CrossRef]
29. Vas, A.; Korpics, E.; Dernovics, M. Follow-up of the fate of imazalil from post-harvest lemon surface treatment to a baking experiment. *Food Addit. Contam.* **2015**, *32*, 1875–1884. [CrossRef]
30. Palou, L.; Smilanick, J.L.; Crisosto, C.H.; Mansour, M. Effect of Gaseous Ozone Exposure on the Development of Green and Blue Molds on Cold Stored Citrus Fruit. *Plant Dis.* **2001**, *85*, 632–638. [CrossRef] [PubMed]
31. Xue, W.; Macleod, J.; Blaxland, J. The use of ozone technology to control microorganism growth, enhance food safety and extend shelf life: A promising food decontamination technology. *Foods* **2023**, *12*, 814. [CrossRef] [PubMed]
32. Karaca, H. Use of ozone in the citrus industry. *Ozone Sci. Eng.* **2010**, *32*, 122–129. [CrossRef]
33. Liew, C.L.; Prange, K. Effect of Ozone and Storage Temperature on Postharvest Diseases and Physiology of Carrots (*Daucus carota*, L). *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 563–567. [CrossRef]
34. Palou, L.; Smilanick, J.L.; Crisosto, C.H.; Mansour, M.; Plaza, P. Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. *Crop Prot.* **2003**, *22*, 1131–1134. [CrossRef]
35. Guzel-Seydim, Z.B.; Greene, A.K.; Seydim, A.C. Use of ozone in the food industry. *Swiss Soc. Food Sci. Technol.* **2004**, *37*, 453–460. [CrossRef]
36. Karaca, H.; Velioglu, Y.S. Ozone Applications in Fruit and Vegetable Processing. *Food Rev. Int.* **2007**, *23*, 91–106. [CrossRef]
37. Tzortzakis, N.; Singleton, I.; Barnes, J. Deployment of low-level ozone-enrichment for the preservation of chilled fresh produce. *Postharvest Biol. Technol.* **2007**, *43*, 261–270. [CrossRef]
38. Sharpe, D.; Fan, L.; McRae, K.; Walker, B.; MacKay, R.; Doucette, C. Effects of Ozone Treatment on *Botrytis cinerea* and *Sclerotinia sclerotiorum* in Relation to Horticultural Product Quality. *J. Food Sci.* **2009**, *74*, 250–257. [CrossRef] [PubMed]

39. Ozkan, R.; Smilanick, J.L.; Karabulut, O.A. Toxicity of ozone gas to conidia of *Penicillium digitatum*, *Penicillium italicum* and *Botrytis cinerea* and control of gray mold on table grapes. *Postharvest Biol. Technol.* **2011**, *60*, 47–51. [CrossRef]
40. Hibben, C.R.; Stotzky, G. Effects of ozone on the germination of fungus spores. *Can. J. Microbiol.* **1969**, *15*, 1187–1196. [CrossRef]
41. Achen, M.; Yousef, A.E. Efficacy of ozone against Escherichia coli O157:H7 on apples. *J. Food Sci.* **2001**, *66*, 1380–1384.
42. Palou, L.; Ali, A.; Fallik, E.; Romanazzi, G. GRAS, plant-and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biol. Technol.* **2016**, *122*, 41–52. [CrossRef]
43. Özen, T.; Koyuncu, M.A.; Erbaş, D. Effect of ozone treatments on the removal of pesticide residues and postharvest quality in green pepper. *J. Food Sci. Technol.* **2021**, *58*, 2186–2196. [CrossRef] [PubMed]
44. Li, C.; Chi, K.; Yu, H.; Guo, Y.; Ya, W.; Qian, H. Degradation, migration, and removal of trichlorfon on harvested apples during storage at room temperature. *Food Chem.* **2022**, *381*, 132243. [CrossRef]
45. Garcia-Martin, J.F.; Olmo, M.; Garcia, J.M. Effect of ozone treatment on postharvest disease and quality of different citrus varieties at laboratory and at industrial facility. *Postharvest Biol. Technol.* **2018**, *137*, 77–85. [CrossRef]
46. Smilanick, J.L.; Margosan, D.M.; Gabler, F.M. Impact of Ozonated Water on the Quality and Shelf-life of Fresh Citrus Fruit, Stone Fruit and Table Grapes. *J. Int. Ozone Assoc.* **2002**, *24*, 343–356. [CrossRef]
47. Tzortzakis, N.; Chrysargyris, A. Postharvest ozone application for the preservation of fruits and vegetables. *Food Rev. Int.* **2017**, *33*, 270–315. [CrossRef]
48. Mustapić, L.; Šimunac, K.; Ivić, D.; Novak, A.; Popović, L. Mandarin plant health analysis in the Neretva valley and its management. *Glas. Biljn. Zaštite* **2024**, *24*, 373–384.
49. European Food Safety Authority (EFSA). Outcome of the consultation with Member States and EFSA on the basic substance application for approval of ozone to be used in plant protection as a bactericide, fungicide, insecticide, nematicide and viricide. *EFSA Support. Publ.* **2021**, *18*, 3–121.