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ORIGINAL ARTICLE

Postharvest quality of lychee treated with ozone gas or ozonated mist[1]

Qualidade pós-colheita de lichias tratadas com gás ozônio e névoa ozonizada

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HIGHLIGHTS:

Ozone gas preserves the external appearance of lychee better than ozonated mist.
Ozone gas and ozonated mist preserve physicochemical characteristics of lychee pulp.
Ozonated mist treatment has the potential for postharvest fruit preservation.

ABSTRACT: Lychees are perishable fruits, and the application of ozone is a promising technology to preserve their quality. This study aimed to determine the effects of ozone gas and ozonated mist on the physicochemical properties of lychees. Two assays were performed using ozone gas and ozonated mist. The experiments were performed using a split-plot scheme, in which the plots corresponded to the treatments (ozone concentration or exposure time) and the subplots to the storage time, following a completely randomized design with three replicates. In the first experiment, ozone gas concentrations were tested (10 and 20 mg L^{-1}) at exposure times of 15, 30, and 45 min. Corresponding control treatments included fruits that were non-exposed to ozone gas. In the second experiment, an ozone concentration was set (20 mg L^{-1}) for exposure times of 3, 5, and 7 min. Exposure to ozone-free mist for 3, 5, and 7 min and control treatment were performed. The quality parameters of lychee fruits were assessed immediately after treatment (day 0) and on days 2, 4, 6, 7, and 8. Ozone gas was more effective for postharvest preservation of lychee than ozonated mist. Ozone gas was also proven to be more efficient than ozonated mist in maintaining the color of lychee peels. The effects of ozone gas and ozonated mist on pulp characteristics (Dif*, h*, and C*) were similar. Ozonated mist is recommended for vegetable species with an outer waxy cuticle or a high tolerance to water.

Key words: Sapindaceae, *Litchi chinensis* Sonn., fruit storage, ozone sanitization, physicochemical analyses

RESUMO: A lichia é uma fruta perecível e a aplicação do ozônio destaca-se como uma tecnologia promissora na preservação da qualidade destes frutos. O objetivo deste estudo foi determinar os efeitos da aplicação do gás O$_3$ e névoa ozonizada na qualidade físico-química de lichia. Foram realizados dois ensaios: gás ozônio e névoa ozonizada. Os experimentos foram conduzidos em esquema de parcelas subdivididas, tendo nas parcelas os tratamentos (concentrações de ozônio e tempos de exposição) e nas subparcelas os tempos de armazenamento, em delineamento inteiramente casualizado (DIC) com três repetições. No primeiro experimento foram testadas duas concentrações do gás ozônio (10 e 20 mg L^{-1}) nos tempos de exposição de 15, 30 e 45 min. O tratamento controle correspondeu a frutas não expostas ao gás. No segundo experimento, foi definida uma concentração de 20 mg L^{-1} de ozônio nos tempos de exposição de 3, 5 e 7 min. Os frutos também foram expostos à névoa sem ozônio por 3, 5 e 7 min. Os parâmetros de qualidade das lichias foram avaliados após o tratamento (dia 0) e nos dias 2, 4, 6, 7 e 8. O gás ozônio foi mais eficiente na preservação da qualidade pós-colheita da lichia e na preservação da cor da casca dos frutos em relação à névoa ozonizada. Os efeitos do gás e névoa sobre as características da polpa (Dif*, h* e C*) foram semelhantes. O uso da névoa ozonizada é recomendado em vegetais com maior presença de cera cuticular ou maior tolerância a água.

Palavras-chave: Sapindaceae, *Litchi chinensis* Sonn., armazenamento de frutas, sanitização com ozônio, análises físico-químicas

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Introduction

Lychee (*Litchi chinensis* Sonn.) is a fruit that belongs to the Sapindaceae family (Pandit & Shukla, 2018). Despite its good added value, lychee is not marketed year-round and has a short shelf life of 72 h at room temperature (Pandit & Shukla, 2018). Among the chemical methods commonly used for the conservation of Sapindaceae fruits, the most notable are fumigation or immersion in a chlorine dioxide (ClO_2) solution and fumigation with sulfur dioxide (SO_2) followed by immersion in a hydrochloric acid (HCl) solution (Joradol et al., 2019; Intarasit et al., 2022).

Ozone gas (O_3) is an alternative to conventional chemicals for treating Sapindaceae fruits destined for export or in the internal market. When applied to longans (Sapindaceae), ozone gas efficiently extends the shelf life of fruits throughout storage (Chamnan et al., 2019). Previous studies suggested that treatment with ozone gas is more efficient for postharvest preservation than submerging fruits in ozonated water (Whangchai et al., 2011; Pandit & Shukla, 2018).

During the post-global outbreak of Covid-19, ozonated mist has been investigated for decontaminating environments (Schroer et al., 2023), but this application strategy also has great potential for agricultural use (Tominaga et al., 2015; Tanuwidjaja & Fuka, 2022; Epelle et al., 2023; Cabral et al., 2024). Studies on the control of aphids in organic tomato crops demonstrated that ozonated mist at 86 mg L^{-1} (Ebihara et al., 2013) and 70 mg L^{-1} (Ebihara et al., 2016) are promising for eliminating insect pests and protecting plants against pathogenic diseases.

These results demonstrate that this application could be expanded to treat fruits and vegetables post-harvest. However, no studies have investigated the use of ozonated mist for this purpose. This study aimed to determine the effects of the application of ozone gas and ozonated mist on the physicochemical properties of lychees.

Materials and Methods

Organic lychee (cultivar Bengal) was purchased from a local family (20° 42' 30.5" S and 42° 56' 43.3" W, 640 m altitude), producer in Viçosa, Minas Gerais (Brazil), and transferred on the same day to the Postharvest Laboratory (Department of Agricultural Engineering, Universidade Federal de Viçosa - UFV). The fruits were in the full-ripeness stage (uniform red peel) and had not received any postharvest treatment. The fruit was measured and the initial soluble solid content (SSC) and color of the peel and pulp were characterized. They were selected for experimentation based on the absence of damage, uniformity of size, and exterior color.

The experiments complied with a split-plot design, and the plots corresponded to the treatments and subplots to the storage times (0, 2, 4, 6, 7, and 8 d). Experiments were performed using a completely randomized design with three replicates. Each experimental assay comprised seven treatments. The experiment with ozone gas tested two concentrations (10 and 20 mg L^{-1}) and exposure times (15, 30, and 45 min). The control treatment consisted of fruits that were not exposed and unexposed to ozone. The ozone-mist experiment analyzed a fixed ozone concentration (20 mg L^{-1}) and exposure times of 3, 5, and 7 min. This case also included treatments with exposure to ozone-free mist for 3, 5, and 7 min, and a control with fruits not exposed to mist, either with or without ozone. The concentrations and exposure times tested in this study were determined based on the results of the preliminary assays.

Experiment 1 – Ozone gas: The treatments consisted of two ozone gas concentrations (10 and 20 mg L^{-1}) for three exposure times (15, 30, and 45 min), and a control with lychee not exposed to ozone or oxygen. Ozone gas was applied to the fruit within a cylindrical polyvinyl chloride (PVC) prototype dimensioned 0.3-meter diameter and 0.3-meter height. Gas inlet and outlet were installed at the bottom, and at the top of the prototype, respectively. A metal screen was fixed 0.10 m from the cylinder bottom to support the fruits and form a plenum, which allowed the flow and uniform distribution of ozone gas. The experimental scheme is shown in (Figure 1A).

Experiment 2 – Ozonated mist: The assays tested ozonated mist at an input concentration of 20 mg L^{-1} for three exposure times (3, 5, and 7 min). We also analyzed lychee exposed to ozone-free mist for 3, 5, and 7 min and a control with fruit not exposed to mist, either with or without ozone. A metallic exhaust hood was used to directly contain the mist. The fruit was laid on a metallic screen placed 0.30 m from the mist inlet. The mist was conveyed from a generator model N10® (myOZONE, Jaguariúna, São Paulo, Brazil) to the exhaust hood through a transparent polyurethane tube reinforced with copper-clad steel. The experimental scheme is shown in (Figure 1B).

In both experiments, the gas was produced with an ozone generator model M10® (myOZONE, Jaguariúna, São Paulo, Brazil) with a production capacity of 10 g h^{-1}, operating in corona dielectric-barrier discharge. Oxygen (90% purity) was obtained with an oxygen concentrator model EverFlo™ OPI 5LPM (Philips Respironics Inc., Mexico), input at a volumetric flow rate of 1.5 L min^{-1} and measured by a model flow meter MF5700 (Siargo Ltd, Chengdu, Sichuan, China). The ozone concentrations used in the trials were quantified using iodometric titration (indirect method) (Rakness et al., 1996) in compliance with the International Ozone Association guidelines.

The lychee samples exposed to ozone gas, ozonated mist, or ozone-free mist and the control were identified, weighed, and put into rectangular polystyrene trays (21.5 cm × 14.5 cm) sealed with PVC film. Each tray contained 150 ± 10 g of fruit. They were stored in a climatized room at 17 °C and 56% relative humidity to simulate typical conditions in farms and supermarkets. The evaluations were performed immediately after treatment (day 0) and on storage days 2, 4, 6, 7, and 8.

The physicochemical analyses followed the methods described by the Adolfo Lutz Institute (IAL, 2008) and Commission Internationale de l'Eclairage (CIE, 2004). To evaluate the parameters of soluble solids content (SSC), pH, total titratable acidity, and pulp color, the samples were processed with a food mixer model RI1341 (Philips Walita, China). The quality analyses of firmness, pH, and total titratable

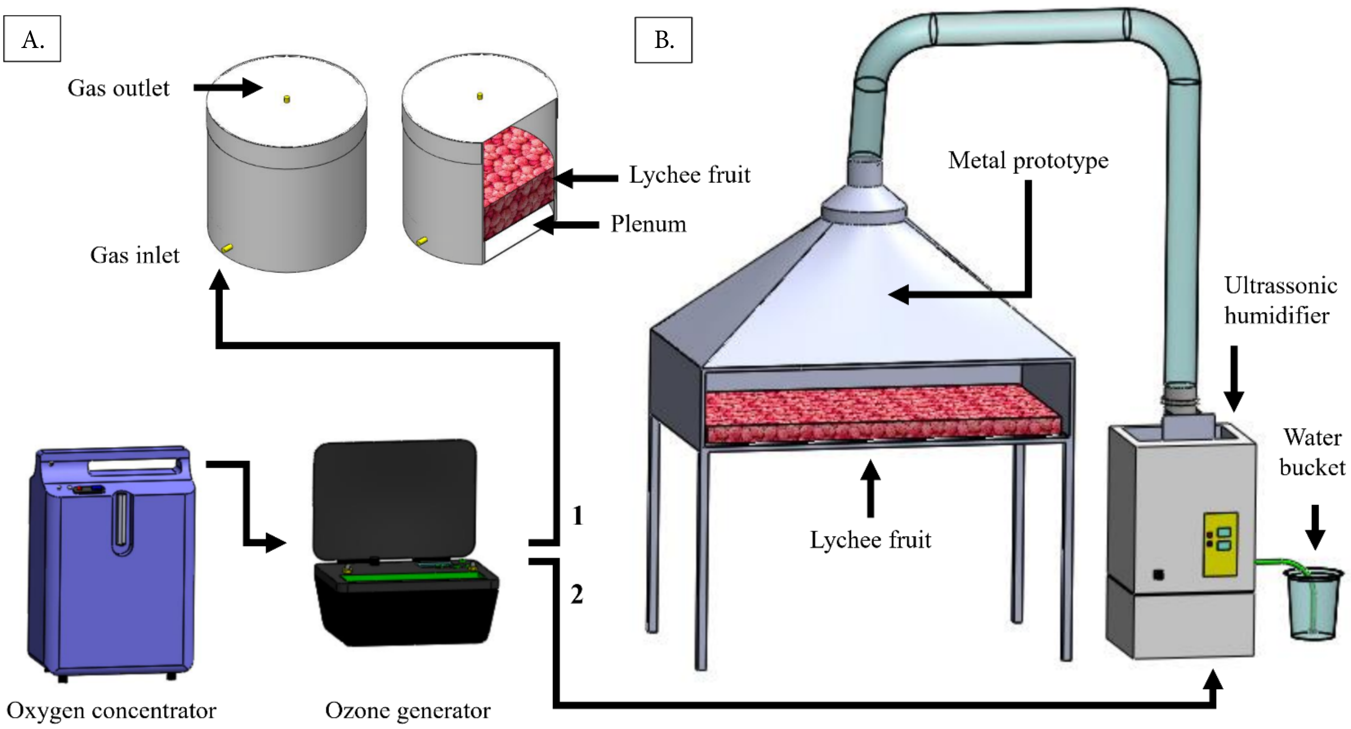

Figure 1. Experimental schemes: application of ozone gas (A) and application of ozonated mist or ozone-free mist (B)

acidity were carried out in triplicate, whereas soluble solids content and pulp color were assessed in quadruplicate.

The samples were mass gauged with a digital semi-analytical balance model BK 8000 with 0.01 g precision (Gehaka, São Paulo, Brazil). The percentage of fresh mass loss corresponds to the difference between the initial mass of each sample (day 0) and the mass on storage days (2, 4, 6, 7, and 8) multiplied by 100 and divided by the initial mass value as given in Eq. 1.

$$\text{WLt} = \frac{W_0 - \text{Wt}}{W_0} \times 100 \qquad (1)$$

where: WLt denotes percentage of mass loss on the analysis day (%); W_0 denotes initial sample mass (day 0) (g); and, Wt denotes sample mass on the analysis day (g).

Firmness was determined using a digital fruit hardness tester (PTR 300; Instrutherm, São Paulo, Brazil). Three fruits from each plot were measured transversely, longitudinally, and diagonally. A 3-millimeter tip was used to pierce the fruit, and the compression force was expressed in Newtons (N).

The concentration of total soluble solids was assessed using a digital pocket refractometer model PAL-3 (Atago, Tokyo, Japan). A drop of crushed pulp was used to determine the concentration of soluble solids.

The pH was determined by submerging a portable digital pH meter (model K39-0014PA; Kasvi, São José dos Pinhais, Brazil) in a solution containing 10 g of crushed lychee pulp and 100 mL of distilled water.

The total titratable acidity (% citric acid $100g^{-1}$ of pulp) was measured via potentiometry of a mixture containing 10 g of crushed lychee pulp and 100 mL of distilled water. A portable digital pH meter (model K39-0014PA; Kasvi, São José dos Pinhais, Brazil) was employed for this purpose, and titration was carried out with a standardized solution of sodium hydroxide (NaOH 0.1N) until the pH reading was 8.2. Eq. 2 was used to obtain the results:

$$\text{Critic acid}\left(\%\ 100\text{g}^{-1}\ \text{of pulp}\right) = \frac{V \times f \times 10}{m} \qquad (2)$$

where: V denotes volume of sodium hydroxide (mL); f denotes correction factor; and m denotes sample mass (g).

The lychee peel color was analyzed by sampling five fruits from each plot and measuring each unit twice. Measurements were taken on opposite sides of the upper part of the fruit (the region with the largest diameter). The pulp color was considered for all fruits from each plot. Pulp from each plot was processed and transferred to a clear plastic bag. Four readings were obtained with two readings on each side of the packaging.

Color was gauged using a digital colorimeter model CR-400 (Konica Minolta, Tokyo, Japan), which provided the L*, a*, and b* coordinates of the Hunter color system. The values of color difference (ΔE*), hue (h*), and chroma (C*) were given by Eq. 4, 5, and 6, respectively. The values L_0, a_0, b_0 were obtained on day 0.

$$\Delta E^* = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \qquad (4)$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \qquad (5)$$

$$C^* = \sqrt{\left(a^{*2} + b^{*2}\right)} \qquad (6)$$

where: L* denotes color intensity in terms of white-black; a* denotes color intensity in terms of red-green; and b* denotes color intensity in terms of yellow-blue.

Data were evaluated using an analysis of variance (ANOVA) and regression analyses. The means were compared using with Tukey's test at a probability level of 0.05, employing the Sisvar 5.6. Dunnett's test was applied using the software Assistat 7.7 to compare the treatments with the control at a significance level of 0.05. The selection of the regression models considered the significance of the regression coefficients determined by the Student's t-test at probability levels of 0.01, 0.05, and 0.10, the coefficient of determination (r^2), and the behavior of the phenomenon were being studied. These analyses were performed using the statistical software SAEG 9.1. The highest degree of interaction was sliced, regardless of its significance, because of the interest in the study.

Results and Discussion

In the test with ozone gas, significant effects ($p \le 0.05$) were observed for in fresh mass loss only on the second day of storage (Table 1). At this storage time, the highest loss percentage (3.12%) corresponded to the treatment with 20 mg L^{-1} for 45 min, which also differed from the control. As for the ozonated mist, this variable was significantly affected ($p \le 0.05$) by the treatments after 2, 4, and 7 storage days. The highest average percentages of fresh mass loss were observed on the second (5.24%) and seventh (9.72%) day of storage in the treatment with ozonated mist at 20 mg L^{-1} for 3 min and on the fourth day of storage in the treatment with ozone-free mist applied for 5 min (6.37%). Differences from the control were also observed for ozone-free mist applied for 5 min, which was observed only on the fourth day of storage. By the end of the experiment (day 8), the average mass loss was 8.88% for the ozone gas and 10.30% for the ozonated mist treatment.

No significant effects ($p > 0.05$) were observed for firmness in the experiment with ozone gas at any storage time (Table 1). Compared to the control, differences only occurred on the fourth day of storage after treatment with at 10 mg L^{-1} for 15 min. In the ozonated mist test, significant effects ($p \le 0.05$) were found only at time 0, with the highest averages observed in the ozone treatments (12.39, 9.41, and 10.03 N). Compared with the control, differences were observed on storage days 0 and 7 in the treatments with ozonated mist at 20 mg L^{-1} applied for 3 and 5 min, respectively. Between the first and seventh storage days, the compression force measured in fruits treated with ozone gas was approximately 6.00 N (Table 1). In fruits subjected to ozonated mist, it was close to 10.00 N in the first evaluation (days 0 and 2). However, on the fourth and seventh storage days, the compression force values did not differ from those observed in fruits treated with ozone gas. In the last evaluation (day 8), the compression force was approximately about 5.5 N for all treatments in both trials.

Table 1 shows the regression equations with the respective coefficients of determination (r^2) describing the behavior of fresh-mass loss (%) and firmness (N) in the experiments with ozone gas or ozonated mist. The loss of fresh mass increased throughout the storage period, whereas firmness decreased. Treatments in which the variables were not significantly affected by storage or exposure time were described as the overall average.

Ozone significantly influenced fresh-mass loss only in the treatments in which it was applied as a mist. In the experiment with ozone gas, only storage time had significant effect ($p \le 0.01$). In the ozone-mist treatments, a linear effect was observed for both storage time ($p \le 0.01$) and exposure time ($p \le 0.01$). The ozone-free mist had a linear effect on storage time ($p \le 0.01$) and a quadratic effect on exposure time ($p < 0.01$). In both cases, the average increase in the fresh-mass loss percentage was close to 1% per evaluation day. The experiment with ozone gas showed a slight increase in the percentage of fresh-mass loss as the concentration of ozone gas increased from 10 to 20 mg L^{-1}. The coefficient of determination (r^2) of fresh-mass loss in both experiments ranged between 0.92 and 0.99.

In this study, fresh mass loss was not altered by ozone gas or ozonated mist treatments compared with the control. A similar behavior was reported by Pandit & Shukla (2018), who washed lychee fruit with ozone water at 0.08 mg L^{-1} for 12 min. Although the present study employed higher concentrations and longer exposure times than those of the authors, there was no influence on mass loss in either ozone treatment (gas or mist). In strawberries, fumigation with ozone gas reduces mass loss (Zhang et al., 2020). Adverse effects of ozone on mass loss were observed in blueberries treated with ozonated water at 10 mg L^{-1} (for 10 or 20 min) and 18 mg L^{-1} (for 10, 15, 20, or 30 min). Ozone caused greater mass loss throughout storage than the control, especially at exposure times longer than 15 min (Jaramillo-Sanchéz et al., 2019). These results indicate that each fruit species can react differently to ozone depending on its concentration and exposure time.

Table 1. Regression equations and determination coefficients (r^2) of fresh-mass loss and firmness of lychee treated with ozone gas or ozone mist

Experiment	Variable	Treatment	Fitted equations	r^2
Ozone gas	Fresh-mass loss (%)	Control	$\hat{y} = 0.26 + 1.10^{**}TE$	0.99
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 0.27 + 1.07^{**}TE$	0.96
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 1.38 + 0.88^{**}TE$	0.95
Ozonated mist		Control	$\hat{y} = 0.26 + 1.10^{**}TE$	0.99
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 2.90 + 1.05^{**}TE - 0.25^{**}TEMP$	0.95
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = -4.88 + 1.17^{**}TE + 2.93^{*}TEMP - 0.34^{**}TEMP^2$	0.92
Ozone gas	Firmness (N)	Control	$\hat{y} = 5.87$	-
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 6.25$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 5.83$	-
Ozonated mist		Control	$\hat{y} = 5.87$	-
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 10.36 - 0.72^{**}TE$	0.77
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 7.93 - 0.36^{**}TE$	0.63

TE - Storage time; TEMP - Exposure time; **Significant at $p \le 0.01$ according to Student's t-test; *Significant at $p \le 0.05$ according to Student's t-test

Regarding firmness, no significant ozone-related effects were noticed in any of the experiments. The effects of the ozone-mist test on this variable were only due to storage time ($p \leq 0.01$), and they exhibited linear behavior. The firmness reduction between the evaluation days was more pronounced when the ozonated mist was used than when the ozone-free mist was used. On each evaluation day, fruits showed an average firmness loss of 0.72 N for ozone-mist treatment, whereas, for ozone-free mist treatment, this value was 0.36 N. The coefficient of determination (r^2) for the ozone-mist experiments were 0.63 and 0.77.

The firmness associated with mass loss is also relevant for marketed fruits because consumers tend to relate firm texture to fresh fruits in their purchasing decision (Soares et al., 2020). In addition, firmness is an essential parameter for designing and dimensioning packaging and is related to resistance against mechanical damage during transport and marketing (Chitarra & Chitarra, 2005). Firmness loss is a natural process expected during the postharvest phase. Over time, changes occur in many compounds, such as cellulose, hemicellulose, and lignin polymers, which are responsible for plant cell structuring and cell wall resistance (Piechowiak et al., 2022). In this study, ozone applied in the form of gas or mist did not alter the firmness of lychee during storage. Previous studies have shown that ozone efficiently preserves the firmness of cantaloupes (Chen et al., 2020) and strawberries (Piechowiak et al., 2022). In longan fruit, a lychee-like species of the same family, a 5-minute exposure to ozone gas at 7.42 mg L^{-1} did not alter fruit firmness (Chamnan et al., 2019).

The total SSC was significantly influenced ($p \leq 0.05$) by ozone gas only at time 0, with the highest averages being observed in the treatments at 10 mg L^{-1} (15, 30, or 45 min), and 20 mg L^{-1} (45 min) (Table 2). These treatments also showed differences compared to the control treatment. In the ozonated mist assay, significant effects ($p \leq 0.05$) were observed on storage days 2, 4, and 8. Immediately after ozonation (day 0), differences were found between the treatments with ozonated mist at 20 mg L^{-1} for 3 min and ozone-free mist for 7 min. Significant effects ($p \leq 0.05$) on pH were observed in the ozone gas treatments on storage days 7 and 8 and in the ozone-mist treatments on storage day 2 (Table 2). As for total titratable acidity, significant effects ($p \leq 0.05$) were verified in the ozone gas treatments on storage day 7 and in the ozone-mist ones on storage day 4.

Table 2 shows the regression equations and the respective coefficients of determination (r^2) which describe the behavior throughout the storage of total soluble solids (°Brix), potential of hydrogen (pH), and total titratable acidity (%) in the experiments with ozone gas or ozonated mist. The hydrogen potential increased during storage, whereas the total soluble solids and total titratable acidity decreased. Treatments in which the variables were not significantly affected by storage or exposure time were described as the overall average. The significant effects observed on these characteristics in both experiments were related only to the storage time.

The total SSC showed a linear effect on the storage time in the ozone gas ($p \leq 0.01$) and ozone-free mist treatments ($p \leq 0.01$), whereas it exhibited a quadratic effect in the ozonated mist treatments ($p \leq 0.01$). In the ozonated mist experiment, the SSC was significantly reduced between the evaluation days compared to the ozone gas assays. The average loss in the ozone gas treatments was close to 0.30 °Brix on each evaluation day, whereas in the ozone-mist and ozone-free mist samples, it was approximately 0.80 °Brix. The coefficients of determination (r^2) obtained for the total SSC ranged between 0.59 to 0.83. Regarding pH and total titratable acidity, significant effects were found only in the ozone gas treatments at 20 mg L^{-1}. Quadratic effects were observed for storage time ($p \leq 0.01$) for both variables. On each evaluation day, the average pH values of these treatments increased by 0.16, and the average citric acid content decreased by 0.06%.

The SSC, pH, and total titratable acidity are crucial for the flavor of lychees, and consumers often prefer sweet and less astringent flavors. Non-climacteric fruits, such as lychee, naturally decline in dissolved solids after harvesting because of the reduction in dominant carbohydrates, such as glucose

Table 2. Regression equations and determination coefficients (r^2) of total soluble solids content, potential of hydrogen, and total titratable acidity in lychee treated with ozone gas or ozone water

Experiment	Variable	Treatment	Fitted equations	r^2
Ozone gas	Total soluble solids content (°Brix)	Control	$\hat{y} = 18.70 + 2.70^{*}\sqrt{TE} - 1.04^{*}TE$	0.83
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\gamma = 20.38 - 0.34^{**}TE$	0.71
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 20.11 - 0.286^{**}TE$	0.62
Ozonated mist		Control	$\hat{y} = 18.70 + 2.70^{*}\sqrt{TE} - 1.04^{*}TE$	0.83
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 19.16 + 0.76^{\circ}TE - 0{,}16^{**}TE^2$	0.65
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 20.11 - 0.79^{**}TE$	0.59
Ozone gas	Potential of hydrogen (pH)	Control	$\hat{y} = 4.47 + 0.10^{*}TE - 0.01^{*}TE^2$	0.87
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\gamma = 4.61$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 4.42 + 0.16^{**}TE - 0.02^{**}TE^2$	0.63
Ozonated mist		Control	$\hat{y} = 4.47 + 0.10^{*}TE - 0.01^{*}TE^2$	0.87
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 4.49$	-
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 4.42$	-
Ozone gas	Total titratable acidity (% 100 g-1 of pulp)	Control	$\hat{y} = 0.45$	-
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 0.40$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\gamma = 0.49 - 0.06^{**}TE + 0.007^{**}TE^2$	0.53
Ozonated mist		Control	$\hat{y} = 0.45$	-
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 0.42$	-
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 0.42$	-

TE - Storage time; TEMP - Exposure time; **Significant at $p \leq 0.01$ according to Student's t-test; *Significant at $p \leq 0.05$ according to Student's t-test; °Significant at $p \leq 0.10$ according to Student's t-test and fructose (Chitarra & Chitarra, 2005; Lima et al., 2005). The increase in soluble solids during ripening is related to the conversion of starch into sugar, however, process does not occur in non-climacteric fruits because they do not ripen after harvesting (Chitarra & Chitarra, 2005).

The SSC, pH, and titratable total acidity values were consistent with those reported in literature. In lychee treated with ozone gas (80 - 240 mg L^{-1}) or ozone water (2.2 - 3.2 mg L^{-1}) for exposure times of 10 to 60 min, SSC ranged from 16.80 to 18.60, and titratable total acidity from 0.39 to 0.51 (Whangchai et al., 2011). Lychee submerged in ozone water at 0.08 mg L^{-1} for 12 min exhibited a pH variation between 4.2 and 4.5 (Pandit & Shukla, 2018). In this study, the soluble solids, pH, and titratable total acidity of the samples treated with ozone gas or ozonated mist were within or close to these ranges. The total SSC ranged from 16.04 to 20.65 °Brix in the lychees treated with ozone gas and from 12.13 to 20.35 °Brix in those subjected to ozonated mist. The pH oscillated from 4.32 to 4.97 in the fruits subjected to ozone gas and 4.00 to 4.83 in those treated with ozonated mist. Regarding total titratable acidity, the percentages of citric acid in fruits treated with ozone gas and ozonated mist were between 0.27 to 0.57% and 0.31 to 0.61%, respectively.

The ozonated mist caused greater red discoloration and peel browning than the control and ozone gas treatments. The application of ozone gas at 10 mg L^{-1} proved to be more efficient in preserving the peel color of lychee than at a concentration of 20 mg L^{-1}, regardless of the exposure time. As for the pulp color, few variations in color difference, hue, and saturation were detected among the ozone-mist treatments and between them and the control.

The three lychee peel color variables were significantly affected ($p \leq 0.05$) by ozone gas treatment (Table 3). Substantial peel color differences were observed after 8 days of storage in fruits treated with ozone gas, with the highest averages recorded in the tests at 20 mg L^{-1} for 30 min and 45 min (11.30 and 11.06 Dif*). These treatments also differed from the control. Lychees treated with ozone gas exhibited significant effects ($p \leq 0.05$) on peel hue on storage days 2, 7, and 8 and peel saturation on days 2, 4, 6, and 8.

Among the peel color variables, only the color differences were significantly affected ($p \leq 0.05$) in the experiment with ozonated mist. Differences were found among the treatments compared with the control at all storage times. The highest averages were observed at the three ozone exposure times (20 mg L^{-1} for 3, 5, or 7 min; 9.73 - 13.11 Dif*), and the lowest averages at all ozone-free mist exposure times (3, 5, and 7 min) (5.28 to 10.88 Dif*).

Table 3 contains the regression equations and respective coefficients of determination (r^2) that describe the behavior of the color difference (Dif *), h*, and saturation (C*) of the peel and pulp of the lychees subjected to ozone gas and ozonated mist treatment throughout storage. Treatments in which the variables were not significantly affected by storage or exposure time were described as the overall average.

Ozone gas significantly affected the three-color variables of lychee peel. Color difference varied only in the treatments at 20 mg L^{-1}, displaying a quadratic effect regarding storage time ($p \leq 0.01$) and exposure time ($p \leq 0.10$). This ozone gas concentration (20 mg L^{-1}) also significantly affected lychee peel hue, exhibiting a quadratic effect for storage time ($p \leq 0.05$) and a linear effect for exposure time ($p \leq 0.10$). Peel color saturation was significantly influenced by ozone gas only in the treatments at 10 mg L^{-1}, with a linear effect observable in storage time ($p \leq 0.01$) and exposure time ($p \leq 0.05$). The peel color variables were not significantly influenced by the exposure times to ozonated mist, and the significant effects of these treatments were exclusively a consequence of storage time. The ozone-free mist significantly affected only the peel color difference, resulting in a quadratic effect of storage time ($p \leq 0.01$) and exposure time ($p \leq 0.01$).

Visual quality is an essential attribute for marketing fresh products. Consumers typically associate alterations in external appearance with fruit senescence or quality loss (Barboza et al., 2022). In the present study, the application of ozonated mist caused more visible changes in the peel color of lychee than ozone gas. Whangchai et al. (2011) found similar results when testing the efficiency of fumigation with ozone gas and the immersion of lychee in ozone water. They verified that the use of ozone water caused a more significant loss of visual quality than the use of ozone gas.

Oxidative stress in ozone-treated fruits is one of the main causes of quality loss during storage (Piechowiak et al., 2021). During ozonation, reactive oxygen species are produced, which can oxidize biomolecules essential for the functioning of cellular compounds, such as proteins, lipids, and DNA, resulting in the loss of antioxidants, enzymatic disruption, changes in cell membrane permeability, and senescence (Piechowiak et al., 2020; Piechowiak et al., 2021).

The major color changes observed verified in the peels of lychees treated with ozonated mist may be linked to the degradation of anthocyanins, pigments abundant in the peel that are responsible for its red color. When applied in aqueous medium, ozone decomposes into free radicals, such as hydroxyl ($OH^{\cdot}$), hydroperoxyl (HO_2), and superoxide ($O_2^{\cdot}$), which may be toxic to the product (Piechowiak et al., 2020). Hydroxyl radicals ($OH^{\cdot}$) cause electrophilic or nucleophilic reactions and produce ozonide, a byproduct that breaks down aromatic rings and accelerates anthocyanin degradation (Tiwari et al., 2009).

The use of water in the treatments of lychees appears to be a complex process for preserving the peel because of its morphology. The lychee has an irregular and rough peel, with micro-fissures that become more conspicuous during storage. This makes the fruit more susceptible to physical changes when exposed to ozone in water (Whangchai et al., 2011). This may explain the differences in the peel color variables of fruits treated with ozonated mist compared with those treated with ozone gas. Satisfactory peel preservation when ozone gas is used has also been observed in other fruits. In raspberries exposed to ozone gas at 8 to 0 mg L^{-1} for 30 min every 12 hours of storage, anthocyanins built up more significantly due to increased antioxidant activity, which contributed to maintaining quality during storage (Piechowiak et al., 2021).

Table 3. Regression equations and determination coefficients (r^2) for color difference, color hue, and color saturation of the peel and pulp of lychee treated with ozone gas or ozone mist

Experiment	Variable	Treatment	Fitted equations	r^2
Ozone gas	Peel color difference (Dif*)	Control	$\hat{y} = 5.41$	-
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 56.09 - 0.74**TE + 0.11**TE^2$	0.53
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 1.58 - 0.36^{\circ}TE + 0.11**TE^2 + 0.21*TEMP - 0.36^{\circ}TEMP^2$	0.87
Ozonated mist		Control	$\hat{y} = 5.41$	-
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 11.24$	-
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = -5.98 - 0.43**TE + 0.06**TE^2 + 6.61**TEMP - 0.70**TEMP^2$	0.93
Ozone gas	Peel hue (h*)	Control	$\hat{y} = 32.08 - 2.49^{\circ}TE + 0.38*TE^2$	0.67
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 31.68$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 28.85 - 0.45^{ns}TE + 0.19*TE^2 + 0.05^{\circ}TEMP$	0.75
Ozonated mist		Control	$\hat{y} = 32.08 - 2.49^{\circ}TE + 0.38*TE^2$	0.67
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 29.62 - 1.51^{\circ}TE + 0.28*TE^2$	0.51
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 30.49 - 1.66*TE + 0.31**TE^2$	0.57
Ozone gas	Peel saturation (C*)	Control	$\hat{y} = 14.46 + 0.99*TE - 0.19*TE^2$	0.92
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 17.07 - 0.54**TE - 0.04*TEMP$	0.70
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 16.47 - 0.99**TE$	0.84
Ozonated mist		Control	$\hat{y} = 14.46 + 0.99*TE - 0.19*TE^2$	0.92
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 12.55 + 1.62**TE - 0.24**TE^2$	0.63
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 14.08 + 1.08*TE - 0.21**TE^2$	0.75
Ozone gas	Pulp color difference (Dif*)	Control	$\hat{y} = 1.36 + 0.2*TE$	0.63
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 2.64$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 2.70$	-
Ozonated mist		Control	$\hat{y} = 1.36 + 0.2*TE$	0.63
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 3.14$	-
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 2.81$	-
Ozone gas	Pulp hue (h*)	Control	$\hat{y} = 125.46 - 3.42**TE$	0.84
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 107.94$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 107.14$	-
Ozonated mist		Control	$\hat{y} = 125.46 - 3.42**TE$	0.84
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 124.68 - 4.16**TE$	0.65
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 124.93 - 3.77**TE$	0.66
Ozone gas	Pulp saturation (C*)	Control	$\hat{y} = 2.46 + 0.17*TE$	0.66
		O_3 10 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 3.31$	-
		O_3 20 mg L^{-1} (15, 30, or 45 min)	$\hat{y} = 3.71$	-
Ozonated mist		Control	$\hat{y} = 2.46 + 0.17*TE$	0.66
		O_3 20 mg L^{-1} (3, 5, or 7 min)	$\hat{y} = 4.19$	-
		O_3 free mist (3, 5, or 7 min)	$\hat{y} = 4.05$	-

TE - Storage time; TEMP - Exposure time; **Significant at $p \le 0.01$ according to Student's t-test; *Significant at $p \le 0.05$ according to Student's t-test; °Significant at $p \le 0.10$ according to Student's t-test

Positive effects on preserving visual quality have also been verified in papaya continuously subjected to ozone gas at 0.04, 1.6, or 4 ppm for 48, 96, or 144 hours (Ong et al., 2013). However, papaya showed external changes associated with surface oxidation during treatment at 4 ppm for 144 hours (Ong et al., 2013).

Other possible interfering factors include the concentration, exposure time, and morphological characteristics of the product. High ozone levels and inadequate exposure times may cause the oxidation and discoloration of vegetable surfaces (Rodrigues et al., 2021). Nonetheless, setting an optimal ozone concentration can be challenging, and the effects of this compound on species or members of the same family may vary considerably. The ozone performance also depends on other fruit factors, including cuticle thickness, composition, cultivar, maturation phase, and growing season (Souza et al., 2018).

Regarding the pulp color variables, the color difference had no significant effect ($p > 0.05$) in the tests with ozone gas. Pulp hue and saturation exhibited significant effects ($p \le 0.05$) on ozone gas treatment after with 6 and 4 days of storage. In the ozone-mist experiments, the three pulp color variables were significantly affected ($p \le 0.05$) on the fourth day of storage. At this storage time point (day 4), the ozonated mist treatment at 20 mg L^{-1} for 3 min delivered the highest pulp color difference and saturation averages. Significant effects ($p \le 0.05$) on pulp color saturation were also immediately observable shortly after the fruit treatment (time 0). Compared to the control, alterations were also noticed in the color difference of lychee pulp on storage day 4 and in color saturation on storage days 0 and 4.

In both trials, significant effects on lychee pulp color variables occurred solely as a function of storage time. The treatments significantly influenced only the pulp hue, and these effects were also observed in the ozone-mist experiments. For this variable, linear effects were observed for storage time ($p \le 0.01$) in both the ozone and ozone-free mist treatments. This variable behaved in a decreasing manner. In the presence of ozone, the h* values showed a greater reduction between evaluation days. In the ozone-mist treatment, the average h* reduction was 4.16 per evaluation day, whereas, in the ozone-free mist treatment, the average h* reduction was 3.77. Both treatments scored higher than the control, with an average h* reduction of 3.42. The coefficient of determination (r^2) obtained for the lychee pulp color hue ranged between 0.65 to 0.84.

Ozone did not alter the pulp color, regardless of the application method. Compared to other results in the literature, ozonated mist proved to be more efficient than ozone water. The only study analyzing this characteristic in lychee has reported that ozone water leads to pulp darkening and more intense deterioration (Whangchai et al., 2011). However, this was not observed in the present study. The authors associated the deterioration of fruits treated with ozonated water with microcracks in their pericarp. The same results were not observed in the present study, which used an ozonized mist.

The potential of ozonated mist is worth exploring in other products, adapting concentrations and exposure times to different agricultural species, their morphologies, and their application purposes. Moreover, the effects of ozonated mist on the metabolism of the product may vary depending on storage conditions.

Conclusions

1. Ozone gas is more effective than ozonated mist for the postharvest preservation of lychee.
2. Ozone gas was also proven to be more efficient than ozonated mist in maintaining the color of lychee peels. The effects of ozone gas and ozonated mist on pulp characteristics (Dif*, h*, and C*) were similar.
3. Ozonated mist is recommended for vegetable species with an outer waxy cuticle or high tolerance to water.

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