

# Influence of pH on the efficacy of ozonated water to control microorganisms and its effect on the quality of stored strawberries (*Fragaria x ananassa* Duch.)

Influência do pH na eficácia da água ozonizada no controle de microrganismos e efeito na qualidade de morango (*Fragaria x ananassa* Duch.) armazenado

Wallas Felipe de Souza Ferreira<sup>1</sup>, Ernandes Rodrigues de Alencar<sup>1\*</sup>, Hanna Alves<sup>1</sup>,  
Jaqueline Lamounier Ribeiro<sup>1</sup>, Caroline Rosa da Silva<sup>1</sup>

<sup>1</sup>Universidade de Brasília/UnB, Faculdade de Agronomia e Medicina Veterinária, Brasília, DF, Brasil

\*Corresponding author: ernandesalencar@unb.br

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## ABSTRACT

Ozonation has been proposed as an alternative for the post-harvest treatment of plant products. Therefore, the objective of this study was to evaluate the influence of the pH on the efficacy of ozonated water to control microorganisms and to determine the possible effects of ozone on the quality of stored strawberries. To evaluate the influence of pH on ozonated water in Portola variety strawberries, the strawberries were divided into six batches. Three batches were exposed to ozonated water and three batches were exposed to non-ozonated water with different pH levels and an immersion time of 5 min. The pH values used were 3.0, 6.5 and 8.7. After the ozonation, the fruits were stored in a cold chamber at 5 °C for 6 days, and analyses were performed every two days. A completely randomized design was adopted using a 6x4 factorial scheme with three replications. The aerobic mesophiles, molds and yeasts, total coliforms and *Escherichia coli* were quantified, and the presence of *Salmonella* spp. was assessed. For the quality evaluation, the mass loss, titratable acidity (TA), pH, soluble solids (SS), SS/TA ratio and color were analyzed. The pH of the water was verified to influenced the effectiveness of the ozonated water to control the aerobic mesophiles and molds and yeasts. The ozonated water can retard fresh mass losses and maintain the pH, soluble solids, titratable acidity, SS/TA ratio and color variables.

**Index terms:** Ozone; microbiological quality; qualitative changes.

## RESUMO

A ozonização tem sido proposta como alternativa para o tratamento pós-colheita de produtos de origem vegetal. Então, objetivou-se com este trabalho avaliar a influência do pH na eficácia da água ozonizada no controle de microrganismos e determinar possíveis efeitos na qualidade de morangos armazenados. Para avaliar a influência do pH na água ozonizada em morangos da variedade Portola, dividiu-se os morangos em seis lotes, sendo três lotes expostos a água ozonizada e três lotes em que a água não foi ozonizada, adotando-se diferentes valores de pH e tempo de imersão de 5 min. Os valores de pH adotados foram 3,0, 6,5 e 8,7. Depois da ozonização, os frutos foram armazenados em câmara fria a 5 °C por 6 dias, sendo realizadas análises a cada dois dias. Adotou-se delineamento inteiramente casualizado em esquema fatorial 6x4, com três repetições. Quantificaram-se os aeróbios mesófilos, bolores e leveduras, coliformes totais e *Escherichia coli* e verificou-se a presença de *Salmonella* spp. Na avaliação da qualidade, analisaram-se perda de massa, acidez titulável (AT), pH, sólidos solúveis (SS), relação SS/AT e cor. Verificou-se que o pH da água influenciou a eficácia da água ozonizada no controle de aeróbios mesófilos e bolores e leveduras. A água ozonizada foi capaz de retardar a perda de massa fresca e manter os níveis de pH, sólidos solúveis, acidez titulável, relação SS/AT e das variáveis referentes à cor.

**Termos para indexação:** Ozônio; qualidade microbiológica; alterações qualitativas.

## INTRODUCTION

The strawberry (*Fragaria x ananassa* Duch.) is a highly perishable product *in natura* on the market and requires the use of appropriate technology for better conservation and reduction of post-harvest losses. Sanitation methods are used as part of the production chain to conserve and sanitize the product until it reached the consumer. Chlorine and its by-products are currently the most used products for

the cleaning of vegetables and fruits. Brazilian legislation has accepted the use of chlorine for food sanitization in the post-harvest stage, but in some European countries, the use of chlorinated products in fresh food has been banned. Chlorinated compounds have some disadvantages for the treatment of water and food because they can lead to the formation of organochlorine, trihalomethane and haloacetic acid compounds, which are mutagenic, toxic and carcinogenic (Lazarova; Savoye; Janex, 1999; Silva et al., 2011).

Studying alternatives to chlorine that efficiently inactivate microorganisms, do not affect physicochemical qualities and do not pose a risk to consumers is important. Ozonation has been proposed as an alternative for the post-harvest treatment of plant products. Ozone gas, i.e., the triatomic molecular form of oxygen, has been recognized as a safe substance since 1982, and the use of this gas as a direct additive to foods was permitted by the FDA a few years later. This allowed for its use as an antimicrobial agent and food sanitizer in the treatment and processing stages, and research on this gas has been increasingly emphasized (Graham, 1997; Kim; Yousef; Dave, 1999; Güzel-Seydim; Greene; Seydim, 2004; FDA, 2013).

The efficiency of ozone gas as a sanitizer is well established, and its role as a potent oxidizer for water treatment has been known since the beginning of the 20<sup>th</sup> century. However, due to their abundance and low price, the use of chlorinated products dominates throughout the world (Khadre; Yousef; Kim, 2001). However, in recent years, ozone has regained importance as sustainability and technologies with less impact on the environment and human health have become priorities. The sustainable use of water is a prime example, and because of the characteristics of ozone, e.g., high oxidative power, no residues and a wide spectrum of antimicrobial action, it is a great solution for the rational use of water (Chiattonne; Torres; Zambiasi, 2008).

The effect of ozone on several groups of microorganisms, such as fungi of the genera *Aspergillus*, *Fusarium*, *Geotrichum*, *Myrothecium*, *Alternaria*, *Penicillium*, *Botrytis* and *Mucor*, viruses and bacteria, is already known (Kim; Yousef; Dave, 1999; Khadre; Yousef, 2001; Raila et al., 2006; Wu; Doan; Cuenca, 2006; Zotti et al., 2008; Oskan; Smilanick; Karabulut, 2011; Alencar et al., 2013; Alexopoulos et al., 2013). However, the use of ozonated water for the sanitization of fruits and vegetables is still less evident when compared to the applications of ozone in its gaseous form. There are many uses for ozonated water in the strawberry post-harvest process and for other fruits and vegetables, and these include treatments to control pathogen infections and plant propagules, water sanitation in washing systems, discharge tanks or channels, sanitation of equipment surfaces and packaging, and alternatives to chlorinated products (Di Bernardo; Dantas, 2005; Silva et al., 2011).

The ozonation of water depends on several factors, including the ozone decomposition kinetics in an aqueous medium, the organic matter content in water, the water temperature and the pH of the medium. When there is a significant variation in the pH of the medium, the changes in the efficiency during the disinfection process are related

to the changes in the ozone decomposition rate (Kim; Yousef; Khadre, 2003). In view of the above, the purpose of this study was to evaluate the influence of pH on the efficacy of ozonated water to control microorganisms in the strawberry sanitization process and to evaluate possible changes in the quality of the product during storage.

## MATERIAL AND METHODS

Strawberries of the cultivar "Portola" were cultivated in the city of Brazlândia, DF, Brazil. The cultivation area is located at an elevation of 1,200 m, and the geographic coordinates are 15°65'25" S latitude and 48°10'92" W longitude. The fruits were harvested by hand at their commercial ripening point and properly selected, and fruits with lesions were discarded. The fruits were then taken to the Laboratory of Pre-Processing and Storage of Vegetable Products at the University of Brasília - DF in August of 2016.

Ozone gas was obtained via an ozone generator (Model 0&L 5.0 RM) based on the Dielectric Barrier Discharge (DBD) - corona effect method. In the ozone generation process, oxygen (O<sub>2</sub>) was used as the input with a purity of approximately 90%, was free of moisture, and was obtained from an oxygen concentrator coupled to the ozone generator.

Distilled water was used at three different pH levels: pH 3.0 with citric acid (p.a.), pH 6.5, and pH 8.7 with sodium bicarbonate (p.a.). The pH measurements were performed using a Digimed Mod. DM21 potentiometer. To obtain ozonated water at different pH levels, the gas was bubbled for 15 min at a concentration of 21 mg L<sup>-1</sup> with a 1.0 L min<sup>-1</sup> flow at 25 °C in glass containers with a capacity of 1.5 L using a porous plate.

The fruits were divided into six batches, and each batch corresponded to one treatment. For three treatments, ozonized distilled water was used, and for the other three treatments, non-ozonized distilled water was used. The treatments were at the different pH levels. The quantification of the ozone dissolved in the water was carried out using a SAM CHEMetrics photometer, Model I-2019, with a measurement range of 0.01 to 5.0 mg L<sup>-1</sup>. The fruits were packed in 3.0 L glass containers and immersed in ozonated or non-ozonated distilled water at the different pH levels. The immersion time was 5 min for all the treatments. After this period, the water was drained, and the fruits were packaged in rectangular, transparent polyethylene packages (18 cm x 12 cm) and identified according to each treatment. Each treatment had 3 replicates. Each of the packages had approximately 100 g of strawberries. They were stored in a climatic B.O.D. chamber at a temperature of 5 ± 1 °C.

Microbiological and physicochemical analyses were carried out immediately before and after immersion of the fruits in the ozonated or non-ozonated distilled water and every two days for a total of six days of storage. The microbiological analyses of the fruits were carried out before immersion in the ozonated or non-ozonated distilled water. Each sample (25 g) was aseptically transferred to sterile plastic bags and homogenized in 225 mL of sterile peptone water. Serial decimal dilutions of each sample were made in the peptone water. Dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  were used.

The Petrifilm™ system (3M Company, St. Paul, MN, USA) was used according to the manufacturer's guidelines to count the yeasts and molds (YM), aerobic mesophiles (AC), total coliforms and *Escherichia coli* (EC 6404). This technique was tested on fresh strawberries by Jordano et al. (1995) for these microorganisms, and satisfactory results were obtained. The results are expressed on a log basis (CFU  $g^{-1}$ ). For the assessment of *Salmonella* spp., the protocol utilized was that reported by the Normative Instruction number 62 from the Brazilian Ministry of Agriculture (Brasil, 2003).

For the quality evaluation of the strawberries, the variables assessed were the fresh weight loss, pH, titratable acidity, soluble solids (°Brix), soluble solids to titratable acidity ratio (SS/TA) and color. The fresh mass loss was determined as the difference between the initial mass and the final mass using a digital scale and was expressed as a percentage of the initial mass. The pH was determined using a Digimed Mod. Potentiometer DM21. Approximately 10 g of the crushed and homogenized samples was used in 100 mL of distilled water for the titratable acidity analysis, and the analysis was performed according to the standards described by the Instituto Adolfo Lutz (2008). Titrations with a standardized 0.1 N sodium hydroxide solution (NaOH) were performed to the turning point equivalent of pH 8.2 using the Digimed Mod. DM21 potentiometer. The results were expressed as the citric acid percentage. The soluble solids were determined using an Atago digital refractometer (Model 1T), and the results are expressed as °Brix according to the AOAC (2002). Using the values obtained for the soluble solids (SS) and the titratable acidity (TA), the SS/TA (ratio) was obtained.

The color of the strawberries was evaluated using the ColorQuest® XE colorimeter from HunterLab. The equipment was duly calibrated, and the values were taken from the pulp of the fruits. Two sample readings were performed for each repetition. Using the values of the coordinates L, a and b, it was possible to obtain the parameters related to the chroma or color saturation (C)

(Equation 1), the hue angle ( $h^\circ$ ) (Equation 2), and the color difference ( $\Delta E$ ) (Equation 3) (McLellan; Lind; Kime, 1995; Maskan, 2001).

$$C = \sqrt{(a^2 + b^2)} \quad (1)$$

$$h = \arctan(b/a) \quad (2)$$

$$\Delta E = \sqrt{[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]} \quad (3)$$

Where

$h$  = the hue angle

$C$  = chroma or color saturation

$\Delta E$  = color difference

$L$  = measurable in terms of intensity from white to black

$a$  = measurable in terms of the red and green intensity

$b$  = measurable in terms of the yellow and blue intensity

$L_0$ ,  $a_0$  and  $b_0$  are the values obtained at time zero.

A completely randomized design was used in a 6x4 factorial scheme, i.e., six treatments and four storage periods (0, 2, 4 and 6 days) with three replicates. Initially, an analysis of variance was performed and was followed by the Tukey mean test. ASSISTAT 7.7 was used in the analysis of variance, and the software SigmaPlot v. 10 was used to obtain the equations and plot the graphs.

## RESULTS AND DISCUSSION

Before immersing the strawberries in ozonated distilled water, the residual ozone was quantified, and values of 0.11 mg  $L^{-1}$ , 0.08 mg  $L^{-1}$  and 0.04 mg  $L^{-1}$  were obtained for the pH levels of 3.0, 6.5 and 8.7, respectively. According to Kim, Yousef and Khadre (2003), aqueous media with higher pH values cause rapid decomposition of ozone and the formation of hydroxyl radicals (OH). Therefore, when the pH of the medium changes, the changes in the efficacy of ozone during the sanitization process are related to changes in the ozone decomposition rate (Kim; Yousef; Khadre, 2003; Di Bernardo; Dantas, 2005). The stability of ozone in water decreases as the pH of the medium increases, and when the pH is higher than 8.0, nearly half of the introduced ozone decomposes into several intermediate oxygen forms within 10 min (Kim; Yousef; Dave, 1999; Wysok; Uradziński; Gomóka-Pawlichka, 2006).

The decomposition of ozone in aqueous media is characterized by a rapid decrease in the initial concentration and a later phase in which the ozone concentration decreases via a first order kinetic rate with hydroxyl radicals (OH) are

the main decomposition products (Kim; Yousef; Khadre, 2003; Almeida et al., 2004). Ozone can react with organic compounds in aqueous solutions via a direct reaction. Molecular ozone participates in reactions, and indirect reactions that involve the hydroxyl radicals (OH) formed from the decomposition of ozone in aqueous media can also occur. These indirect reactions are not selective because hydroxyl radicals are capable of promoting an attack on organic compounds that is  $10^6$ - $10^9$  times faster than some oxidizing agents, such as  $H_2O_2$  and molecular ozone (Kim; Yousef; Dave, 1999; Almeida et al., 2004; Di Bernardo; Dantas, 2005). The solubility of ozone gas in the aqueous medium also depends on the organic matter content in the medium because a lower organic matter concentration results in a longer ozone half-life in water. Most disinfection processes occur via a molecular ozone ( $O_3$ ) direct route. However, oxidation processes predominantly occur via the hydroxyl radical indirect pathways (Khadre; Yousef; Kim, 2001; Silva et al., 2011).

Regarding the microbiological quality of the strawberries prior to immersion in the ozonated or non-ozonated distilled water, counts equivalent to  $6.70 \pm 0.10$  and  $5.20 \pm 0.20$  log CFU  $g^{-1}$  were obtained for the aerobic mesophiles and molds and yeasts, respectively.

The estimated value of *E. coli* in the strawberries was  $<1.00$  log CFU  $g^{-1}$ . There was no evidence of *Salmonella* spp. for the  $10^{-1}$  dilution. The total coliform count did not allow for the capacity of the ozonated water to control the microorganisms of this group to be determined under the different conditions.

There was a significant difference ( $p < 0.01$ ) in the count of the aerobic mesophiles and the yeasts and molds due to the treatment interactions and storage periods of the strawberries immersed for 5 min in ozonated or non-ozonated distilled water at different pH levels. A lower aerobic mesophiles count was observed in the strawberries immersed in ozonated distilled water (ODW), except for the fruits immersed in ozonated distilled water at pH 8.7 relative to the fruits immersed in non-ozonated distilled water (NODW) at the same pH (Table 1). The greatest difference estimated on the sixth day of storage was verified by comparing the aerobic mesophiles count in fruits immersed in ODW at pH 3.0 (3.67 log CFU  $g^{-1}$ ) with the results from the fruits immersed in NODW at pH 6.5 (6.33 log CFU  $g^{-1}$ ), resulting in a significant difference ( $p < 0.01$ ) of 2.66 log CFU  $g^{-1}$ . It should be noted that all the treatments resulted in aerobic mesophile counts lower ( $p < 0.01$ ) than that obtained for the fruits prior to immersion in ODW and NODW, which was equivalent to 6.70 log (CFU  $g^{-1}$ ).

**Table 1:** Mean values and standard deviation for the log count (CFU  $g^{-1}$ ) of aerobic mesophiles and molds and yeasts in strawberries immersed for 5 min in non-ozonated distilled water (NODW) and ozonated distilled water (ODW) at different pH levels and stored at 5 °C.

Treatment	Time (days)			
	0	2	4	6
Aerobic mesophiles				
NODW - pH 3.0	4.81 ± 0.46 aA	4.15 ± 0.46 aA	4.94 ± 0.46 aA	5.08 ± 0.46 bA
NODW - pH 6.5	4.80 ± 1.24 aB	4.64 ± 1.24 aBC	3.61 ± 1.24 bC	6.33 ± 1.24 aA
NODW - pH 8.7	4.50 ± 0.37 aA	4.57 ± 0.37 aA	5.28 ± 0.37 aA	4.82 ± 0.37 bcA
ODW - pH 3.0	4.16 ± 0.39 aA	3.91 ± 0.39 aA	4.15 ± 0.39 abA	3.67 ± 0.39 cA
ODW - pH 6.5	3.88 ± 0.60 aA	3.56 ± 0.60 aA	3.61 ± 0.60 bA	4.58 ± 0.60 bcA
ODW - pH 8.7	4.30 ± 0.52 aA	4.42 ± 0.70 aA	4.35 ± 0.70 abA	5.26 ± 0.70 abA
Molds and yeasts				
NODW - pH 3.0	3.71 ± 0.92 aB	5.40 ± 0.92 aA	5.65 ± 0.92 abA	5.00 ± 0.92 abA
NODW - pH 6.5	3.38 ± 0.95 aB	4.99 ± 0.95 abA	4.87 ± 0.95 bcA	5.74 ± 0.95 aA
NODW - pH 8.7	3.33 ± 1.10 aC	5.01 ± 1.10 abB	5.98 ± 1.10 aA	5.80 ± 1.10 aAB
ODW - pH 3.0	3.87 ± 0.42 aA	3.94 ± 0.42 cA	4.20 ± 0.42 cA	4.47 ± 0.42 bA
ODW - pH 6.5	3.37 ± 0.53 aB	4.39 ± 0.53 bcA	4.16 ± 0.53 cAB	4.25 ± 0.53 bA
ODW - pH 8.7	3.35 ± 0.70 aB	4.73 ± 0.70 abcA	4.43 ± 0.70 cA	4.82 ± 0.70 abA

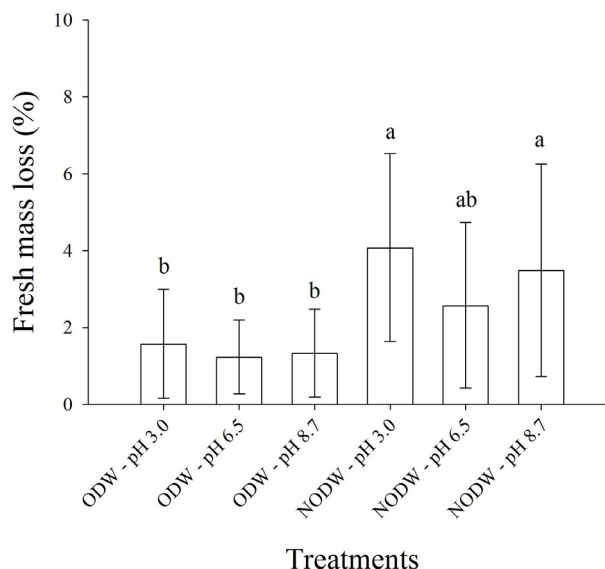
Means followed by the same lower-case letters in a column and capital letters on the lines do not differ significantly based on the Tukey test ( $p < 0.05$ ).

According to the results, ODW at different pH levels resulted in lower increases in the counts of molds and yeasts in the fruits during storage, as shown in Table 1. When ODW at pH 6.5 was used, a count of the molds and yeasts of 4.25 log CFU g<sup>-1</sup> was obtained on the sixth day of storage, whereas the count was 5.74 log CFU g<sup>-1</sup> for the NODW sample at this pH, which indicated a difference of 1.49 log CFU g<sup>-1</sup>. It is important to note that immersion in NODW or ODW reduced the counts of the molds and yeasts at the beginning of storage. A count equivalent to 5.20 log CFU g<sup>-1</sup> was obtained for the untreated strawberries, while those immersed in NODW or ODW had counts below 3.90 log CFU g<sup>-1</sup>. The difference obtained when comparing the counts of the molds and yeasts in the non-ozonated samples and those immersed in NODW or ODW was significant ( $p < 0.05$ ).

There are several studies in the literature that have evaluated the efficacy of gaseous ozone dissolved in water to control microorganisms. Pang and Hung (2016) demonstrated that a combination of UV radiation and ozonated water can achieve a reduction of 5 log cycles in the *E. coli* O157:H7 count in romaine and iceberg lettuces, whereas the treatment with UV radiation only obtained a reduction of 2.1 log cycles and treatment with a chlorine solution a reduction of 2.5 log cycles. Aguayo et al. (2013) reported that ozonated water with a concentration of 0.4 mg L<sup>-1</sup> and three immersion times of 1, 3 and 5 min could effectively reduce the concentration of mesophilic bacteria in tomato fruits. On the 5<sup>th</sup> and 14<sup>th</sup> day of storage, the immersion time of 3 min was more effective than that of 1 and 5 min. Martínez, García and Sánchez (2002) demonstrated that using ozonated water at a concentration of 2.2 mg L<sup>-1</sup> and a 15 min immersion time was able to significantly inhibit the germination of *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and, to a lesser extent, *Lasiodiplodia theobromae* in mangos of the Haden cultivar. Alencar et al. (2014) ozonated pears with ozone gas at a concentration of 100 ppm for 60 min and did not observe a significant increase in the counts of the molds and yeasts for up to 13 days of storage. However, the authors obtained counts equivalent to 3.0 log CFU g<sup>-1</sup> after 13 days of storage in fruits that were not subjected to ozonation.

There was no significant difference for the variable pH ( $p > 0.05$ ), and the pH values during the storage period remained between 3.50 and 3.20. There was a significant difference between the treatments

( $p < 0.01$ ), independent of the storage period, for the variable fresh mass loss (Figure 1). The mean values of the fresh mass loss do not include the initial time. The percent mass loss in the fruits immersed in NODW at pH 3.0 and pH 8.7 statistically differed from the treatments with the ODW. The lower loss of the fresh mass in the ozonated fruits may be associated with a reduction in the respiratory rate and the lower microorganism count. Nadas, Olmo and García (2003) observed a lower percentage of fresh mass loss in strawberries treated with ozone gas and stored at 2 °C for three days than those not submitted to ozonation. According to these authors, ozone reduced the loss of fresh mass due to a decrease in the fruit respiration rate. Zhang et al. (2011) observed a significant decrease in the strawberry mass loss over 20 days of storage. The authors obtained lower values for the fresh mass loss and respiratory rate in strawberries when a concentration of 4 ppm of ozone was used. Liu et al. (2016) observed that when using ozonated water at a concentration of 1.4 mg L<sup>-1</sup> and a contact time of 5 and 10 min, there was a reduction in the fresh mass loss and microorganism count in apples after two days of storage.



**Figure 1:** Mean values and standard deviation for the fresh mass loss (%) of strawberries immersed for 5 min in ozonated distilled water (ODW) and non-ozonated distilled water (NODW) at different pH levels and stored at 5 °C.

Means followed by the same letter do not statistically differ from each other based on the Tukey test at a 5% probability.

Significant variations ( $p < 0.01$ ) were obtained for the soluble solids (SS; °Brix), titratable acidity (TA, %) and SS/TA ratio. In the case of the fruits immersed in ODW at different pH levels, the soluble solids contents ranged between 6.38 and 7.12 °Brix on the sixth day of storage, while for those immersed in NODW at pH 3.0, the value was 4.70 °Brix, as shown in Table 2. Higher titratable acidity variation was observed in fruits immersed in NODW at pH 8.7. However, the values of the titratable acidity remained higher than 0.75% in all the treatments (Table 2). A more pronounced SS/TA reduction trend was observed in the fruits immersed in NODW at pH 3.0, and the values were lower than 4.85 on the sixth day of storage. However, for the fruits immersed in ozonated water at different pH levels, the SS/TTA values on the sixth day were greater than 6.80 (Table 2).

In relation to the pulp color of fruits immersed in NODW or ODW at different pH levels, a significant difference ( $p < 0.05$ ) was obtained for the variable hue angles and color differences as a result of the interaction between the treatment and the storage period. The color saturation did not present a significant difference ( $p > 0.05$ ). Regarding the hue angle, on the sixth day of storage, the pulps obtained from the fruits immersed in NODW at pH 3.0 and 8.7 had values of 26.96 and 27.70, respectively, while those obtained from the fruits immersed in ODW were greater than 30.20, as shown in Table 3. It is important to mention that this difference was significant at a 5% probability. Nadas, Olmo and García (2003) obtained lower color saturation and hue angles in ozone-treated strawberries at a concentration of 1.5  $\mu\text{L L}^{-1}$  and stored at 2 °C compared to non-ozonated fruits.

**Table 2:** Mean values and standard deviations for the soluble solids (SS, °Brix), titratable acidity (TA, %) and SS/TA in strawberries immersed in non-ozonated distilled water (NODW) and ozonated distilled water (ODW) and stored at 5 °C.

Treatment	Time (days)			
	0	2	4	6
	SS (°Brix)			
NODW - pH 3.0	7.08 ± 1.07 abA	7.10 ± 1.07 aA	6.70 ± 1.07 aA	4.70 ± 1.07 cB
NODW - pH 6.5	6.52 ± 0.31 abA	6.90 ± 0.31 aA	6.77 ± 0.31 aA	6.55 ± 0.31 abA
NODW - pH 8.7	7.01 ± 0.81 abA	7.30 ± 0.81 aA	6.95 ± 0.81 aAB	6.10 ± 0.81 bB
ODW - pH 3.0	7.25 ± 0.35 aA	6.75 ± 0.35 aA	6.63 ± 0.35 aA	7.02 ± 0.35 abA
ODW - pH 6.5	7.10 ± 0.28 abA	6.75 ± 0.28 aA	7.05 ± 0.28 aA	7.12 ± 0.28 aA
ODW - pH 8.7	6.15 ± 0.49 bB	7.20 ± 0.49 aA	6.83 ± 0.49 aAB	6.38 ± 0.49 abAB
	TA (%)			
NODW - pH 3.0	0.99 ± 0.03 aA	1.01 ± 0.03 aA	1.04 ± 0.03 aA	0.98 ± 0.03 aA
NODW - pH 6.5	0.93 ± 0.07 aA	0.90 ± 0.07 abA	0.96 ± 0.07 aA	1.00 ± 0.07 aA
NODW - pH 8.7	0.93 ± 0.11 aA	0.80 ± 0.11 bB	0.94 ± 0.11 aA	1.03 ± 0.11 aA
ODW - pH 3.0	0.95 ± 0.06 aAB	0.91 ± 0.06 abB	1.05 ± 0.06 aA	0.96 ± 0.06 aAB
ODW - pH 6.5	0.99 ± 0.05 aA	0.93 ± 0.05 aA	1.03 ± 0.05 aA	0.99 ± 0.05 aA
ODW - pH 8.7	0.93 ± 0.05 aA	0.97 ± 0.05 aA	1.01 ± 0.05 aA	0.93 ± 0.05 aA
	SS/TA			
NODW - pH 3.0	7.18 ± 1.01 aA	7.03 ± 1.01 bA	6.43 ± 1.01 aA	4.81 ± 1.01 cB
NODW - pH 6.5	7.03 ± 0.77 aA	7.71 ± 0.77 bA	7.12 ± 0.77 aAB	6.53 ± 0.77 abB
NODW - pH 8.7	7.26 ± 1.33 aA	9.12 ± 1.33 aA	7.37 ± 1.33 aB	5.93 ± 1.33 bcC
ODW - pH 3.0	7.64 ± 0.64 aA	7.41 ± 0.64 bAB	6.31 ± 0.64 aB	7.33 ± 0.64 aAB
ODW - pH 6.5	7.21 ± 0.45 aAB	7.29 ± 0.45 bA	6.85 ± 0.45 aA	7.17 ± 0.45 abA
ODW - pH 8.7	6.62 ± 0.49 aB	7.42 ± 0.49 bA	6.72 ± 0.49 aA	6.82 ± 0.49 abA

Means followed by the same lower-case letters in a column and capital letters on the lines do not differ significantly based on the Tukey test ( $p < 0.05$ ).

**Table 3:** Mean values and standard deviation for the hue angle (°h) and color difference in strawberries immersed in non-ozonated distilled water (NODW) and ozonated distilled water (ODW) and stored at 5 °C.

Treatment	Time (days)			
	0	2	4	6
	Hue angle (°h)			
NODW - pH 3.0	30.13 ± 1.65 aA	29.04 ± 1.65 bAB	30.64 ± 1.65 aA	26.96 ± 1.65 bB
NODW - pH 6.5	29.30 ± 1.67 aB	31.63 ± 1.67 aA	31.53 ± 1.67 aA	28.31 ± 1.67 abB
NODW - pH 8.7	29.74 ± 1.05 aA	29.71 ± 1.05 abA	29.19 ± 1.05 aA	27.70 ± 1.05 bA
ODW - pH 3.0	29.05 ± 1.12 aA	30.87 ± 1.12 abAB	31.35 ± 1.12 aA	30.23 ± 1.12 aAB
ODW - pH 6.5	28.91 ± 1.16 aB	30.11 ± 1.16 abA	30.10 ± 1.16 aA	28.29 ± 1.16 abA
ODW - pH 8.7	29.51 ± 1.76 aA	30.50 ± 1.76 abAB	31.59 ± 1.76 aA	30.28 ± 1.76 aB
	Color difference			
NODW - pH 3.0	0.0 ± 2.18 aC	2.62 ± 2.18 bB	6.22 ± 2.18 aA	5.20 ± 2.18 abA
NODW - pH 6.5	0.0 ± 1.01 aB	5.09 ± 1.01 abA	6.41 ± 1.01 aA	6.33 ± 1.01 abA
NODW - pH 8.7	0.0 ± 2.38 aC	2.57 ± 2.38 bB	6.71 ± 2.38 aA	6.61 ± 2.38 abA
ODW - pH 3.0	0.0 ± 1.22 aB	4.19 ± 1.22 abA	6.53 ± 1.22 aA	7.13 ± 1.22 abA
ODW - pH 6.5	0.0 ± 2.41 aC	3.47 ± 2.41 abB	6.52 ± 2.41 aA	7.48 ± 2.41 aA
ODW - pH 8.7	0.0 ± 0.87 aB	4.91 ± 0.87 aA	5.45 ± 0.87 aA	4.43 ± 0.87 bA

Means followed by the same lower-case letters in a column and capital letters on the lines do not differ significantly based on the Tukey test ( $p < 0.05$ ).

Barth et al. (1995) found a significantly lower hue angle in blackberries treated with ozone after 5 days of storage at 2 °C.

Regarding the color difference in the fruit pulp, a tendency to increase that was independent of the ozonation was obtained throughout the storage. It is noteworthy that only the pulp of the fruits immersed in ozonated water at pH 8.7 showed a color difference of less than 4.50 after six days of storage (Table 3). Changes in the color of food have been explained by the degradation of pigments by ozone. Ozone has a high oxidative potential and can degrade several types of organic compounds, including pigments (Güzel-Seydim; Greene; Seydim, 2004; Tiwari et al., 2009). Alencar et al. (2011) and Sanchez et al. (2016) explained the change in peanut color as depigmentation of the skin, i.e., the reddish color, by ozone gas. In the present research, the observed differences in the strawberry color are associated with the capacity of ozone to retard the deterioration process.

## CONCLUSIONS

The use of ozonated water at different pH levels can be an important alternative to maintain the post-harvest quality of strawberries. The pH influenced the efficiency of the ozonated water to control undesirable microorganisms in strawberries during storage. With regards to the strawberry quality during storage, ozonated water was able to retard the

loss of the fresh mass and maintain the pH, soluble solids, titratable acidity, SS/TA ratio and color variables.

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