

Ozonated water for the control of banana rot during storage

Água ozonizada no controle de podridões em bananas durante o armazenamento

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Highlights

Temperature affected the final concentration of dissolved ozone in drinking water.

Organic matter reduced ozone half-life in water by 128 times.

Ozonated water was not efficient in controlling banana rot during storage.

Abstract

Banana is a crop of great economic and socio-environmental importance, with significant losses occurring throughout the production chain, particularly during the post-harvest phase. The use of ozone has emerged as a promising and safe alternative to conventional chemical treatments to minimize these losses. This study aimed to evaluate the kinetic models of ozone reactions in water at different temperatures and to determine the effectiveness of immersion in ozonated water for controlling banana rot during storage. Five treatments were tested on the 'Nanicão' banana variety: control (no immersion), immersion in water without ozone (two and three cycles), and immersion in ozonated water (two and three cycles). Each cycle lasted 15 min. After treatment, the fruits were stored for 9 days at 25 °C and 75% RH. The results showed that the highest ozone concentration and the longest half-life (117.6 min) were achieved at 15 °C. However, in the presence of bananas, the half-life was drastically reduced to only 0.9 min. No significant effect of ozonated water treatment was observed in controlling rot during storage. It is concluded that, although the ozone half-life is longer at 15 °C, the organic matter present in bananas substantially reduces this time, and immersion in ozonated water was not effective in controlling post-harvest rot.

Key words: *Musa* spp. Ozone. Kinetic models. Saturation. Decay. Post-harvest.

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Resumo

A banana é uma cultura de grande relevância econômica e socioambiental, com perdas significativas ocorrendo ao longo da cadeia de produção, especialmente na fase de pós-colheita. O uso de ozônio surge como uma alternativa promissora e segura aos tratamentos químicos convencionais para minimizar essas perdas. Este estudo teve como objetivo avaliar os modelos cinéticos da reação do ozônio em água em diferentes temperaturas e determinar a eficácia da imersão em água ozonizada no controle de podridões em bananas durante o armazenamento. Desta forma, foram testados cinco tratamentos na variedade de banana 'Nanicão': controle (sem imersão), imersão em água sem ozônio (dois e três ciclos), e imersão em água ozonizada (dois e três ciclos). Cada ciclo durou 15 min. Após o tratamento, os frutos foram armazenados por 9 dias a 25 °C e 75% UR. Os resultados mostraram que a maior concentração de ozônio e o tempo de meia-vida mais prolongado (117,6 min) foram alcançados a 15 °C. No entanto, na presença de bananas, a meia-vida foi drasticamente reduzida para apenas 0,9 min. Não houve efeito significativo do tratamento com água ozonizada no controle das podridões durante o armazenamento. Conclui-se que, embora a meia-vida do ozônio seja maior a 15 °C, a matéria orgânica presente na banana reduz substancialmente esse tempo, e a imersão em água ozonizada não foi eficaz no controle de podridões pós-colheita.

Palavras-chave: *Musa* spp. Ozônio. Modelos cinéticos. Saturação. Decaimento. Pós-colheita.

The banana (*Musa* spp.) is one of the most popular and widely consumed fruits in the world. Its accessibility, practicality, and nutritional quality make it attractive to consumers, generating increasing demand and reinforcing its economic and social importance (Organisation for Economic Co-operation and Development/Food and Agriculture Organization of the United Nations [OECD/FAO], 2021). In the international fruit trade, Brazil has potential for the export market but does not occupy top positions as an exporter. Despite the extensive planted area and the volume harvested, the lack of price transparency, limited use of classification criteria, inadequate packaging, and, above all, post-harvest losses significantly affect the supply of this product (Instituto Brasileiro de Geografia e Estatística [IBGE], 2021). Bananas are harvested while still green and are particularly susceptible to mechanical damage, which serves as an entry point for

fungi in the field and leads to physiological changes, premature ripening, and increased weight loss.

The main agent associated with post-harvest rots in bananas is the fungus *Colletotrichum* sp., frequently related to anthracnose. Post-harvest losses are estimated to range from 25% to 50%, with a substantial portion of production lost due to this disease (Al-Dairi et al., 2023). Contamination generally occurs in the field, although symptoms become evident during fruit ripening.

With the growing demand for sustainable agricultural practices and alternative treatment methods that do not leave harmful residues, new technologies have been developed to optimize production. Ozone has been used as a sustainable and safe alternative for pest and disease control in post-harvest systems, increasing the

shelf life of numerous products. Coelho et al. (2015), in an integrative review, concluded that ozone is an eco-friendly technology that offers savings in energy, transportation, and storage costs, as it can be generated at the site of use. Ozone is a compound capable of oxidizing organic substances and exhibits antimicrobial and antifungal activity, as demonstrated in some studies (Chen et al., 2020; Alencar et al., 2013). Its mechanism of action against microorganisms involves the generation of free radicals during ozone decomposition in solution. Because oxygen is its degradation product, ozone represents an alternative to conventional pest control methods, minimizing negative impacts on human health and leaving no environmental residues (Premjit et al., 2022). However, the high reactivity of ozone in aqueous media, particularly in the presence of organic matter, may limit its practical effectiveness, highlighting the need for kinetic studies under realistic conditions.

This study was motivated by the findings of Alencar et al. (2013), who evaluated the effects of gaseous and aqueous ozone in the post-harvest treatment of Cavendish bananas and reported positive results for aqueous ozone. Therefore, the objective of this study was to determine the kinetic models of ozone reactions in water and to evaluate the effectiveness of immersion in ozonated water for controlling banana rot during storage.

Gaseous ozone was generated using an O&L3 ORM ozone generator (Ozone & Life, São Jo-sé dos Campos, São Paulo, Brazil) coupled to a Mark 5 Plus oxygen concentrator (NIDEK Medical, Birmingham, Alabama, USA), which uses ambient air as its source.

To produce ozonated water, water from the municipal distribution network of Viçosa, Minas Gerais, was used at temperatures of 15 °C, 20 °C, and 25 °C. A 70-L stainless steel tank equipped with a refrigeration and recirculation system was employed. After the desired temperature was reached, ozone gas was injected at an initial concentration of 30 mg L⁻¹ and an airflow rate of 2 m³ min⁻¹. The ozone gas was introduced into the tank through a Venturi-type injector, where it was mixed with the water and continuously recirculated throughout the system.

To determine reaction kinetics, dissolved ozone in water was quantified using the iodometric method. Initially, measurements were taken at 3-min intervals, and as the ozone concentration approached stabilization, the intervals were progressively extended. Determination of O₃ concentration was completed after three identical measurements were obtained at 30-min intervals.

The decay curve was determined after interruption of the ozone supply and shutdown of the circulation pump. The O₃ concentration was measured every three minutes, with intervals progressively increased as the decay stabilized, adopting the criterion of three identical determinations at 30-min intervals to conclude the process. The same procedure was applied in the presence of bananas: 6 L of ozonated water were transferred to a 10 L container containing 10 bananas (approximately 600 mL of water per fruit). O₃ concentration determinations were performed every five minutes until the compound became undetectable.

For the study of O₃ decomposition kinetics, the data were fitted to zero-, first-,

and second-order models. The best-fitting model was selected based on comparison of the coefficients of determination (R^2) and analysis of the residuals of the kinetic models.

The 'Nanicão' banana variety (AAA group) was obtained from the Department of Agronomy, Fruit Growing Experimental Field, at the Federal University of Viçosa, Viçosa campus. The fruits were harvested at the pre-climacteric stage, peel color 1, according to a color scale ranging from 1 to 7. In the field, the fruits were sanitized with a 2.0 mL L⁻¹ detergent solution and running water to remove latex residues.

Five treatments were adopted: control (T - control) without immersion; two immersion cycles in tap water (without ozone); three immersion cycles in tap water (without ozone); two immersion cycles in ozonated water; and three immersion cycles in ozonated water.

The ozonated water used for fruit treatment was obtained as previously described, at a temperature of 15 °C, until gas saturation in the water was reached. The inlet ozone gas concentration was 30 mg L⁻¹ and the airflow was 2 m³ min⁻¹. After reaching an ozone concentration in the water of 5 mg L⁻¹, 6 L were removed from the tank and transferred to a 10 L container, into which the bananas were placed. Each immersion cycle lasted 15 min, as determined in preliminary tests.

After completion of the treatments, the bunches were placed in expanded polystyrene trays and stored in climate chambers for nine days at 25 °C and 75% RH. As pathogen isolation and identification were not performed in this study, the

observed symptoms are generically referred to as banana rot. To assess the area affected by rot, a diagrammatic scale developed by Moraes et al. (2008) for bananas was used, with damaged areas ranging from 0 to 64%. Analyses were performed at the beginning of storage and after 3, 6, and 9 days.

A completely randomized design was adopted in a 5x4 factorial arrangement, consisting of five treatments and four storage periods, with nine replications, each represented by one fruit. The data were subjected to analysis of variance (ANOVA), followed by regression analysis or Tukey's test, at a 5% significance level. StatPlus 5.0 software (AnalystSoft Inc, Canada) was used for analysis of variance, and SigmaPlot version 14.0 software (Systat Software Inc, Germany) was used to obtain equations and plots.

Dissolved ozone concentrations in water, obtained from an inlet concentration of 30.0 mg L⁻¹ (C_0), were evaluated at temperatures of 15, 20, and 25 °C. The highest concentration was observed at 15 °C, reaching 5.23 mg L⁻¹ after 84.3 min. The longest stabilization time was recorded at 20 °C, corresponding to 99.5 min, with a concentration of 4.23 mg L⁻¹. The lowest concentration, 2.95 mg L⁻¹, was observed at 25 °C.

It should be noted that water from the municipal distribution network was used in this study, in accordance with Ordinance GM/MS No. 888 of May 4, 2021, due to its greater applicability under real agricultural conditions. This represents a differentiated approach, as many studies employ distilled water under controlled conditions, which differ from the practical scenarios evaluated

here. The temperature range was defined considering the need to maintain values above 10 °C to avoid chilling injury to the banana peel (Chitarra & Chitarra, 1984).

One of the major limitations of using ozone dissolved in water is the concentration achievable in the aqueous medium (Premjit et al., 2022). Although the inlet concentration was 30 mg L⁻¹, the saturation concentration at 15 °C was 5.23 mg L⁻¹. At 25 °C, the saturation concentration corresponded to 56.4% of that obtained at 15 °C. This phenomenon is

attributed to the higher molecular energy, which facilitates ozone decomposition reactions (Galdeano et al., 2018).

The ozone decay kinetics model is essential for optimizing its effectiveness as an antimicrobial agent. Table 1 presents the equations for zero-order, first-order, and second-order kinetic models fitted to residual ozone concentration data in water as a function of time at temperatures of 15, 20, and 25 °C.

Table 1

Regression equations of zero-, first-, and second-order kinetic models describing the residual concentration of aqueous ozone as a function of time, along with their respective coefficients of determination (R²), standard errors of estimate (SEE) and half-lives, in the absence and presence of bananas, for an inlet ozone gas concentration of 30.0 mg L⁻¹ (C₀) at different temperatures

Temperature (°C)	Order	Fitted equations	R ²	SEE	Half-life (min)
Absence of bananas					
15	0	$C = 4.9500 - 0.0188t$	0.9356	0.2604	131.6
	1	$C = 1.6283 - 0.0054t$	0.9716	0.0470	128.4
	2	$C = 0.1850 + 0.0016t$	0.9866	0.099	115.6
20	0	$C = 4.0353 - 0.0229t$	0.9315	0.2492	88.1
	1	$C = 1.4396 - 0.0087t$	0.9847	0.0434	79.7
	2	$C = 0.2112 + 0.0036t$	0.9884	0.0155	58.7
25	0	$C = 2.4357 - 0.0147t$	0.8534	0.2490	82.8
	1	$C = 0.9303 - 0.0093t$	0.9501	0.0877	74.5
	2	$C = 0.3490 + 0.0065t$	0.9885	0.0288	53.7
Presence of bananas					
15	0	$C = 4.4274 - 0.3312t$	0.8871	0.9342	6.7
	1	$C = 2.2905 - 0.3935t$	0.8550	1.2814	1.8
	2	$C = 0.1177 + 0.1173t$	0.9424	0.2049	0.9

The literature indicates that O_3 decomposition in water follows kinetics between first-order and second-order models, with some studies adopting a fractional order of 1.5, indicating intermediate behavior (Gardoni et al., 2012). This approach suggests that the reaction rate is more sensitive to concentration than in first-order reactions but less sensitive than in second-order reactions. In the present study, the model with the highest coefficient of determination (R^2) was selected, which was the second-order kinetic model. The curves obtained from the experimental data corroborate reports in the literature regarding the sensitivity of ozone decomposition to temperature variation, as described by Gardoni et al. (2012).

Regarding ozone half-life in water, a value of 115.6 min was obtained at 15 °C (Table 1). On the other hand, as the temperature increased to 20 and 25 °C, half-life values decreased to 58.7 and 53.7 min, respectively. Previous studies have shown that lower temperatures result in longer ozone half-lives (Premjit et al., 2022), similar to the results observed in this experiment.

With the addition of the fruit, an abrupt reduction in ozone half-life was observed (Table 1). Under this condition, the half-life decreased from approximately 116.0 min to less than 1.0 min. This change can be

explained by the high reactivity of ozone in the presence of organic matter (Gardoni et al., 2012). Banana peel contains antioxidant compounds, organic acids, and volatile constituents that react with ozone molecules, thereby accelerating the decomposition of the gas dissolved in water (Al-Dairi et al., 2023).

Regarding the effect of ozonated water on the control of post-harvest rot in bananas, no significant difference ($P > 0.05$) occurred among the tested treatments, as an increase in the percentage of damaged area was observed during storage in all treatments (Figure 1). The ineffectiveness of ozone in controlling banana rot under the conditions adopted in this study can be attributed to several factors, with ozone concentration in the water being the primary one. Feng et al. (2018), when analyzing the interaction among concentration, exposure time, and microorganism population, concluded that ozone concentration has the greatest impact on microbial control, as low concentrations do not disrupt bacterial membranes, leaving them intact. Furthermore, a review evaluating ozone application in the juice industry showed that its efficiency depends on the inherent sensitivity of each microorganism, with fungi, yeasts, and spore-forming bacteria requiring higher ozone concentrations (Akbas & Ozdemir, 2008).

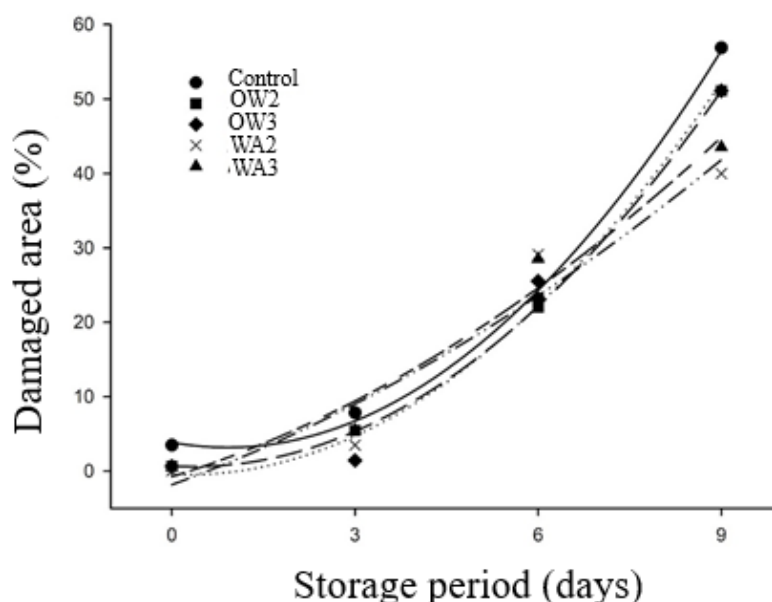


Figure 1. Evolution of the damaged area of bananas stored for 0, 3, 6, and 9 days. OW2: two immersions in ozonated water; OW3: three immersions in ozonated water; WA2: two immersions in water; WA3: three immersions in water.

The experimental conditions, which involved the use of water directly from the distribution network, resulted in a pH close to neutral, a condition that accelerates ozone decomposition compared to more acidic waters (Wang et al., 2019). The pH affects the chemical form of ozone and the generation of free radicals, which can directly influence its ability to inactivate microorganisms.

Although more than one immersion cycle was applied, the total ozonation time may not have been sufficient. Evidence suggests that continuous ozonation is more effective than immersion in pre-saturated water (Alexopoulos et al., 2013). This can be explained by the half-life of ozone in the presence of organic matter, as residence time in systems without a continuous ozone supply is a limiting factor. Although the selected temperature favored a longer half-life, the addition of bananas

drastically reduced this time, such that all available ozone was consumed within 6.7 and 0.9 min according to the zero-order and second-order kinetic models, respectively. The volume of ozonated water per banana may have also been another limiting factor. Increasing the ratio of ozonated water to fruit would raise the number of ozone molecules in the medium, enhancing oxidation reactions, activation of reactive oxygen species (ROS), and free radical production, thereby improving microbial control (Alexandre et al., 2011).

This study was limited to evaluating an efficient means of extending fundamental knowledge of ozone gas kinetics, its effects on pathogen control, and the post-harvest treatment of bananas toward the development of an eco-friendly technique for field application, with potential replacement of active ingredients harmful

to the environment and human health. The results obtained are essential for guiding future strategies for the efficient application of ozone. Generating ozone via electrolytic decomposition of water, using equipment with a continuous ozone supply, and increasing the ratio of ozonated water per banana are promising approaches for future investigations.

Based on the experimental conditions evaluated in this study, the main conclusions can be summarized as follows:

- i. The decay of dissolved ozone in drinking water followed a second-order kinetic model ($R^2 > 0.98$), adequately describing ozone decomposition at three different temperatures and in the presence of fruit.
- ii. Lower temperatures imply a longer ozone half-life in water, whereas the presence of organic matter drastically reduces this time and accelerates the degradation rate, a critical factor in the design of aqueous ozone-based disinfection systems.
- iii. Immersion in pre-ozonated water was not effective in controlling banana rot, even when two or three immersion cycles were applied at a saturation concentration of 5 mg L^{-1} .

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