Effect of Some Sanitizing Treatments on Strawberry Fruit Quality during Cold Storage

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RESEARCH ARTICLE

Abstract
The present research was conducted to evaluate the effect of dipping treatments with 2% citric acid, 0.2% benzoic acid, 0.2% sorbic acid and acidic electrolyzed water on the quality of strawberries ("Malvina" cultivar) during 21 days storage at 8 °C. The following analyses were performed on the control and sanitized strawberry fruits at 1, 7, 14 and 21 days of storage: weight loss, firmness, decay incidence, soluble solids content, titratable acidity, total phenolics content, total anthocyanins content and DPPH antioxidant activity. All sanitizing treatments significantly (P<0.05) reduced weight loss and fruit decay during strawberry storage. Dipping strawberries in 0.2% sorbic acid or 0.2% benzoic acid aqueous solutions were the most effective treatments for maintaining firmness and phytochemical content and for delaying the decay of strawberries during cold storage.

Keywords: citric acid; benzoic acid; sorbic acid; acidic electrolyzed water; dip wash treatments; phenolic compounds; antioxidant activity.

INTRODUCTION
Increasing the shelf life of fruits and vegetables represents an interest of researchers in the current overpopulation as well as against the background of global climate change which led to a decrease in production. This can be done by developing the methods of fresh preservation or by decreasing the microbiota on the surface of products using sanitizers washing treatments. Strawberry is a fruit belonging to several plant species of the genus Fragaria (Rosaceae family), the most popular being Fragaria x ananassa Duch. Strawberry occupies an important place among the small fruit plants and it is grown throughout the world (Sharma et al., 2009). Strawberry fruits are widely consumed all over the world, both fresh and processed, due to their attractive color, flavor and aroma. Strawberries are a relevant source of bioactive compounds including vitamins (such as vitamin C, folate), -carotene and phenolic compounds (phenolic acids, flavonoids and anthocyanins) (Ariza et al., 2016), most of which express relevant antioxidant capacities in vitro and in vivo (Giampieri et al., 2012). Kähkönen et al. (2001) reported the highest antioxidant capacity in strawberries among twelve fruit species. Anthocyanins are very abundant in mature strawberries while ellagitannins were the second largest group of phenolic compounds after anthocyanins (Cordenunsi et al., 2005; Kähkönen et al., 2001). These phenolic compounds exert major effects on human health, preventing cardiovascular diseases, cancer and age-induced oxidative stress (Olsson et al., 2004).
Strawberries have a high perishability and a short postharvest life. The short growing season and ripening of strawberries, sensitivity to mechanical injury during transport and microbiota infection during storage reduce significantly their shelf life. The strawberries spoilage losses may reach as much as 40% (Trinetta et al., 2020).

Various sanitization methods have been studied for microbial decontamination of fruits. Chlorine based sanitizers are the most commonly used because of their efficiency and low cost. However, aiming to avoid the risks associated with exposure to chlorinated organic by-products and to meet current safety standards, in the past years, some other sanitizers, such as organic acids or acidic electrolyzed water have been tested in the postharvest disinfection of fresh fruits (Ma et al. 2017; Pablos et al. 2018; Nicolau-Lapeña et al. 2019; Lepaus et al., 2020). Dipping of iceberg lettuce in 0.5% citric acid or 0.5% lactic acid solution for 2 min proved to be as effective as chlorine for reducing microbial populations on fresh-cut iceberg lettuce (Akbas and Ölmel, 2007).

The antimicrobial action of organic acids is based on their capacity to reduce the pH of the environment, to depress the internal cellular pH by ionization of the undissociated acid molecule, or to disrupt the substrate transport by alteration of cell membrane permeability (Raybaudi-Massilia et al., 2009).

Application of organic acids or acidic electrolyzed water (alone or in combination with other preservation methods) was used in recent years as alternative sanitization methods for improving the microbiological and physicochemical qualities of strawberries (van de Velde et al., 2013; Lafarga et al., 2019). The acidic electrolyzed water treatment of strawberries for 10 min achieved a significant reduction of naturally present aerobic bacteria, coliforms, and fungi (Koseki et al., 2004). Ding et al. (2015) tested the combination effect of ultrasonic and slightly acidic electrolyzed water (SAEW) on microbial loads and quality of strawberries and found that ultrasonic treatment can improve the disinfection effect of SAEW. Hung et al. (2010) reported also that electrolyzed oxidizing water (EO) was either more than or as effective as chlorinated water in killing E. coli O157:H7 cells on strawberries.

Wei et al. (2017) reported that the treatment with 2% organic acids, 0.02% sodium hypochlorite, 0.1% sodium chlorite, and 0.1% acidified sodium chlorite reduced the background microbiota on fresh strawberry. In this study, the acidified sodium chlorite method had the best sterilizing effect and also required the shortest sanitizing time and low chloride content. The heat shock and/or salicylic acid applied postharvest have been studied on shelf-life extension of strawberry as these techniques have been related to their effect on inducing physiological defense responses against the oxidative stress and pathogen development. However, these treatments did not influence the incidence of pathogens or chemical variations in stored strawberry (Coltro et al., 2014).

Do Rosário et al. (2017) evaluated the effects of acetic acid, peracetic acid, and sodium dodecylbenzenesulfonate isolated or combined with 5 min of ultrasound treatment (40 kHz, 500 W) on strawberry quality, natural contaminant microbiota (molds and yeasts, mesophilic aerobic and lactic acid bacteria) and inactivation of Salmonella enterica intentionally inoculated onto strawberries over 9 days of storage at 8 °C. They found that ultrasound combined with peracetic acid was the best treatment for those groups of microorganisms. Also, important reductions of natural microbiota were obtained in the sanitization process of strawberry by combining ultrasound with acetic acid, lactic acid, peracetic acid, sodium dichloroisocyanurate and hydrogen peroxide treatments (Pelissari et al., 2021). The combination of ultrasound and peracetic acid treatment promoted a significant reduction in the molds, yeasts and Escherichia coli count without causing significant changes in the physicochemical characteristics of strawberries (Donatti Leão Alvarenga et al., 2021).

The aim of the present study was to investigate the effects of dip wash treatments with organic acids and acidic electrolyzed water on weight loss, firmness, titratable acidity, total soluble solids, total phenolic content, total anthocyanin content and DPPH antioxidant activity of strawberries cv. "Malvina" during 21 days storage at 8 °C. The effectiveness of these treatments in reducing decay of strawberry fruits was also examined.

MATERIALS AND METHODS

Plant material

Strawberry (Fragaria x ananassa Duch.) fruits belonging to the "Malvina" cultivar, harvested at mature stage (at full red color), were sorted and conditioned by removing the calyx and peduncle. Then the strawberry fruits were washed and wilted and divided into five groups (60 fruits per group and three replicates per sanitizing treatment), each group being subjected to a sanitizing treatment.

The treatments consisted in immersing the fruits for 5 min at room temperature in: tap water (control, C); 2% citric acid (CA); acidic electrolyzed water (AEW); 0.2% benzoic acid (BA); 0.2% sorbic acid (SA). Fruits were allowed to dry and then packed in plastic containers (500 ml capacity), each containing approximately 250 g, covered by a lid and stored at 8 oC and 85% relative humidity for 21 days. During the cold storage period, at 1, 7, 14 and 21 days of storage, weight loss, firmness, soluble solids content, titratable acidity, total phenolic content, total anthocyanins content and DPPH antioxidant activity were determined.

Chemicals

The reagents used were of analytical grade: benzoic acid, sorbic acid, and sodium carbonate from Merck (Darmstadt, Germany); Folin-Ciocalteu reagent, gallic acid, sodium acetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Sigma-Aldrich (Germany), and acidic electrolyzed water
produced from Kangen Water Type LeveLuk SD 501 apparatus (Enagic, Dusseldorf, Germany).

Weight loss
During storage, weight loss was determined by weighing the fruits with a sensitive digital scale (Sartorius CP124S, UK, accuracy = 0.01 g) both at the beginning of the experiment and at the end of each storage period and was expressed as a percentage of weight loss to initial weight (Garcia et al., 1998).

Firmness
The strawberry fruits firmness was measured using a GY-3 fruit penetrometer (Sundoo Instruments, Zhejiang, China) with a 6 mm diameter plunger (Devassy and George, 2021). Firmness of six fruits from each replicate was determined for each sanitizing treatment at the end of each storage period and the mean value was reported in kg/cm².

Titratable acidity
The titratable acidity was determined by the titrimetric method (Sadler and Murphy, 2010). 10 g of fruit homogenate was made up to 100 ml with deionized water and titrated with 0.1 M NaOH solution to pH 8.2. The results were expressed as grams of citric acid per 100 g fresh weight.

Total soluble solids
The total soluble solids content (TSS) was determined by using a digital refractometer (Hanna Instruments, Woonsocket, USA) from a homogenous sample, obtained by blending strawberry fruits in an electrical blender (Kafkas et al., 2007). The results were expressed as percentage of soluble solids.

Extraction
Three grams of strawberry homogenates were extracted with 10 ml methanol for 60 minutes in an ultrasonic bath at room temperature, then centrifuged at 6000 rpm for 15 min. The supernatants were collected and stored at –40 °C. Subsequently the extracts were used for the analysis of total phenolic content and DPPH free radical-scavenging activity.

Total phenolic content
The total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965); 10 μL of extract was mixed with 5 ml of water and 500 μL of Folin-Ciocalteu reagent, after 3 min 1.5 ml of sodium carbonate solution (20% w/v) were added and the final mixture was made up to a volume of 10 ml with distilled water. The vials were shaken vigorously and kept at 40 °C in the dark for 30 min, then the absorbance was measured at 765 nm on a Varian Cary 50 UV spectrophotometer (Varian Co., USA). Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 fresh weights according to a pre-determined calibration curve made with standard solutions of gallic acid.

Total anthocyanin content
Total anthocyanins were determined by extracting them from 2 g of strawberry homogenate with 20 mL of methanol acidified with 0.1% HCl (v/v) and their quantification by the pH differential spectrophotometric method described by Giusti and Wrolstad (2001). Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5, with a Varian Cary 50 UV spectrophotometer (Varian Co., USA). The total anthocyanin content was expressed as milligrams of cyanidin 3-O-glucoside equivalents (CGE) per 100 g of fresh weight (fw) and calculated using the following formula:

\[
\text{Total anthocyanin content} = (A \times MW \times DF \times 1,000)/\varepsilon
\]

where \(A = (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 4.5\); \(MW\) (molecular weight) = 449.2 g/mol for cyanidin-3-O-glucoside; \(DF\) = dilution factor of the samples; \(\varepsilon\) (molar absorbptivity of cyanidin-3-glucoside) = 29,600 L/(mol·cm).

DPPH free radical-scavenging activity
The antioxidant activity was determined according to the method described by Oliveira et al. (2008). Briefly, 50 μl of strawberry extract was mixed with 3 mL of 0.004% DPPH methanolic solution. After vigorously shaken, the mixture was kept 30 min in darkness then absorbance of the mixture was measured at 517 nm on a Varian Cary 50 UV-VIS spectrophotometer. The blank sample was produced by mixing methanol with DPPH solution instead of extract. The results were calculated according to the formula:

\[
\text{DPPH scavenging activity (\%) = } [1 - \text{absorbance of sample/absorbance of blank}] \times 100
\]

The standard reference was Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and results were expressed as mmol Trolox equivalents (TE) per 100 g fresh weight (fw).
Fruit decay
The external appearance of strawberries was visually evaluated after 7, 14 and 21 days of storage. The fruits showing macroscopic fungal growth or injuries on the fruit surface were considered decayed. Fruit decay was expressed as percent of fruit showing decay symptoms.

Statistical analysis
Results were expressed as means±standard deviations. The effect of treatment was analyzed using the least significant difference (LSD) test and differences at p < 0.05 were considered to be significant. The statistical analysis was carried out using Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA).

RESULTS AND DISCUSSIONS
Weight loss
The variation of fruit weight loss as influenced by treatments is shown in Table 1.

Table 1. Effect of various sanitizing treatments on weight loss (%) of strawberries during storage at 8 °C for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.04 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AEW</td>
<td>0.05 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.71 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CA</td>
<td>0.04 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.63 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BA</td>
<td>0.00 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.49 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.94 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA</td>
<td>0.00 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.46 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, P < 0.05).

Nunes and Emond (2007) mention that a loss of 2.5%-3% in weight of strawberries is the maximum acceptable loss for fresh strawberry kept at 20°C. The weight loss was significantly lower in treated fruits than in the control fruits. The higher weight loss was registered between 14 and 21 days of storage for all fruits. All sanitizing treatments significantly (P<0.05) reduced weight loss during strawberry storage. At the end of storage, the highest loss was found in control fruits (1.65%) and the lowest in SA (0.46%), followed by CA (0.86%), AEW (0.90%) and BA (0.94%) treated fruits. The weight loss of fruits is mainly due to transpiration, respiration and to other metabolic activities. The reducing weight loss of fruits dipped in organic acids may be attributed to the reducing of the respiration rate and to the slowing of the evapotranspiration and maturation during storage (Ehteshami et al., 2020).

Firmness
Strawberries soften considerably during short storage or transportation and thus, the texture of the strawberry represent an important quality parameter (Velickova et al., 2013). Both the control and each of the four sanitizing treatments resulted in a significant firmness decrease during the storage period (table 2). Similar trends of firmness associated with the weakening of cell walls which is due to the decrease in pectin solubility and its hydrolysis, associated with pectin esterase and polygalacturonase activities (Ehteshami et al., 2022). The better maintaining of firmness in BA and SA treated samples may be due to the inhibitory influence of sorbic and benzoic acids on the enzymatic activity (Wedzicha et al., 1990; Chipley, 2005; Stopforth et al., 2005).

Table 2. Effect of various sanitizing treatments on firmness (kg/cm²) of strawberries during storage at 8 °C for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.76 ± 0.13</td>
<td>1.42 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.27 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14 ± 0.11&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>AEW</td>
<td>1.80 ± 0.12</td>
<td>1.77 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45 ± 0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.12 ± 0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CA</td>
<td>1.82 ± 0.12</td>
<td>1.58 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.27 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BA</td>
<td>1.72 ± 0.11</td>
<td>1.63 ± 0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.58 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA</td>
<td>1.82 ± 0.14</td>
<td>1.67 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.57 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, P < 0.05).
Titratable acidity

Fruit acidity increased in the first 7 days after treatments and decreased afterwards in all samples. A similar trend of variation in strawberry titratable acidity during storage was reported by Koyuncu and Dilmacunal (2010). The titratable acidity recorded the lowest decrease in SA treated strawberries as compared with the controls while dipping in 2% citric acid led to a sharper decline in it. A lower decrease in the titratable acidity may be attributed to a reduced respiratory rate as the decline of the titratable acidity in fruits has been attributed to the use of organic acids as respiratory substrates (Kaur et al. 2019).

Total soluble solids

TSS content is an important index that affects strawberry quality, a higher TSS value being preferred (Aday et al., 2013). The evolution of total soluble solids of strawberry fruits during storage is shown in Table 4. TSS decreased slowly in the first 14 days in all treatments, followed by a sudden decline in the last 7 days of fruits storage. This evolution may be related to the high physiological activity and respiration in the last 7 days of storage, resulted in fast hydrolysis of sucrose (Zhang et al., 2007). Strawberries dipped in citric acid recorded the lowest TSS content while fruits dipped in acidic electrolyzed water, benzoic acid and sorbic acid presented significantly higher values of TSS than controls. The highest TSS content was recorded in SA treated strawberries. A possible explanation for this might be that sorbic acid delayed the hydrolysis of sugars by inhibiting the hydrolytic enzymes. It has been suggested that sorbic acid inhibits enzymes by reacting with sulphydryl groups (Wedzicha et al., 1990).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 (g)</th>
<th>7 (g)</th>
<th>14 (g)</th>
<th>21 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.58 ± 0.04</td>
<td>0.61 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.44 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>AEW</td>
<td>0.58 ± 0.03</td>
<td>0.61 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CA</td>
<td>0.61 ± 0.04</td>
<td>0.63 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.48 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BA</td>
<td>0.61 ± 0.04</td>
<td>0.64 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.48 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA</td>
<td>0.61 ± 0.03</td>
<td>0.67 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, P < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>7 (g)</th>
<th>14 (g)</th>
<th>21 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>9.92 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.80 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.44 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.92 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AEW</td>
<td>10.54 ± 0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.64 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.12 ± 0.24&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.14 ± 0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CA</td>
<td>10.12 ± 0.28&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.52 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.82 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.54 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BA</td>
<td>10.74 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.46 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.06 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.36 ± 0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA</td>
<td>10.62 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.38 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.98 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.62 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, P < 0.05).

Total phenolic content

The phenolic compounds are major antioxidants in fruits (Méndez-Lagunas et al., 2017). The average total phenolic content found in control strawberries was 73.27 mg GAE/100 g fw, which is similar with those reported in previous studies (Nicolau-Lapeña et al., 2019; Perin et al., 2019). One day after the sanitizing treatments, the highest total phenolic content was found in BA and AEW followed by SA treated fruits while the lowest in control fruits (Table 5). The total phenolic content increased in the first week of storage, thereafter it decreased during the remainder period of storage in control, AEW and CA treated fruits probably due to the physiological collapse of the fruit, the largest decrease been found in the CA treated fruits. In BA and SA treated fruits, the total phenolic content increased in the first two weeks and dropped further in the last week of storage. After 21 days of storage, the highest total phenolic content was found in BA and AEW, followed by SA treated fruits. The lower decrease of phenolic content in BA treated samples may be due to the ability of benzoic acid to inhibit polyphenoloxidases, which has been demonstrated in previous studies (Neves et al., 2009). Regarding the effect of acidic electrolyzed water, Chen et al. (2019) reported that AEW treated-bluberry...
exhibited higher total phenolics content and concluded that AEW treatment for enhancing storability of blueberries during storage may be mediated by regulating the metabolism of reactive oxygen species (ROS) metabolism, manifested by increasing ROS scavenging capacity and reducing ROS accumulation.

Table 5. Effect of various sanitizing treatments on total phenolic content (mg GAE/100 g fw) of strawberries during storage at 8°C for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>53.08 ± 1.26</td>
</tr>
<tr>
<td>AEW</td>
<td>53.03 ± 1.46</td>
</tr>
<tr>
<td>CA</td>
<td>52.49 ± 1.34</td>
</tr>
<tr>
<td>BA</td>
<td>52.49 ± 0.88</td>
</tr>
<tr>
<td>SA</td>
<td>50.93 ± 1.84</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, <sup>P < 0.05</sup>).

Total anthocyanin content

The average total anthocyanin content of strawberry fruits before sanitizing treatments was 53.08 mg CGE/100 g fw. Even if the total anthocyanin content recorded a slightly decrease after the sanitizing treatments, there were no significant differences between treatments (p < 0.05) at this point (table 6). After 7 days of storage at 8°C, the total anthocyanin content decreased in all samples while after 14 days of storage it recorded a sharp decrease, the highest decline been registered in the control samples followed by AEW treated samples. Results showed that under refrigerated storage the anthocyanin content was better retained if strawberries were previously dipped in 0.2% benzoic acid, 2% citric acid or 0.2% sorbic acid. The sanitizing treatments have been also shown to determine changes in anthocyanin content in strawberries in other previous studies (Nicolau-Lapeña et al., 2019).

Table 6. Effect of various sanitizing treatments on total anthocyanin content (mg CGE/100 g fw) of strawberries during storage at 8°C for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>4.80 ± 0.28</td>
</tr>
<tr>
<td>AEW</td>
<td>4.90 ± 0.15</td>
</tr>
<tr>
<td>CA</td>
<td>5.00 ± 0.28</td>
</tr>
<tr>
<td>BA</td>
<td>4.93 ± 0.22</td>
</tr>
<tr>
<td>SA</td>
<td>4.83 ± 0.24</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, <sup>P < 0.05</sup>).

DPPH free radical-scavenging activity

The antioxidant activity of fresh strawberries before sanitizing treatments was 4.8 mmol Trolox/100 g fw and no significant differences were found between samples immediately after treatments (Table 7).

Table 7. Effect of various sanitizing treatments on DPPH free radical-scavenging activity (mmol Trolox/100 g fw) of strawberries during storage at 8°C for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>4.80 ± 0.28</td>
</tr>
<tr>
<td>AEW</td>
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<tr>
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<td>SA</td>
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</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, <sup>P < 0.05</sup>).
**Fruit decay**

Acidic electrolyzed water significantly delayed the decay in strawberries during cold storage as compared to the control samples (Table 8). Many previous studies reported on the efficacy of acidic electrolyzed water in inactivation of microorganisms of fresh and fresh-cut fruits and vegetables (Park et al., 2009; Navarro-Rico et al., 2014). This effect is attributed to its low pH, high oxidation–reduction potential, and the presence of active oxidizers such as hypochlorous acid (Ramos et al., 2013).

CA treatment was more effective than acidic electrolyzed water in retarding decay of fresh strawberries during storage. The antimicrobial action of citric acid has been attributed to pH reduction, disturbance of membrane transport and/or permeability, anion accumulation and reduction in internal cellular pH (Parish et al., 2003). Wei et al. (2017) reported that 2% citric acid solution significantly reduced aerobic bacteria, *E. coli* O157:H7, yeast, and *S. typhimurium* but not mold in strawberries while Chen et al. (2016) found that 0.5% citric acid reduced the bacterial count of fresh-cut apples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0.00 ± 0.00</td>
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<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Note: Means followed by different superscript letter in a column are significantly different (LSD test, *P* < 0.05).

BA and SA treatments were the most effective in reducing strawberry decay. Sorbic and benzoic acids are food additive preservatives, able to inhibit yeasts, molds and bacterial growth. They have been used to control spoilage or pathogenic bacteria on fresh and fresh-cut fruits and vegetables by disturbing their ionic permeability across the membrane, anion accumulation and decreasing the internal cellular pH (Parish et al. 2003). Montesinos-Herrero et al. (2016) found that sodium benzoate satisfactorily controlled green and blue mold on oranges, even when treated fruit were cold-stored, while Al-Kuraieef et al. (2019) reported that dipping strawberries in a solution of potassium sorbate delayed mould growth and extended the shelf life by approximately 7 days compared to the shelf-life of the control strawberries.

**CONCLUSIONS**

Dip wash treatments of fresh strawberries with 2% citric acid, 0.2% benzoic acid, 0.2% sorbic acid and acidic electrolyzed water delayed the physiological collapse of fruits during storage, slowing down the water losses. Dipping strawberries in 0.2% sorbic acid or 0.2% benzoic acid aqueous solutions retarded the loss of firmness in fresh strawberries. In addition, the sanitizing treatments maintained a higher level of the anthocyanin and phenolic content, the most effective being the benzoic and sorbic acid treatments. As a result, the fruits dipped in benzoic or sorbic acid maintained higher antioxidant activity during storage than control samples. The sanitizing treatments consisting of dipping in 0.2% benzoic acid or 0.2% sorbic acid solutions may extend the postharvest life of strawberry fruit with 7 days in cold storage conditions.

**Author Contributions:** V.N. Conceived and designed the analysis; A.M.P. Collected the data; M.E.I. Contributed data or analysis tools; V.N. and A.M.P. Performed the analysis; M.E.I. Wrote the paper; V.N. Revised the paper.

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**Conflicts of Interest**

The authors declare that they do not have any conflict of interest.
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