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# The effectiveness of ozone in post-harvest preservation of citrus fruits: Preliminary research

# Učinkovitost ozona u očuvanju agruma nakon berbe: Preliminarno istraživanje

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## ABSTRACT

Citrus fruits are among the most widely cultivated crops globally. Recently, exotic species and hybrids like kumquat, limequat, and lime have gained popularity due to their rich content of antioxidants, polyphenols, essential oils, minerals, and vitamins. With growing consumer demand for safe and organic produce, especially for fruits consumed with their peel, extending the shelf life of fresh produce while preventing mould contamination is crucial. Traditionally used pesticides, such as imazalil, often leave residues and face increasing resistance, prompting the need for more environmentally friendly methods. Ozone treatment is being explored as a viable alternative to slow down fruit decay and extend shelf life. This research aimed to evaluate the effectiveness of ozone gas treatments on kumquat, limequat, and lime fruits inoculated with blue mould (*Penicillium italicum*). The study also monitored the effectiveness of imazalil fungicide in extending the shelf life of infected fruits. Ozone treatment variants included single, double, and triple exposures lasting 10, 30, and 60 minutes, with ozone concentrations ranging from 3.3 to 20 ppm. Results showed that ozone treatments were successful in extending shelf life. Specifically, 30-minute treatments with 20 ppm ozone reduced infection rates to 10-50% of fruits. Compared to conventional fungicide methods, ozone treatments produced equal or superior outcomes, suggesting that ozone could partially or fully replace traditional fungicidal treatments. These findings provide valuable insights into extending the shelf life of exotic citrus fruits using ozone, offering a promising alternative to chemical fungicides.

**Keywords:** exotic citrus fruits, ozone treatments, shelf life, mould infestation, fungicide resistance

## SAŽETAK

Agrumi su među najraširenijim poljoprivrednim kulturama na svijetu. U posljednje vrijeme, egzotični plodovi poput kumkvata, limkvata i limete postali su popularni zbog visokog sadržaja antioksidansa, polifenola, esencijalnih ulja, minerala i vitamina. S rastućom potražnjom potrošača za sigurnim i ekološki uzgojenim proizvodima, posebno kod onih koji se konzumiraju s korom, produženje roka trajanja svježih proizvoda i sprječavanje kontaminacije fitopatogenim gljivama postaje sve važnije. Tradicionalno korišteni pesticidi, poput imazalila, često ostavljaju rezidue i suočavaju se s rastućom rezistentnošću, što iziskuje potrebu za ekološki prihvatljivijim metodama. Tretmani ozonom istraženi su kao održiva alternativna metoda za usporavanje propadanja voća i produženje roka trajanja. Ovo istraživanje imalo je za cilj utvrditi učinkovitost tretmana plinovitim ozonom u usporedbi s fungicidom imazalilom na kumkvatu, limkvatu i limeti zaraženima plavom plijesni (*Penicillium italicum*). Također je praćena učinkovitost u produženju roka trajanja zaraženog voća. Varijante tretmana ozonom uključivale su jednostruko, dvostruko i trostruko izlaganje u trajanju od 10, 30 i 60 minuta, s koncentracijama ozona od 3,3 do 20 ppm. Rezultati su pokazali da su tretmani ozonom uspješni u produženju roka očuvanja agruma. Tretmani od 30 minuta s 20 ppm ozona smanjili su zarazu na 10-50% plodova. U usporedbi s konvencionalnim metodama zaštite fungicidima, tretmani ozonom postigli su jednake ili bolje rezultate, što sugerira da bi ozon mogao djelomično ili potpuno zamijeniti tradicionalne tretmane. Ovi rezultati pružaju vrijedne uvide u produženje roka trajanja egzotičnih plodova citrusa pomoću ozona, nudeći obećavajuću alternativu kemijskim tretmanima.

**Ključne riječi:** agrumi, ozoniranje, skladištenje, patogeni, rezistentnost

## INTRODUCTION

Citrus fruits are one of the most widely grown agricultural crops in the world. They are cultivated on a large scale and are a popular choice in people's daily diets. Citrus fruits are grown in over 140 countries, with the majority being produced in the Northern Hemisphere in tropical and subtropical regions such as Brazil, China, India, USA, Mexico and Spain (Liu et al., 2012; Agusti et al., 2014; Cheng et al., 2020). The global export of citrus fruits is estimated at around 11 million tons, with oranges accounting for more than 40% (Bahtta, 2022). Their importance lies in their diversified use, as they are consumed either as fresh fruit or as fruit juice (Talibi et al., 2014).

Some of the popular choices by consumers in recent years include exotic citrus fruits like kumquat, limequat and lime. They contain potent antioxidants, polyphenols, essential oils, minerals and vitamins, all of which have health-promoting benefits. Unlike most, these citrus fruits are eaten whole, with their peel (Agalar et al., 2021; Li et al., 2022; Karahuseyin and Nenni, 2023; Qi et al., 2023).

In recent years, consumer demand for safe and organically grown produce has increased (Shah et al., 2019), especially for food eaten with peel. The shelf life of fresh produce is limited and is determined by the initial quality of the product at harvest and the storage conditions (Glowacz et al., 2014; Shah et al., 2019). Studies show that between 30 and 50% of all food produced worldwide is lost and not consumed (Zainuri and Hasim, 2021). The losses occur at various stages of production, storage, packaging, retail and consumption. For citrus fruits, losses due to fruit rot typically range from 10-30% but can escalate to as much as 50% under severe conditions (Bahtta, 2022).

Rot caused by green mould (*Penicillium digitatum*) and blue mould (*Penicillium italicum*) is the most important post-harvest disease in citrus fruit (Di Renzo et al., 2005; Ashebre, 2015). It is stated that *P. italicum* and *P. digitatum* are together responsible for 80% of total post-harvest citrus fruit decay in Mediterranean climates (Nunes et al., 2007). Their spores are present on the fruit surface and become active when the peel is damaged. Injuries to the fruit tissue can be caused by insects, branches or improper handling of the fruit during harvest (Droby et al., 2008; Papoutsis et al., 2019). The severity of losses caused by these fungi depends on the growing region, variety, climatic conditions and post-harvest handling (Moscoso-Ramirez et al., 2013; Sukorini et al., 2013; Papoutsis et al., 2019). *Penicillium digitatum* and *P. italicum* have a relatively short infection cycle, which lasts 3 to 5 days at 25 °C (Bahtta, 2022). On infected fruits, these fungi can produce up to two billion conidia which are spread by the wind. Under favorable conditions, green and blue mould can cause 60-80% rot. After harvest, citrus fruits are stored and handled in packing houses to preserve their shelf life and post-harvest quality and reduce the risk of rot caused by post-harvest pathogens. In untreated fruit, the loss due to fungal attack during post-harvest treatment and commercialization is estimated to be up to 90% (Bahtta, 2022).

The loss indicates the need for post-harvest treatments to prevent pathogenic and phytotoxic damage. A widely used method of controlling green and blue mould is the use of post-harvest fungicides, such as o-phenylphenole, imazalil or thabendazole (Suckorini et al., 2013). One of the most used agents to protect citrus fruit is imazalil (Smilanick et al., 1997; Moscoso-Ramirez et al, 2013; Sukorini et al, 2013; Erasmus et al., 2015; Vass et al, 2015; Papoutsis et al, 2019). Although it is effective, the problem with the use of imazalil is the accumulation of its residues in citrus peel, especially since citrus peel is often used in food preparation. Kruve et al. (2007) has shown that a small amount of imazalil residues were present in both the pulp and the peel. There are various washing methods used to remove phytochemicals from the fruit, but none of them were able to remove imazalil to any significant extent (Vass et al., 2015).

In light of the concentration of residues, the increasing development of resistance, and the negative effects of pesticides on human health should be carefully considered (Palou et al., 2001). Alternative methods are needed to protect citrus plants from mould infestation (Palou et al., 2001; Glowacz et al., 2014; Shah et al., 2019). New methods to reduce unwanted microbiological contamination and spoilage and to preserve the freshness and nutritional value of products are critical factors in production and distribution (Glowacz et al., 2014; Shah et al., 2019). Non-thermal methods that are becoming increasingly popular include the use of ozone, ultraviolet radiation (UV-C), pulsed electric fields (PEF) and high-pressure processing (HPP) (Shah et al., 2019).

Ozone is a triatomic oxygen molecule and a powerful antimicrobial substance due to its oxidative properties. It decomposes to oxygen either spontaneously or after coming in contact with oxidizable surfaces (Liew and Prange, 1994). Ozone is used for water disinfection, swimming pool cleaning and wastewater treatment. Since 1997, it has been classified as GRAS (Generally Recognized as Safe) by the US FDA (Food and Drug Administration) (USDA, 1997) and is therefore approved as a disinfectant for food production (Palou et al., 2003; Guzel-Seydim et al., 2004).

Ozone has an antimicrobial effect on bacteria, fungi, parasites and viruses. It rapidly inactivates microorganisms by reacting with intracellular enzymes, nucleic acids, components of cell membranes, spore envelopes or viral capsids. Its high oxidation potential enables the oxidation of substances in air and water. The oxidation of substances in the air is of great importance, as the spores of *Penicillium* species are primarily transmitted through airborne spores (Palou et al., 2001; Karaca and Velioglu, 2007; Tzortzakis et al., 2007; Sharpe et al., 2009; Ozkan et al., 2011). Ozone treatment of fruit delays the development of blue and green mould (Boonkorn et al., 2012; Horvitz and Cantalejo, 2014). Previous studies have shown that ozone inhibits spore germination of *Botrytis cinerea* by 99.5% and reduces spore viability from 99% to 0.3% (Sharpe et al., 2009). It also delays the production of conidia on fruit infected with *P. digitatum* and *P. italicum* (Hibben and Stotzky, 1969; Palou et al., 2003; Ozkan et al., 2011).

Karaca (2010) states that ozone exposure to cold-stored oranges and lemons did not prevent the occurrence of postharvest mould but influenced its development delay. Additionally, a study was conducted to explore the use of ozone in treating clementines, lemons, oranges, and tangerines, specifically investigating the effect of gaseous ozone on sporulation and mould development paper highlights the successful reduction in sporulation and delayed mould development through gaseous ozone treatment, as well as ozone-treated water for oranges (Horvitz and Cantalejo, 2014).

The use of ozone has considerable potential for reducing microbiological contamination during the processing of citrus fruits. It is essential to fully understand the possible advantages and disadvantages of ozone application in order to conduct further experiments in the production of citrus fruits and improve their shelf life (Glowacz et al., 2014).

The aim of this study was to evaluate the effectiveness of gaseous ozone in delaying the appearance of blue and green mould on exotic citrus fruits grown in Croatia, including kumquat, limequat and limes.

## MATERIALS AND METHODS

This study was conducted at the storage facility in Opuzen, Croatia, in the period from February to March 2022. The controlled storage temperature was 10-12 °C, and the humidity was 50–60% throughout the study period. The volume of the storage facility was 12 $m^2$.

### *Fruits in the experiment*

In the study, a total of three types of fruit (kumquat, limequat and lime) were tested, which were purchased directly from the orchard by a local producer. The fruits were not treated with anything before the experiment. An isolate of *P. italicum* PI-M3, originating from Satsuma mandarin, was used for inoculation. It was grown on potato-dextrose agar (PDA) for 10 days at 25 °C, after which conidia were harvested and suspended in water. The final spore concentration for inoculation was adjusted with a hemocytometer to about $10^5$ condia/ml. One day before conducting the experiment, all fruits were artificially infected with a suspension of *P. italicum* in water.

Two variants of the experiment were treated with imazalil in accordance with standard treatments of citrus fruit on recommendation in industrial warehouses/refrigerators. Fungicidal variant 1 was treated once with imazalil, while the fungicidal variant 2 was treated twice with imazalil, half an hour apart. The control variant was not treated with anything. The imazalil (NEOZIL 50C) agent was applied in a concentration of 0.1% (100 mL agent/100 L water) by soaking the fruits with the "drencher" system.

### *Ozonation*

An ozonator of the OZ-10 series, 130 W ozone generator was used for ozonation. This device uses an electrical charge to convert $O_2$ from the air into $O_3$ ozone. The ozone output of 20 mg/$m^3$ was used. The ozone output could not be changed, and the time of exposure to ozone also defined the amount of ozone used as the test variable.

Considering the test parameters, we initiated the study to determine the ozone production per hour and then determine the ozone concentration in parts per million (ppm) within the storage chamber.

Ozonation was carried out in three groups. The first group consisted of the 10-, 30- and 60-minute variants and they were ozonated only once. The second group consisted of the 10-, 30- and 60-minute variants, which were ozonated on two consecutive days with an interval of 24 hours between treatments. The third group consisted of the 10-, 30- and 60-minute variants that were ozonated on three consecutive days with an interval of 24 hours between treatments.

There were a total of 12 variants in the trial (two treated with fungicide, three treated with ozone in three ozonation groups and the control variant). Each variant was planted in four replicates. In each replicate, there were five fruits of a single citrus species. A total of 20 fruits per variant were treated so a total of 240 fruits per citrus species were tested in this study. During the experiment, the fruits (per replicate) were stacked in cardboard boxes (standard for citrus storage), with the minimized possibility of contact between the fruits to exclude the spread of infection through contact.

### *Readings and data analysis*

The measurements were carried out four times (7, 14, 21 and 28 days after the start of the experiment). Each individual fruit was analyzed and the presence of infection with *P. italicum* was determined. The percentage of infected fruit (disease incidence: %) per variant to determine the effect of ozone on disease development at different exposure times was subjected to an analysis of variance ANOVA (ARM 2023.6 GDM software), with the mean distance estimated using the Duncan Multiple Range Test (MRT).

## RESULTS

At the first reading, seven days after treatment, the differences between the variants are visible (Table 1).

**Table 1.** Infected kumquat fruits (% ± SE) after treatments

| Imazalil | No. of treatments | Duration of ozonation (min) | Infected fruits (%) kumquat | | | |
|---|---|---|---|---|---|---|
| | | | Reading 1 | Reading 2 | Reading 3 | Reading 4 |
| Fungicide | 1 | - | 5.0 ± 5.0[bc*] | 15.0 ± 5.0[bc] | 15.0 ± 5.0[cd] | 40.0 ± 0.0[ef] |
| | 2 | - | 5.0 ± 5.0[bc] | 5.0 ± 5.0[c] | 15.0 ± 5.0[cd] | 35.0 ± 5.0[f] |
| | 1 | 10 | 5.0 ± 5.0[bc] | 40.0 ± 8.3[a] | 55.0 ± 5.0[ab] | 80.0 ± 0.0[ab] |
| | 1 | 30 | 1.0 ± 0.0[c] | 45.0 ± 5.0[a] | 60.0 ± 8.2[a] | 75.0 ± 5.0[ab] |
| | 1 | 60 | 5.0 ± 5.0[bc] | 5.0 ± 5.0[c] | 35.0 ± 5.0[bc] | 50.0 ± 5.8[def] |
| | 2 | 10 | 15.0 ± 5.0[ab] | 40.0 ± 0.0[a] | 50.0 ± 5.8[ab] | 55.0 ± 5.0[cde] |
| | 2 | 30 | 0.5 ± 1.0[c] | 40.0 ± 14.1[a] | 55.0 ± 15.0[ab] | 65.0 ± 12.6[bcd] |
| | 2 | 60 | 5.0 ± 5.0[bc] | 5.0 ± 5.0[c] | 10.0 ± 5.8[d] | 50.0 ± 5.8[def] |
| | 3 | 10 | 10.0 ± 5.8[bc] | 30.0 ± 5.8[ab] | 55.0 ± 5.0[ab] | 70.0 ± 5.8[bc] |
| | 3 | 30 | 15.0 ± 5.0[ab] | 25.0 ± 5.0[abc] | 35.0 ± 5.0[bc] | 35.0 ± 5.0[f] |
| | 3 | 60 | 5.0 ± 5.0[bc] | 5.0 ± 5.0[c] | 5.0 ± 5.0[d] | 15.0 ± 5.0[g] |
| Control | - | - | 25.0 ± 5.0[a] | 45.0 ± 5.0[a] | 60.0 ± 8.2[a] | 90.0 ± 5.8[a] |
| LSD *P* = 0.05 | | | 12.5 | 19.1 | 61.1 | 60.0 |
| Standard Deviation | | | 8.6 | 13.3 | 14.6 | 11.8 |
| CV | | | 109.3 | 55.0 | 40.7 | 20.8 |
| P (Shapiro-Wilk) | | | 0.0005 | 0.0056 | 0.008 | 0.0095 |
| Treatment F | | | 2.7 | 5.9 | 7.4 | 14.1 |
| Treatment Prob(F) | | | 0.0114 | 0.0001 | 0.0001 | 0.0001 |

*Means followed by the same letter or symbol do not significantly differ (*P* = 0.05, Duncan's New MRT)

The control variant without treatment had a significantly higher percentage of infected fruit (25%; $P \leq .0$). The lowest infection was found in the one-time ozone treatment of 30 minutes (1%) and the two-time same treatment of only 0,5%. Two-time treatment with 10 minutes resulted in 15% which was the same as the triple ozone treatment of 30 minutes. The rest of the treatments showed a result of 5%. After 14 days, a significant infection was found in the control variant (45%) and the ozone-treated variants of 10 and 30 minutes (30-45%). The significantly lowest infection was found in the variant treated twice with fungicide (5%), which did not differ from the infection in all ozonated variants after 60 minutes. In the third assessment, the highest infestation was again found on the control variant (60%) and all ozonated variants (10 and 30 minutes). The lowest proportion of infected fruit was found in the triple ozonated variant (60 minutes) (5%). Four weeks after the start of the trial, a significant infection of the fruit with *P. italicum* was detected in all varieties. In the control variant, 90% of the fruits were infected, while the significantly lowest infection was found in the 60-minute triple ozonated variety (15%). No significant differences were found between the other variants of the experiment.

In the case of limequat, seven days after treatment, significant differences among the variants were not observed except for the control, which showed the highest fruit infection rate (32,5%) (Table 2). The lowest fruit infection rates were 5% with fungicide treatment, one-time ozone treatment for 10 and 30 minutes, two-time ozone treatment for 60 minutes, and triple ozone treatment for 30 and 60 minutes. Other variants showed fruit infection rates of 10%. After 14 days post-treatment, the highest infection was observed in the one-time ozone treatment for 10 minutes (25%) and the control variant (35%). The lowest infection rates were observed in the one-time 30-minute, two-time 60-minute, and three-time 60-minute variants (5%). Other variants showed 10% infection rates, except for the two-time and triple-time ozone treatment for 30 minutes, where the infection rate was 15%. After 21 days, the highest infection was again found in the control variant (45%) and the lowest (10%) in the fungicide-treated variant, one-time ozone treatment for 60 minutes, two-time ozone treatment for 60 minutes, and triple ozone treatment for 60 minutes. Other variants showed disease incidence ranging from 20-30%. After four weeks, the highest fruit infection rate was observed in the control variant (70%). The lowest infection was detected in all three 60-minute variants (10%). Other variants resulted in fruit infection rates ranging from 20 to 35%.

**Table 2.** Infected limequat fruits (% ± SE) after treatments

| Variant | No. of treatments | Duration of ozonation (min) | Infected fruits (%) limequat | | | |
|---|---|---|---|---|---|---|
| | | | Reading 1 | Reading 2 | Reading 3 | Reading 4 |
| Imazalil | 1 | - | 5.0 ± 5.0$^{b*}$ | 5.0 ± 5.0$^{c}$ | 10.0 ± 5.8$^{b}$ | 30.0 ± 5.8$^{bc}$ |
| | 2 | - | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{c}$ | 15.0 ± 9.6$^{b}$ | 30.0 ± 12.9$^{bc}$ |
| Ozone | 1 | 10 | 5.0 ± 5.0$^{b}$ | 25.0 ± 5.0$^{ab}$ | 30.0 ± 5.8$^{ab}$ | 35.0 ± 5.0$^{b}$ |
| | 1 | 30 | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{c}$ | 25.0 ± 9.6$^{ab}$ | 30.0 ± 10.0$^{bc}$ |
| | 1 | 60 | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{bc}$ | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{c}$ |
| | 2 | 10 | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{bc}$ | 20.0 ± 11.5$^{b}$ | 30.0 ± 10.0$^{bc}$ |
| | 2 | 30 | 10.0 ± 5.8$^{b}$ | 15.0 ± 5.0$^{bc}$ | 25.0 ± 5.0$^{ab}$ | 25.0 ± 5.0$^{bc}$ |
| | 2 | 60 | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{c}$ | 10.0 ± 5.8$^{d}$ | 10.0 ± 5.8$^{c}$ |
| | 3 | 10 | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{bc}$ | 30.0 ± 5.8$^{ab}$ | 35.0 ± 5.0$^{b}$ |
| | 3 | 30 | 5.0 ± 5.0$^{b}$ | 15.0 ± 5.0$^{bc}$ | 20.0 ± 8.2$^{b}$ | 20.0 ± 8.2$^{bc}$ |
| | 3 | 60 | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{c}$ | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{c}$ |
| Control | - | - | 32.5 ± 4.8$^{a}$ | 35.0 ± 5.0$^{a}$ | 45.0 ± 5.0$^{a}$ | 70.0 ± 5.8$^{a}$ |
| LSD *P* = 0.05 | | | 14.71 | 14.33 | 16.68 | 18.75 |
| Standard Deviation | | | 10.2 | 9.9 | 12.9 | 13.0 |
| CV | | | 114.1 | 82.4 | 62.3 | 46.7 |
| P (Shapiro-Wilk) | | | 0.006 | 0.0874 | 0.1694 | 0.1943 |
| Treatment F | | | 2.3 | 3.5 | 2.7 | 6.2 |
| Treatment Prob(F) | | | 0.0303 | 0.0022 | 0.0112 | 0.0001 |

*Means followed by the same letter or symbol do not significantly differ (*P* = 0.05, Duncan's New MRT)

For lime, 7 days after the experiment, the highest fruit infection rate was recorded in the control variant (35%), while the other variants did not exhibit significant differences among themselves, with infection rates ranging from 5% to 15% (Table 3). During the second assessment, the lowest infection was found in variants treated with fungicide and triple ozone treatment for 60 minutes (5%), while the highest infection rate was recorded in the control variant at 45%. For other ozone-treated variants, infection rates ranged from 10 to 20%. In the third assessment, after 21 days, the highest infection was by far detected in the control variant (50%), while the lowest (5) was in the triple ozone treatment for 60 minutes. Fungicide-treated fruits showed a 10% infection rate. Ozone-treated fruits exhibited infection rates from 15 to 25%. After 28 days, the highest infection was recorded in the control group, at 65% of the fruits. The lowest detected infection was with the triple ozone treatment for 60 minutes and fungicide treatment (10-15%). Other variants showed infection rates from 20 to 35%, with 35% detected in the two-time ozone treatment for 10 minutes.

**Table 3.** Infected lime fruits (% ± SE) after treatments

| Variant | No. of treatments | Duration of ozonation (min) | Infected fruits (%) lime | | | |
|---|---|---|---|---|---|---|
| | | | Reading 1 | Reading 2 | Reading 3 | Reading 4 |
| Imazalil | 1 | - | 5.0 ± 5.0$^{b*}$ | 5.0 ± 5.0$^{b}$ | 10.0 ± 5.8$^{cd}$ | 15.0 ± 5.0$^{cd}$ |
| | 2 | - | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{b}$ | 10.0 ± 5.8$^{cd}$ | 10.0 ± 5.8$^{d}$ |
| Ozone | 1 | 10 | 15.0 ± 5.0$^{b}$ | 20.0 ± 0.0$^{b}$ | 25.0 ± 5.0$^{bc}$ | 30.0 ±5.8$^{bc}$ |
| | 1 | 30 | 10.0 ± 5.8$^{b}$ | 15.0 ± 9.6$^{b}$ | 20.0 ± 8.2$^{bcd}$ | 25.0 ± 5.0$^{bcd}$ |
| | 1 | 60 | 5.0 ± 5.0$^{b}$ | 10.0 ± 5.8$^{b}$ | 15.0 ± 5.0$^{cd}$ | 20.0 ± 8.2$^{bcd}$ |
| | 2 | 10 | 10.0 ± 5.8$^{b}$ | 20.0 ± 8.2$^{b}$ | 35.0 ± 5.0$^{b}$ | 35.0 ± 5.0$^{b}$ |
| | 2 | 30 | 5.0 ± 5.0$^{b}$ | 15.0 ± 5.0$^{b}$ | 15.0 ± 5.0$^{cd}$ | 25.0 ± 5.0$^{bcd}$ |
| | 2 | 60 | 5.0 ± 5.0$^{b}$ | 10.0 ± 5.8$^{b}$ | 10.0 ± 5.8$^{cd}$ | 20.0 ± 0.0$^{bcd}$ |
| | 3 | 10 | 10.0 ± 5.8$^{b}$ | 15.0 ± 9.6$^{b}$ | 20.0 ± 8.2$^{bcd}$ | 25.0 ± 9.6$^{bcd}$ |
| | 3 | 30 | 5.0 ± 5.0 $^{b}$ | 10.0 ± 5.8$^{b}$ | 15.0 ± 5.0$^{cd}$ | 20.0 ± 0.0$^{bcd}$ |
| | 3 | 60 | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{b}$ | 5.0 ± 5.0$^{d}$ | 10.0 ± 5.8$^{d}$ |
| Control | - | - | 35.0 ± 5.0$^{a}$ | 45.0 ± 5.0$^{a}$ | 50.0 ±5.8$^{a}$ | 65.0 ± 5.0$^{a}$ |
| LSD *P* = 0.05 | | | 15.0 | 18.3 | 14.82 | 15.3 |
| Standard Deviation | | | 10.4 | 12.7 | 10.3 | 10.6 |
| CV | | | 109.1 | 97.2 | 53.7 | 42.5 |
| P (Shapiro-Wilk) | | | 0.0019 | 0.0111 | 0.0202 | 0.4943 |
| Treatment F | | | 2.7 | 2.9 | 5.9 | 7.5 |
| Treatment Prob(F) | | | 0.0124 | 0.0076 | 0.0001 | 0.0001 |

*Means followed by the same letter or symbol do not significantly differ (*P* = 0.05, Duncan's New MRT)

## DISCUSSION

Through our research, we aimed to demonstrate how the utilization of ozone as a substitute for chemical agents can mitigate pathogens on citrus fruits, thereby mitigating their adverse environmental effects. The study was conducted to assess the effectiveness of gaseous ozone treatment in prolonging the shelf life of exotic citrus fruits. The results we obtained indicate the following: i) treatments with gaseous ozone positively impact the extension of the storage life of exotic fruits, ii) gaseous ozone treatments can partially or completely replace standard fungicide treatments.

In our research, the positive effect of ozone on suppressing blue moulds on kumquats, limequats, and limes was determined. In the case of kumquats, the most effective treatment after 28 days was a triple treatment with gaseous ozone lasting 60 minutes at a concentration of 20 ppm, resulting in only 35% of the fruit being infected. For limequats, the most effective treatments were ozone treatments lasting 60 minutes at 20 ppm. With a single ozone treatment of 60 minutes, only 10% of the limequat fruit was infected. The same result was obtained with double and triple ozone treatments. For limes, the most effective treatment was a triple treatment of 60 minutes at 20 ppm, resulting in only 10% of the fruit being infected. Single and double ozone treatments of 60 minutes resulted in 20% infected lime fruit.

The results obtained aligned with previous studies on the effects of ozone in extending the shelf life of citrus fruits, indicating that ozone effectively slows the progression of post-harvest diseases in clementines, oranges, mandarins, lemons, and tangerines. Studies on mandarins and oranges were conducted with 60 ppm ozone for 28 days. Clementines were initially inoculated with *B. cinerea* spores and then exposed to µmol/mol ozone for 8 days. Inoculated lemons were treated with 0.3 ppm ozone in day-night cycles for 3 weeks. Inoculated oranges were also treated with 0.3 ppm ozone for one week. A slowed mould development was observed in all fruits, but complete prevention did not occur (Palou et al., 2007; Garcia-Martin et al., 2018). Another study on oranges states that a combination of low temperature (5 °C) and high relative humidity (90-95%) with intermittent ozone treatment could reduce postharvest losses in orange fruit during storage (Di Renzo et al., 2005). In our research, no treatment resulted in the complete absence of blue mould, but in all variants, it was significantly lower compared to the control group, where the incidence of infection ranged from 65 to 90% on all fruits. The fact that we used higher temperatures during our research (10-12 °C) and relatively low air humidity (50-60%) was for the purpose of creating ideal environment for intensive mould growth so that we can obtain information on the full potential of ozone treatments.

Given that the fruit in our study was pre-inoculated with mould spores, the infection was stronger and more persistent than it would have been through the development of natural disease. Garcia-Martin et al. (2018), observing the impact of ozone in 12-hour cycles, found that un-inoculated fruit showed no mould infection after 28 days.

Palou et al. (2007) state that exposing oranges and lemons to low levels of gaseous ozone did not reduce the final incidence of post-harvest moulds; however, the infection developed significantly slower in fruit stored under ozonated conditions. They conclude that ozone certainly inhibits sporulation as long as the fruit is stored in an ozonated atmosphere but does not kill fungal spores on the fruit's surface. They note that with higher ozone concentrations (higher than 0.3 ppm), the results could be better in favor of extending the shelf life of citrus fruits. We confirmed such a claim with our research, where the highest success in suppressing the occurrence of green and blue moulds was with the treatment with the longest exposure to ozone (60 minutes). Smilanick et al. (1999) stated that a contact time of two minutes in 1.5 ppm ozone kills 95-100% of eight analyzed fungi, and at three minutes, none survived. They mention that the spore problem is resolved by treating the fruit with ozonated water. Smilanick et al. (2002) stated that green mould and sour rot on citrus caused by *P. digitatum* and

*Geotrichum citri-aurantii* were not reduced when fruit was treated for 20 minutes in 10 ppm aqueous ozone. In our research, treatments of 60 minutes (one-time, two-time, and triple) were applied with 20 ppm ozone, which showed a reduction in the occurrence of blue mould on kumquat, limequat, and lime fruits, but the disease was not completely eliminated. In the case of limequat and lime, all three variants of 60 minutes showed satisfactory success (only 10% infected fruit), while with kumquat, this was the case only with the triple ozone treatment (15% infected fruit).

The use of fungicides on all three citrus fruits resulted in 10-40% infected fruit, correlating with the results obtained using ozone treatments. To reduce the use of fungicides and other chemical agents, especially for fruits consumed with the peel where residues of chemical agents accumulate (Bonkoorn et al., 2012). In relation to that, research was also conducted to study the impact of gaseous ozone on pesticide degradation. Oranges were stored in an ozone atmosphere ranging from 0.18 to 0.20 ppm for 35 days, resulting in reduced levels of imazalil, malathion, and chlorpyrifos (Metzger et al., 2007). This research suggests the possibility of using ozone not only as a pesticide substitute but also as a means of pesticide degradation. Karaca and Velioglu (2007) also state that pesticide and toxin residues followed by fungal and bacterial contamination can also be reduced by applying ozone treatments.

Developing the best method for ozone utilization to extend the shelf life of fruit is a worthwhile investment, considering that ozone leaves no residues, which is crucial, especially for fruits where the peel is consumed, such as citrus fruits. The USDA National Organic Program has classified ozone as organic, and reliable ozone generators are now widely available (Feliziani et al., 2014). Research conducted on strawberries that were ozone-treated for three days in storage showed a threefold increase in vitamin C concentration compared to the control group (Skog and Chu, 2001). Such results could also benefit citrus fruits, given their valued nutritional composition and importance in diets. One of the main benefits of ozone is its rapid decomposition into oxygen, eliminating residues in the space that could be harmful to warehouse workers and economizing food processing time in storage areas (Horvitz and Cantalejo, 2014).

On the other hand, if ozone is improperly used, it can cause some deleterious effects on the treated produce, such as losses in sensory quality and nutritional value. To achieve effective and successful results, optimum conditions such as ozone concentration and treatment time should be separately defined for each application. Also, due to the development stages of ozonation for commercial use in food products, the cost of ozone generators is unfortunately still quite high. Having that in mind, a large amount of capital investment is required before putting ozone into use (Karaca, 2010).

Ozone should be considered as a suitable alternative, especially with the implementation of the European Green Deal, which tends to achieve a mandatory 50% reduction in pesticide use by 2030 (European Commission, 2022). Furthermore, considering that ozone is classified as "organic" and is currently in the process of recognition as a basic substance in plant protection with bactericidal, fungicidal, insecticidal, nematicidal, and viricidal effects (EFSA, 2021), we can conclude that ozone is a substance of the future that will greatly contribute to the process of food protection, storage, and processing. Additional research is needed with different ozone concentrations to determine its definitive potential for wide application in the industry, but there is certainly great potential for extending the shelf life of citrus fruits, all in the spirit of environmentally friendly solutions.

## CONCLUSIONS

This study demonstrates the successful extension of the shelf life of exotic citrus fruits (kumquat, limequat, and lime) using ozone treatments. The effectiveness of the treatments was influenced by the duration and concentration of ozone exposure. Compared to conventional fungicidal methods, ozone treatments provided comparable or superior outcomes, highlighting their potential to partially or fully replace standard fungicides. These findings offer valuable insights into strategies for prolonging the shelf life of exotic citrus fruits, which are widely consumed and integral to the diet of the general population. Given the European Union's emphasis on environmentally friendly and sustainable practices, ozone emerges as a promising alternative that warrants further in-depth investigation.

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