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## Abstract

Strawberries are perishable fruits with high nutritional value and strong consumer appeal. This study aimed to investigate the effectiveness of different postharvest pre-treatments in preserving the overall quality and extending the shelf-life of organic strawberries during cold storage ( $4.0 \pm 0.5$  °C). Four different treatments were tested: carbon dioxide (CO<sub>2</sub>, 30% for 3 h), ozone (O<sub>3</sub>, 5 ppm for 30 min), and two edible coatings (sodium alginate–calcium chloride and sodium alginate–moringa leaf extract). Unlike most previous studies focusing on conventional strawberries or individual treatments, this research provides a comparative evaluation of multiple GRAS-compliant strategies applied specifically to organically grown strawberries. The study aims to identify scalable, safe, and effective alternatives designed for the organic sector’s limited postharvest options. Among the tested treatments, CO<sub>2</sub> treatment was the most effective in maintaining visual appearance, color, and firmness for up to 9 days. In contrast, O<sub>3</sub> treatment led to noticeable early bruising due to skin oxidation and reduced firmness, while edible coatings did not yield significant improvements under the tested conditions. These findings support the use of CO<sub>2</sub> as a simple, safe, and cost-effective postharvest strategy to preserve the commercial quality of organic strawberries.

**Keywords:** Carbon dioxide; edible coating; G.R.A.S.; organic strawberry; ozone; postharvest.

## Introduction

Strawberries (*Fragaria × ananassa* Duch.) are a popular fruit with high economic value due to their delicious taste, numerous health benefits, and consumer preference for organic options. According to the Food and Agriculture Organization of the United Nations, global strawberry production is significant, reaching 8.8 million tonnes annually. This results in an important export market worth 2.9 billion (Zhang *et al.*, 2022).

Despite their appeal, strawberries are highly perishable due to their soft structure, high respiration rate, and sensitivity to fungal growth. Postharvest deterioration, including water loss, softening, and microbial decay, significantly limits their shelf life and marketability. Cold storage (CS) at 0-4 °C is a common method to slow the deterioration essential (Shahzad *et al.*, 2020); however, strawberries continue to lose water and undergo quality loss even under refrigerated conditions (Haider *et al.*, 2022).

Mechanical damage during harvesting and handling is a major issue for strawberries. Their susceptible structure makes them prone to abrasions, cuts, bruises, and juice leakage (Aliasgarian *et al.*, 2015). Bruising is particularly problematic as it reduces shelf life, causes softening, and allows fungal infections (Aliasgarian *et al.*, 2015; Qureshi Quarshi *et al.*, 2023). Proper harvesting to prevent these damages is crucial to avoid infections, preserve strawberries' quality and market value (Azam *et al.*, 2019; Trinetta *et al.*, 2020).

Water loss is another key challenge in the postharvest loss of strawberries due to their permeable skin (Hurtado *et al.*, 2021). Fresh strawberries contain 80-95% water, giving them an attractive appearance and texture. After harvest, water loss leads to wilting, shriveling, browning, and reduced flavor and weight (Peng *et al.*, 2017). Moreover, small changes in humidity can significantly affect water loss; strawberries stored at 76 % relative humidity (RH) lose more water than those at 86% for five days of storage at 5°C. Maintaining high humidity during both cold and shelf storage can help reduce this loss (Lufu *et al.*, 2020). Moreover, fungal pathogens are a major cause of postharvest losses in strawberries. Low pH, high water content, high sugar, and soft texture make strawberries susceptible to infections (Trinetta *et al.*, 2020). These fungi grow in humid, cool storage conditions, starting from wounds on the fruit, where *Botrytis cinerea*, causing grey mold, is the most common

pathogen (Feliziani and Romanazzi, 2016). Effective postharvest control is essential to prevent significant losses (Trinetta *et al.*, 2020).

In traditional practice, sulfur dioxide (SO<sub>2</sub>) is used to slow fungal growth and extend the shelf life of conventional strawberries under storage conditions (Hakimi *et al.*, 2017). However, SO<sub>2</sub> use can lead to bleaching of the fruit, and the residue left on strawberries poses potential health risks to consumers (Daniel-Swartland *et al.*, 2024). Due to increasing concerns, regulatory bodies like the United States Food and Drug Administration (US FDA) have restricted SO<sub>2</sub> use and required labeling of sulfites on fruits with residual levels exceeding 10 µL L<sup>-1</sup>. Similarly, the European Union (EU) prohibits the postharvest use of SO<sub>2</sub> on organic crops (Matera *et al.*, 2024).

As consumers become more aware of pesticide residues and environmental concerns, there is growing interest in safer and more sustainable alternatives for postharvest treatment of organic produce. These alternatives, which have a minimal impact on the environment and human health, include biocontrol agents, natural antimicrobials, and physical methods (Romanazzi *et al.*, 2012).

Efforts to develop alternative strategies for limiting postharvest decay in organic strawberries focus on solutions that are safe, effective, cost-efficient, compatible with commercial handling procedures, and compliant with organic agriculture standards. Biocontrol agents have emerged as a promising alternative to synthetic fungicides, demonstrating significant success in mitigating preharvest and postharvest diseases through the use of antagonistic microorganisms (Sellitto *et al.*, 2021).

Natural antimicrobials, such as ethanol (Feliziani and Romanazzi, 2016), essential oils (Abd-Elkader *et al.*, 2021; Haider *et al.*, 2022), and calcium chloride (CaCl<sub>2</sub>) (Weber, 2020), were also tested to reduce post-harvest decay and maintain quality standards along with marketing. Furthermore, the application of gaseous mixtures or natural components permitted in organic farming, such as natural extracts, are alternatives to control postharvest decay (Admane *et al.*, 2018).

Since ozone (O<sub>3</sub>) was declared a GRAS (generally recognized as safe) compound, the food industry has shown interest in developing processes that involve its applications. O<sub>3</sub> exposure has been a viable option for the postharvest treatment of berries, especially for

strawberries. It has been investigated the effects of pairing modified atmosphere packaging (MAP) with gaseous O<sub>3</sub> pre-treatment for strawberries, raspberries, and blueberries during CS, and results have shown significant reductions in yeasts and other fungi compared to samples stored conventionally, with no adverse impact on berry quality (Fang & Wakisaka, 2021). Hence, O<sub>3</sub> is considered a promising postharvest technology that could extend the shelf life of strawberries (Macías-Gallardo *et al.*, 2023). However, it is essential to note that high concentrations of O<sub>3</sub> and prolonged exposure times can negatively affect strawberry quality, and the effectiveness of gaseous O<sub>3</sub> may vary depending on the strawberry variety being treated, which is important to consider for industrial-level applications (Macías-Gallardo *et al.*, 2023).

Besides, short-term exposure to high-carbon dioxide (CO<sub>2</sub>) treatment is a common strategy for reducing strawberries' postharvest losses. This approach effectively reduces respiration rate, postharvest decay, and maintains fruit firmness. However, high CO<sub>2</sub> concentrations (>20%) are usually more effective but make them susceptible to physiological injuries like fruit discoloration and off-flavors (Eum *et al.*, 2021). Short-term high levels of CO<sub>2</sub> from 10 to 100 kPa for around 3 hr have been shown to increase fruit firmness, while excessive CO<sub>2</sub> concentrations can lead to physical damage, such as discoloration and off-flavors (Eum *et al.*, 2021). High levels of CO<sub>2</sub> can also inhibit the growth of various pathogens, including *B. cinerea*, with a more significant inhibitory effect at lower temperatures (Lurie, 2001). Additionally, short-term exposure to CO<sub>2</sub> during cooling has also been beneficial in maintaining the freshness of strawberries during transportation and local marketing (Jin Choi *et al.*, 2016).

Edible coatings are another promising approach to maintaining strawberry quality and extending shelf life, providing a semi-permeable barrier that reduces microbial attack, moisture and solute movement, gas exchange, and oxidative reaction rates. Studies have proven that edible coatings could improve the visual appeal of fruits and delay spoilage, minimizing decay, softening, and the ripening process (Shafique *et al.*, 2023). Unlike traditional synthetic packaging, edible coatings and films offer a sustainable solution that is both environmentally friendly and consumer-safe (Popescu *et al.*, 2022).

Edible coatings can comprise various components, including proteins, polysaccharides, and essential oils (Riaz *et al.*, 2021). One recent advancement in edible coatings with sodium alginate -  $\text{CaCl}_2$ -based, has demonstrated that it can significantly extend the shelf life of cut strawberry fruits by acting as a physical barrier and a carrier of antimicrobial agents (Alharaty and Ramaswamy, 2020). Moreover, natural plant derivatives, such as Moringa Leaf Extract (MLE), are also used as an edible coating, proposing a cost-effective and eco-friendly approach to improve fruit quality and extend shelf life. Research showed that applying MLE after harvest could delay fruit senescence and improve the overall quality attributes (Shafique *et al.*, 2023).

This study aims to compare the effectiveness of multiple GRAS-compliant postharvest treatments, namely  $\text{CO}_2$ ,  $\text{O}_3$ , and two types of edible coatings (sodium alginate- $\text{CaCl}_2$  and sodium alginate-MLE), in preserving the commercial quality of organically grown strawberries during cold storage. While many previous studies that examined individual treatments or focused on conventional strawberries, this research provides a side-by-side evaluation of alternative treatments specifically for organic fruit. The novelty of the study lies in its comprehensive approach and its relevance to the organic market, where few postharvest treatments are legally permitted. The goal is to identify a safe, effective, and scalable method for reducing postharvest losses in organic strawberries, thereby supporting overall food quality, sustainability, and waste reduction.

## **Materials and Methods**

### **Plant materials**

Fresh organic strawberries (*Fragaria × ananassa* Duch. cv. *Melissa*) with 80-90% red color were hand-harvested from a commercial organic greenhouse farm in Policoro, Italy (APOFRUIT Italia soc. coop. Agricola). The harvest took place in mid-March 2023. The strawberries were placed in open recycled polyethylene terephthalate (R-PET) trays and immediately transported to the postharvest laboratory. After pre-cooling at 4°C for 1 h, the strawberries were sorted for uniform size, absence of physical damage and fungal infection, and over 80% red surface color.

### **Treatment applications**

A randomized complete block design experiment was set, with 5 samples and three replications (i.e., three containers) per sampling. The experiment step aimed to evaluate individual postharvest technologies cited in the literature. Fresh organic strawberries were randomly divided into four treatment groups and two control groups (distilled water and non-treated), as shown in Table 1. Each group consisted of three biological replicates ( $n=3$ ), with 100-120 g of fruit per replicate, packed in commercial macro-perforated recycled PET clamshells. All samples were stored at  $4.0\pm0.5^{\circ}\text{C}$  and 80-90% relative humidity for 15 days. Sampling was performed on days 0, 3, 6, 9, and 15.

### **Preparation and application of edible coating based on sodium alginate and $\text{CaCl}_2$**

The coating solution was prepared following Alharaty and Ramaswamy (2020) with slight modifications. For the coating, 2% sodium alginate and 2%  $\text{CaCl}_2$  solutions were made with distilled water. Two g of sodium alginate was dissolved in 100 ml of distilled water and heated to  $100^{\circ}\text{C}$  while stirring at 300 rpm. Similarly, 2 g of  $\text{CaCl}_2$  was dissolved in 100 mL of water. Strawberries were dipped in the sodium alginate solution for 5 min, drained for 1 min, then dipped in the  $\text{CaCl}_2$  solution for 2 min, and drained again to remove excess coating.

### **Preparation and application of edible coating based on MLE**

MLE was obtained from the University of Bari “Aldo Moro”, Italy. According to Admane *et al.* (2023), fresh moringa leaves were dried and then extracted using microwave-assisted extraction. A 200 mg sample of leaf powder was mixed with 2 mL of water, microwaved for 5 min at  $80^{\circ}\text{C}$ , filtered, and centrifuged. The supernatant was lyophilized and dissolved in 1 L of distilled water, stirred at 300 rpm until fully mixed. The strawberries were dipped in the MLE solution for 5 min, drained for 1 min, then dipped in  $\text{CaCl}_2$  solution for 2 min, and drained again to remove excess coating. Control samples were washed with tap water and drained for 10 min at room temperature.

### **Preparation and application of CO<sub>2</sub> treatment**

Strawberries were packed in macro-perforated (Ø 8 mm) recycled PET clamshells (500 ml capacity, 100-120 g per sample). Afterward, clamshells were placed in sealed barrels with two pipes for air removal and gas treatment. The mixture of 30% CO<sub>2</sub> and 70% N<sub>2</sub> from a tank was used to inject gas into the barrel. Once the barrel was full of the gas mixture, they were placed for 3 hr at 4°C (Jin Choi *et al.*, 2016).

### **Preparation and application of O<sub>3</sub> treatment**

Strawberries were packed in macro-perforated (Ø 8 mm) recycled PET clamshells (500 ml capacity, 100-120 g per sample). Afterward, clamshells were placed in sealed barrels with two pipes for air removal and gas treatment. 5 ppm O<sub>3</sub> was injected into the barrel and then placed for 30 min at 4 °C. O<sub>3</sub> was produced using an O<sub>3</sub> generator (Chen *et al.*, 2019).

### **Control groups setup**

Control groups were made with non-treated (NT) and distilled water-dipped strawberries (Water). Treated samples were compared to untreated controls and stored at 4.0±0.5°C with 80-90% RH for 15 days. Chemical, mechanical, physical-chemical, and microbiological attributes were evaluated on days 0, 3, 6, 9, and 15 of CS.

### **Determination of physicochemical properties**

#### ***Fruit weight loss***

Strawberries were individually weighed at the beginning and at each sampling time using a precision digital scale (±0.01 g) (Gibertini Europe, Novate Milanese, Italy). The percentage of weight loss (WL) was then calculated and expressed as a percentage.

#### ***Appearance***

The visual appearance of samples was evaluated based on the color and the decayed surface area. The macro-perforated recycled PET clamshells of the treated and the control samples were stored at 4.0°C and used to evaluate the visual appearance of treated strawberry fruits



and the control groups during the experiment. Visual appearance photographs of the samples were taken on evaluation days.

### ***Firmness***

Firmness was measured using an Instron Universal 3343 Series Machine (Instron Mechanical Testing Systems, Norwood, MA, USA) with a 500 N load cell and a 3 mm cylindrical probe, following the method of Soazo *et al.* (2014) with slight modifications. Five strawberries per replication were tested at a puncture speed of 1 mm s<sup>-1</sup> to a depth of 6 mm at the fruit's equator. Results were expressed in Newtons (N).

### ***Color***

The external color of five strawberries was assessed using a portable colorimeter (SAMA Tools Colorimetro SA130, Viareggio, LU, Italy). The CIELAB color parameters L\* (brightness: 0 is black, 100 is white), a\* (red trend), and b\* (yellow trend) were recorded. The Total Color Difference ( $\Delta E$ ) was also measured to indicate color changes between stored and control samples.

### ***Total soluble solids and titratable acidity***

Five berries per tray were crunched, and the tissue was used for the chemical assessments [total soluble solids (TSS), titratable acidity (TA), total phenolic compounds (TPC), anthocyanin, DPPH]. TSS was measured using a hand refractometer (Atago Co. Ltd, Tokyo, Japan) and expressed in °Brix, following AOAC procedures.

TA was measured using the AOAC titrimetric method. A 1 g sample diluted in 50 mL of deionized water was titrated with 0.1 M potassium hydroxide (KOH) using phenolphthalein as an indicator. The analysis was performed with a T50 automatic titrator (Titrette®, Germany), and results were expressed as a percentage of citric acid.

## **Determination of bioactive compounds and antioxidant capacity**

### ***Extraction of bioactive compounds***

Anthocyanins were extracted from fruit tissue using 1% hydrochloric acid (HCl) in pure methanol (MeOH). One g of fruit tissue was mixed with 10 mL of this solvent, stirred in the dark for 1.5 h at room temperature, and centrifuged. The supernatant was collected and stored at -18°C for analysis. Two biological replications were performed for each treatment, and the extracts were analyzed for phenolic compounds, anthocyanin content, and antioxidant capacity (Parra-Palma *et al.*, 2020).

### ***Total phenolic compounds, total anthocyanin content and antioxidant capacity***

TPC in strawberries was measured using the Folin-Ciocalteu (FC) reagent method with some modifications. A 100 µL centrifuged fruit extract (13,500 rpm per 10 min) was mixed with 3.25 mL of pre-diluted FC reagent and allowed to stand for 7 min. Then, 750 µL of 20% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added, and the mixture was incubated in the dark for 2 hr. Absorbance was recorded at 760 nm, and results were calculated using a standard gallic acid calibration curve ( $y = 454,22x + 5,5911$ ,  $R^2 = 0,9993$ ). Data are expressed as µg gallic acid equivalent per mL of sample. Each sample was measured in triplicate (Shahinuzzaman *et al.*, 2020).

The TAC in strawberries was measured using a modified method from Singh *et al.* (2022). Methanolic extracts were prepared by mixing 0.1 mL of fruit extract with 0.9 mL of solvent (1% HCl in pure MeOH). The absorbance was read at 536 nm against a blank. Three replicates were taken for each sample. TAC was expressed as nmol cyanidin-3-glucoside per gram of fruit, using a molar extinction coefficient of 34,300 L mol<sup>-1</sup> cm<sup>-1</sup>.

Antioxidant capacity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition method, modified from Shahinuzzaman *et al.* (2020). A UV-visible spectrophotometer (Biochrom Ultraspec 2100 Pro Scanning, United Kingdom) was used at 520 nm. Each sample contained 100 µL of fruit extract mixed with 3.9 mL of 0.1 mM DPPH in ethanol (EtOH). Samples were incubated for 30 min at room temperature and measured in triplicate. Ethanol was used as a blank, and a control sample without antioxidants was also included.

### ***Microbiological analyses***

Strawberries from each pre-treatment were homogenized in a stomacher bag with a filter for 1 min using a Lab-Blender Model 400 stomacher. From each replication, a portion of 2 berries was randomly selected, aseptically mixed 1:9 (w/w) with sterile physiological solution (0.85% NaCl) for 1 min, and then further diluted using the sterile physiological solution.

### ***Total bacteria count***

Total bacteria count (TBC) was determined on Plate Count Agar (PCA) substrate with 0.1 g L<sup>-1</sup> cycloheximide (PCA+C) to inhibit yeast and mold by spiral plating (WASP Spiral Plater, bioMérieux Italia SpA, Bagno a Ripolo, Florence, Italy) and incubated at 30 °C for 48 h. Colonies were counted using a digital colony counter (EasyCount 2, bioMérieux Italy), and results were expressed as log CFU g<sup>-1</sup> fresh product (FP).

### ***Yeast and molds***

Yeasts and molds were determined on Glucose-Yeast Extract Agar (GYEA) with 0.1 g L<sup>-1</sup> chloramphenicol (GYEA+C) to inhibit bacterial growth. Samples were spread and incubated at 28 °C for 48 hr and up to 5 days to check for molds. The pH of the strawberry puree (homogenized from 3 strawberries for 1 min) was measured using a CyberScan pH 110 meter (Eutech Instruments, Thermo Fisher Scientific, Massachusetts, USA) and an electrode for solids (Double PoreSlimelectrode, Hamilton Company, Reno, NE, USA). Results were expressed as log CFU g<sup>-1</sup> FP.

### ***Statistical analysis***

Data were analyzed using Minitab 17 statistical software. A two-way ANOVA was used to assess the effects of storage time and pre-treatment on the strawberries' physicochemical, bioactive, and microbiological properties. Tukey's test ( $\alpha=0.05$ ) identified significant differences between means. Different uppercase letters indicate a significant difference between storage times for each treatment; different lowercase letters indicate a significant difference between treatments at the same storage time. Principal component analysis (PCA)

was also performed to explore relationships amongst experimental samples during storage and quality parameters.

## **Results and Discussion**

### **Determination of physicochemical parameters**

#### ***Weight loss***

A significant increase in WL was observed in all treatments over the 15-day cold storage period ( $p < 0.05$ ) (Figure 1). Strawberries are highly prone to rapid WL during extended storage due to their thin skin, which leads to water migration from the fruit to the atmosphere (Bose *et al.*, 2019). Over time, WL progressively increased in all groups; however, the rate and magnitude of this increase varied significantly by treatment. The highest WL occurred in strawberries coated with MLE, the sodium alginate-based coating, and water-dipped controls each exceeding 25% WL by day 15, indicating their limited barrier effect against moisture loss. In contrast, the CO<sub>2</sub>, NT, and O<sub>3</sub>-treated groups consistently demonstrated lower WL across all time points. Notably, the CO<sub>2</sub>-treated samples maintained ~10% WL until day 9, after which a moderate increase was observed, reaching ~20% on day 15. These findings align with Blanch *et al.* (2012), who noted that CO<sub>2</sub> can reduce WL by increasing cellular water retention linked to osmolyte accumulation. Likewise, Vazquez-Hernandez *et al.* (2018) showed that CO<sub>2</sub> helps maintain the structural and cellular homeostasis of the fruit, thereby limiting senescence-related WL. This trend is further supported by Jin Choi *et al.* (2016), who found that short-term CO<sub>2</sub> exposure significantly reduced WL while preserving freshness.

O<sub>3</sub>-treated samples showed intermediate performance, effective until day 9 but slightly less so thereafter. These results are consistent with Macías-Gallardo *et al.* (2023), who noted that O<sub>3</sub> application at moderate doses can delay WL but may lose efficacy over extended storage durations due to surface oxidation. These findings show that CO<sub>2</sub>, followed by O<sub>3</sub>, can effectively mitigate WL in organic strawberries during cold storage. However, post day-9, structural and metabolic changes may reduce treatment efficacy, pointing to the importance of combining treatments with humidity control or MAP systems for long-term storage.

Unexpectedly, the NT group also exhibited relatively low WL (~18%) by day 15, lower than some treated groups (CO<sub>2</sub>, O<sub>3</sub>), but not significantly different. While most literature reports higher WL in untreated controls predominantly, this deviation may be attributed to optimized cold storage conditions in our study (e.g., stable high humidity, minimal handling damage), which may have helped maintain cuticle integrity and reduce transpiration. These results highlight the need for further studies to better understand whether this effect is specific to our storage conditions or can be reproduced more broadly.

### ***Appearance***

In this study, pre-treated and non-treated (NT) strawberry fruits were stored at  $4.0 \pm 0.5$  °C to visually monitor the decayed surface area and color alterations. No signs of decay were observed in pre-treated and NT strawberries during the CS (Figure 2). This supports the findings by Nayik and Muzaffar (2014) and Lufu *et al.* (2020), who suggested that careful harvesting and consistent, rapid CS are necessary to maintain the high quality of harvested strawberries. On days 0 and 6, control and pre-treated strawberries maintained a bright red color with no visible defects or mold growth. However, by day 9, small brown spots due to oxidative stress appeared on the control samples, while pre-treated strawberries showed minimal changes, with only slight softening and no progress to mold. By day 15, the control group demonstrated noticeable browning and tissue softening, indicating advanced senescence. In contrast, pre-treated strawberries showed only minor softening and slight color darkening, remaining mostly free from mold growth. These results demonstrate that the pre-treatments used in this study effectively preserved visual quality and delayed browning and softening during CS. The lack of significant fungal growth in all samples underscores the role of controlled CS in maintaining the quality of strawberries.

### ***Firmness***

Firmness is a key quality attribute for fruits, often influencing consumer choices. In soft fruits like strawberries, a decrease in firmness during storage is a major factor affecting overall quality and shelf life (Jin Choi *et al.*, 2016; Panou *et al.*, 2021). Statistical analysis showed that pre-treatments, storage duration, and their interaction significantly affected strawberry

firmness ( $p < 0.05$ ) (Figure 3). Firmness decreased in all samples over time due to water loss and tissue softening, with fresh strawberries showing an initial firmness of 3.66 N. Manning (1993) attributed fruit softening to changes in cell wall components, mainly pectin, driven by enzymes such as polygalacturonase (Tanada-Palmu and Grosso, 2005). Gaseous exposure, especially to  $O_3$ , determine a rapid firmness decline after 3 days' storage, further reduced during and at end storage. The berries significantly reduce firmness after  $CO_2$  treatment, but when was used  $CO_2$  firmness was retained. Notably,  $CO_2$  pre-treatment maintained highest firmness (2.8 N) at the end of CS compared to the other pre-treatments. Similarly, Harker *et al.* (2000) observed enhanced texture retention in  $CO_2$ -treated strawberries. Scanning Electron Microscopy images suggested that the increased firmness in  $CO_2$ -treated samples might result from more pectin-to-pectin interactions facilitated by soluble pectin and surface juice, promoting gelation.

### **Color**

Color is a critical quality attribute for strawberries, influencing consumer acceptance. Statistical analysis showed a significant decrease in  $L^*$  values across all samples over storage time, reflecting ripening and potential senescence (Figure 4) (Chandra *et al.*, 2019). However,  $CO_2$ ,  $O_3$ , and coating pre-treatments slowed the decline, with  $CO_2$  pre-treatment maintaining the highest brightness ( $L^* = 32.6$ ) after 15 days, followed by coating (31.7) and  $O_3$  (31.5). Color change measurements ( $\Delta E$ ) showed significant increases, indicating remarkable color changes in all pre-treatments over time. By the 15th day of CS, most pre-treatments showed similar levels of color change, with no significant differences except in  $O_3$  and Water pre-treatments.  $CO_2$  and NT samples showed the lowest  $\Delta E$  values, suggesting better color retention.

Similarly, Petriccione *et al.* (2015) observed that strawberry color, particularly  $L^*$  and hue angle ( $H^*$ ), decreased during storage, causing the fruit to darken. In their study, uncoated fruits experienced faster color changes, while coatings slowed this process. Our findings also show that  $CO_2$  and coating treatments delayed brightness loss and minimized color changes, which is crucial for maintaining marketability.

The  $a^*$  parameter (redness) is also crucial for strawberry marketing. In this study, CO<sub>2</sub> treatment significantly preserved the  $a^*$  value of the strawberries, showing higher values compared to other pre-treatments and approaching NT samples, which surprisingly had the highest L\* and  $a^*$  values.

These findings align with Chandra *et al.* (2019), who found that CO<sub>2</sub> treatment effectively delayed senescence in "Charlotte" strawberries, preserving their visual appeal. Although Moringa and O<sub>3</sub> pre-treatments also delayed changes in  $a^*$  value, their effects were less distinct compared to CO<sub>2</sub>. On the other hand, coating treatments resulted in the lowest  $a^*$  value (16.4) after 15 days of storage. These observations align with previous studies, such as Khodaei *et al.* (2021), who found that edible coatings (carboxymethyl cellulose, pectin, persian gum, and tragacanth gum) had minimal effects on color characteristics in strawberries. Meanwhile, Nadim *et al.* (2014) reported that methylcellulose-based coatings reduced lightness in strawberries, supporting our findings for a lower  $a^*$  value with coatings. The parameter of the  $b^*$  value did not show any differences between the control and treated samples.

### ***Total soluble solids and titratable acidity***

Changes in TSS content are often linked to fruit ripeness and metabolic processes that increase sugar accumulation, enhancing sweetness (Treviño-Garza *et al.*, 2015). Fresh strawberries had an initial TSS of 8.9%. After 3 days' storage, the average TSS of control samples (NT) was the lowest (7.8%) and remained at this value up to the end of the storage. Throughout storage, no consistent variation in TSS evolution was observed across experimental samples, with no statistically significant differences (Figure 5). However, CO<sub>2</sub>-pre-treated strawberries maintained a TSS value closest to the fresh fruit (8.8%) by the end of the storage period, suggesting a stabilizing effect on sugar metabolism. This is consistent with the findings of Chandra *et al.* (2019), who reported a significant increase in TSS values in 'Charlotte' strawberries following short-term CO<sub>2</sub> treatments (50–70 kPa for 3 h), with final values rising from 6.90 to 8.65 °Brix. Although our study did not observe significant increases, the stability of TSS in CO<sub>2</sub>-treated samples supports the hypothesis that such

treatments can help preserve sweetness by limiting respiration-driven sugar degradation during cold storage.

The initial TA of strawberries was 0.76%, considered optimal by Gross *et al.* (2016). TA was only slightly affected by storage duration, with a general decrease observed after 15 days, attributed to the use of organic acids in respiration and conversion to sugars (Ornelas-Paz *et al.*, 2013). There were no significant differences in TA among pre-treatments. However, a statistically significant decrease was observed only in the NT group by day 15 ( $p < 0.05$ ), suggesting faster senescence and acid degradation in the absence of any protective treatment. These findings are consistent with those of Shin *et al.* (2019), who reported a comparable decline in TA during storage of 'Goha' strawberries, attributing it to natural ripening processes. Similar trends were also described by Han *et al.* (2004) in coated strawberries, where coatings helped mitigate rapid acidity loss. Notably, the initial TA value of 0.76% in our study is nearly identical to the 0.79% reported by Shin *et al.* (2019), suggesting physiological similarity between the two cultivars and lending further support to the observed trends under comparable postharvest conditions.

## **Determination of bioactive compounds and antioxidant capacity**

### ***Total phenolic compounds, total anthocyanin content and antioxidant capacity***

Phenolic compounds are essential plant components with antioxidant properties and associated health benefits. During storage, bioactive compounds like ascorbic acid, flavonoids (including anthocyanins), and phenolics can degrade due to oxidation, often caused by exposure to oxygen, light, and high temperatures (Farida *et al.*, 2023). The initial average TPC in fresh strawberries was 187.68 GAE/g (Figure 6). TPC after a short period (3 days) drops in all samples, following different trends during the cold storage. A bell-shaped trend with significant reduction was detected in CO<sub>2</sub> and water-treated samples, whilst a drastic reduction from the beginning of the storage was detected in coated samples. On the contrary, a notable increase was observed in O<sub>3</sub>-treated samples by day 15 ( $p < 0.05$ ). This aligns with Chen *et al.* (2019), who reported that O<sub>3</sub> exposure (5 ppm) stimulates phenylpropanoid metabolism, enhancing phenolic compound synthesis in strawberries. The increase in TPC and anthocyanins in the O<sub>3</sub> group may thus result from oxidative stress-



induced defense mechanisms. These findings support the hypothesis that moderate oxidative stimuli can elicit secondary metabolite production during C. In fact, on the contrary, the coated samples in this experiment (sodium alginate–CaCl<sub>2</sub> and sodium alginate–MLE) experienced the lowest phenolic content during storage, due to the lower oxidative stress. The anthocyanin biosynthesis process can continue in strawberries even after harvest and during storage at low temperatures, as Colussi *et al.* (2021) reported. This may explain the slight increase in anthocyanin content observed up to day 3 or, in some conditions, up to day 6. However, no significant differences in anthocyanin levels were found between samples from harvest to the end (day 15) of the storage period, except for Moringa and O<sub>3</sub> treated samples, where was found a significant reduction and increasing of anthocyanin, respectively. With the exception of the final values, this parameter's progression aligns with TPC evolution, that is, treatment-dependent, confirming that O<sub>2</sub> exposure during storage affects the synthesis of phenolic compounds, including anthocyanins.

Antioxidant activity, measured by DPPH radical scavenging rates, increased consistently over the storage period regardless of pre-treatment. However, this trend does not align directly with total phenolic and anthocyanin content. For example, Moringa- and coating-treated samples showed the highest (%) inhibition despite the lower TPC and anthocyanin values. This suggests that additional antioxidant compounds or surface interactions from the applied treatments, such as external phenolics or structural residues, may have contributed to the radical scavenging activity observed (Ezz El-Din Ibrahim *et al.*, 2022).

## **Determination of microbiological properties**

### ***Total bacteria count and yeast and mould***

Initial average TBC, yeast, and mould levels in freshly harvested strawberries were 4.39, 3.4, and 2.88 log CFU/g, respectively (Figure 7). TBC levels slightly decreased in both pre-treated and NT samples during the first 3 days of cold storage, although these changes were not statistically significant ( $p>0.05$ ). Microbial levels remained relatively stable in most samples, shifting of  $\pm 1$  log CFU/g was detected between the treatments.

After 3 days' storage, due probably the thermal shock of cold environment, all the microbial count of those species investigates, were reduced with characteristic dependent by the treatments.

Cold storage affected the TBC evolution as a reduction was found in all the samples along with the storage, however after 9 days the CO<sub>2</sub> and MLE showed a marked increase in TBC and moulds.

Among all treatments, O<sub>3</sub> application was the most effective in reducing TBC and yeast counts over time. These results align with Macías-Gallardo *et al.* (2023), who showed that low-dose gaseous O<sub>3</sub> (0.3-1.0 ppm) significantly inhibited mesophilic bacteria, molds, and yeasts in strawberries. Their findings suggested a bacteriostatic effect induced by oxidative stress, particularly during the early stages of storage. In our study, O<sub>3</sub>-treated strawberries similarly showed suppressed microbial growth, supporting their potential as a non-thermal antimicrobial strategy. Interestingly, O<sub>3</sub>-treated samples also maintained moderate weight loss and firmer texture by the end of storage, underlining the link between microbiological stability and physicochemical preservation.

Yeast and mould count initially decreased slightly after 3 days of storage but gradually increased over time. However, there were no significant differences between fresh and stored samples, and the levels remained within acceptable limits. NT samples also maintained lower microbial loads compared to treated samples, particularly at the end of the storage period. This may be attributed to minimal postharvest handling, which likely reduced the risk of cross-contamination introduced during treatment applications. These findings emphasize that while pre-treatments are designed to inhibit microbial growth, they may incorrectly compromise microbiological safety if not properly managed. Moreover, the microbial stability observed in NT strawberries throughout storage highlights the critical role of cold storage (4°C) in suppressing microbial growth. This finding aligns with previous studies, such as Ragaert *et al.* (2006), which reported that strawberries stored at slightly higher temperatures (>5°C) also maintained acceptable microbial stability, indicating that temperature control is a crucial factor in preserving microbial safety during postharvest storage. Conversely, while CO<sub>2</sub> is known to inhibit microbial growth, a notable increase in yeast and bacterial counts was observed in CO<sub>2</sub>-treated strawberries after day 9. This may

be explained by the microaerophilic nature of certain yeasts, which are capable of adapting to CO<sub>2</sub>-enriched environments and even using CO<sub>2</sub> in their metabolic pathways, as supported by Crancer *et al.* (2018) and Guadalupe-Daqui *et al.* (2023). These findings suggest that while CO<sub>2</sub> may suppress bacterial activity, it can paradoxically create a niche for yeast proliferation under certain nutrient-rich conditions.

Overall, these findings emphasize that postharvest strategies should be assessed using both microbial and physicochemical parameters. Although treatments such as CO<sub>2</sub> and O<sub>3</sub> can effectively alter microbial populations, their success depends on their interaction with fruit physiology, storage conditions, and handling practices. A comprehensive approach that balances microbial control with minimal quality loss is crucial to enhance the shelf life and safety of strawberries.

### **Multivariate analysis**

Multivariate analysis was performed using PCA. The biplot graph in Figure 8 (score plot + loading plot) illustrates the multivariate statistical processing obtained from the relationship matrix, containing the measured parameters and the product at each sampling time. Components 1 and 2 explain approximately 70% of the variance, effectively separating the samples based on the applied pre-treatments and storage times. The samples on day 6, particularly those with higher water content, acidity, L\* value, and TPC were positioned in the first dial, clearly separated from the other samples. Conversely, samples from day 15 were located in the opposite dial, characterized by a greater variation in weight loss, mold, and yeast. The last dial, which includes the FP, also contains the strawberries pre-treated with CO<sub>2</sub> on day 6. This dial is evident by the highest values of TPC and water content. In particular, the samples moved along the PC1 axis in correlation with storage duration and quality changes. Specifically, color and acidity were the most affected by storage, while weight loss and water content were more effective in discriminating between the pre-treatments. From the PCA results, the space coordinates of the samples were obtained, and their distance from the FP in the Cartesian plane was evaluated using the Euclidean distance (Table 2). According to this score, CO<sub>2</sub>-pre-treated samples, at 6 and 15 days of CS, were

closest to the FPs, suggesting that CO<sub>2</sub> is the most effective pre-treatment in maintaining strawberry overall quality during CS.

## **Conclusions**

This study showed that short-term postharvest treatments, particularly CO<sub>2</sub> exposure at 30% for 3 hours at 4 °C, effectively preserved the physicochemical, visual, and microbiological quality of organic strawberries during cold storage. Among all treatments, CO<sub>2</sub> consistently showed the highest performance in minimizing weight loss, preserving firmness and brightness (L\*), and maintaining bioactive compounds such as phenolics and anthocyanins. O<sub>3</sub> pre-treatment also contributed to bioactive compound retention, potentially through oxidative stress-induced activation of phenylpropanoid pathways. Although edible coatings such as Moringa extract (MLE) showed antioxidant benefits, their performance was inferior in terms of preserving firmness, color, and microbial quality compared to gas-based treatments. These findings suggest that the use of CO<sub>2</sub> pre-treatment, as an alternative to SO<sub>2</sub>, is a promising and non-destructive method for extending the cold storage life of organic strawberries while maintaining key quality attributes. The use of CO<sub>2</sub> treatment can be practically applied within existing cold storage systems and scaled up for commercial use. It may offer a feasible, clean-label option for reducing postharvest losses in both organic and conventional strawberry production. However, postharvest storage alone may not fully reflect commercial scenarios where fruits are subjected to fluctuations in temperature, handling, and retail conditions. Therefore, future studies should explore on evaluating the combined effects of the most effective treatment with innovative and sustainable packaging technologies. In particular, assessing biodegradable packaging materials under MAP conditions could help minimize postharvest losses during storage and distribution, simulating commercial shelf-life more accurately. This integrated approach will contribute to improving both the shelf life and environmental sustainability of organic strawberry supply chains.

## **Contributions**

**Burcu Aykanat:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization, Visualization. **Francesco Genovese:** Writing – review & editing, Supervision, Validation, Investigation, Data curation, Resources. **Naouel Admane:** Writing – original draft, Writing – review & editing, Methodology, Supervision, Validation, Investigation, Data curation, Resources. **Vincenzo Verrastro:** Writing – review & editing, Resources. **Simona Marianna Sanzani:** Writing – review & editing, Resources, **Maria Maddalena Cavalluzzi:** Writing – review & editing, Resources, **Attilio Matera:** Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Resources, Software.

## **Conflict of interest**

The authors declare no potential conflict of interest.

## **Data Availability**

Data will be made available on request.

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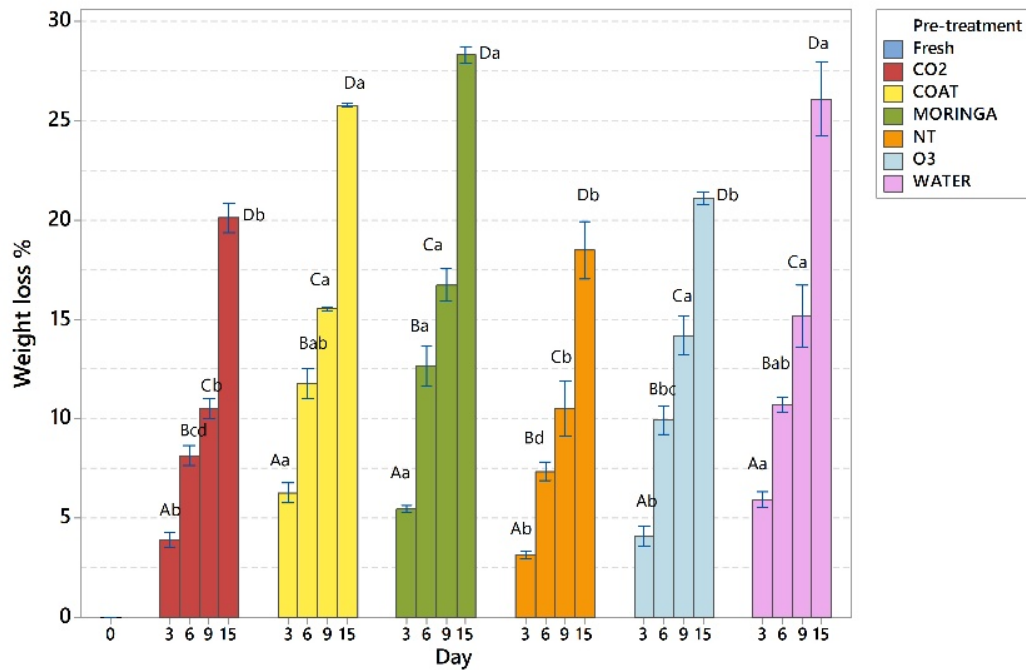
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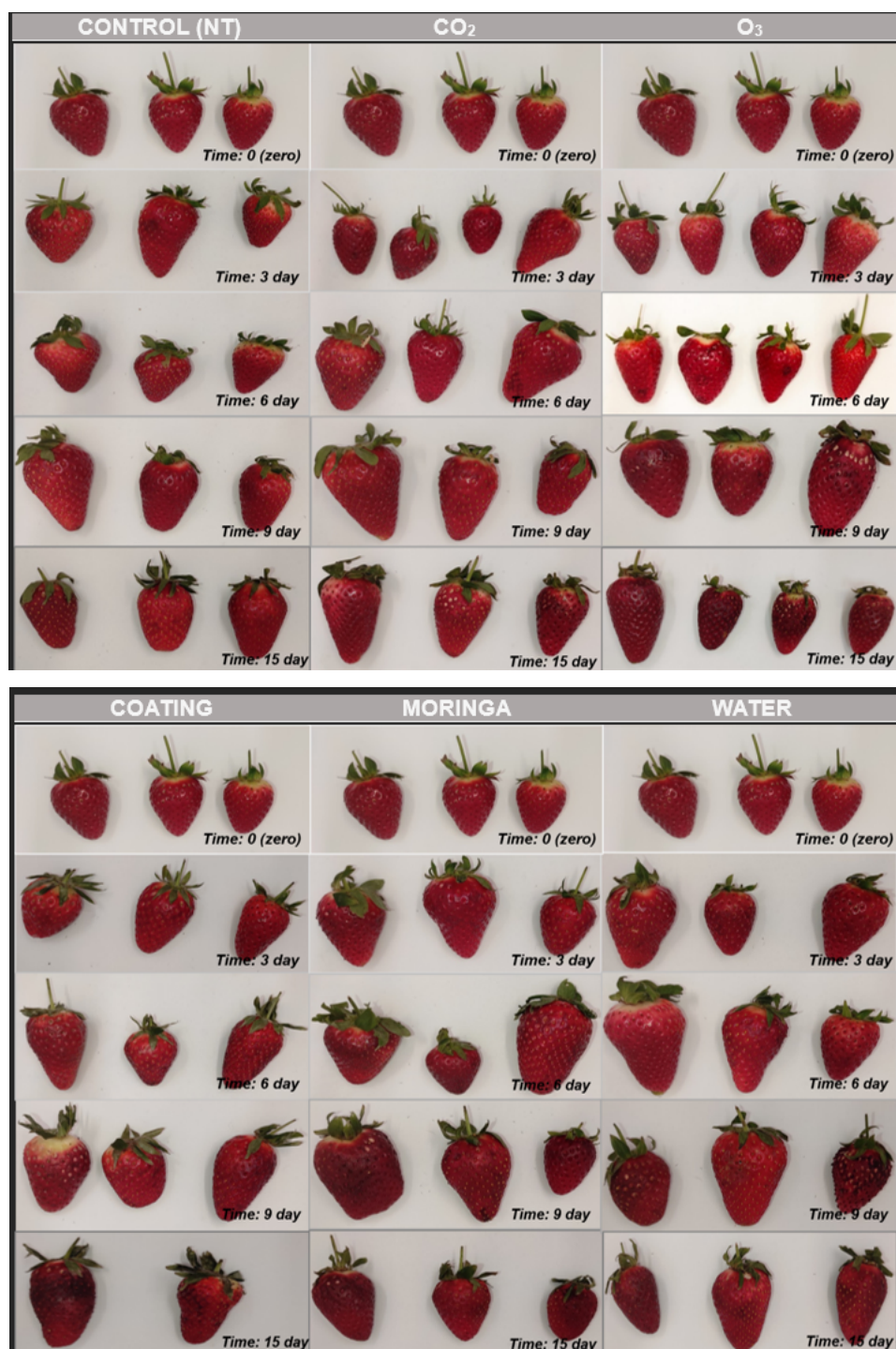
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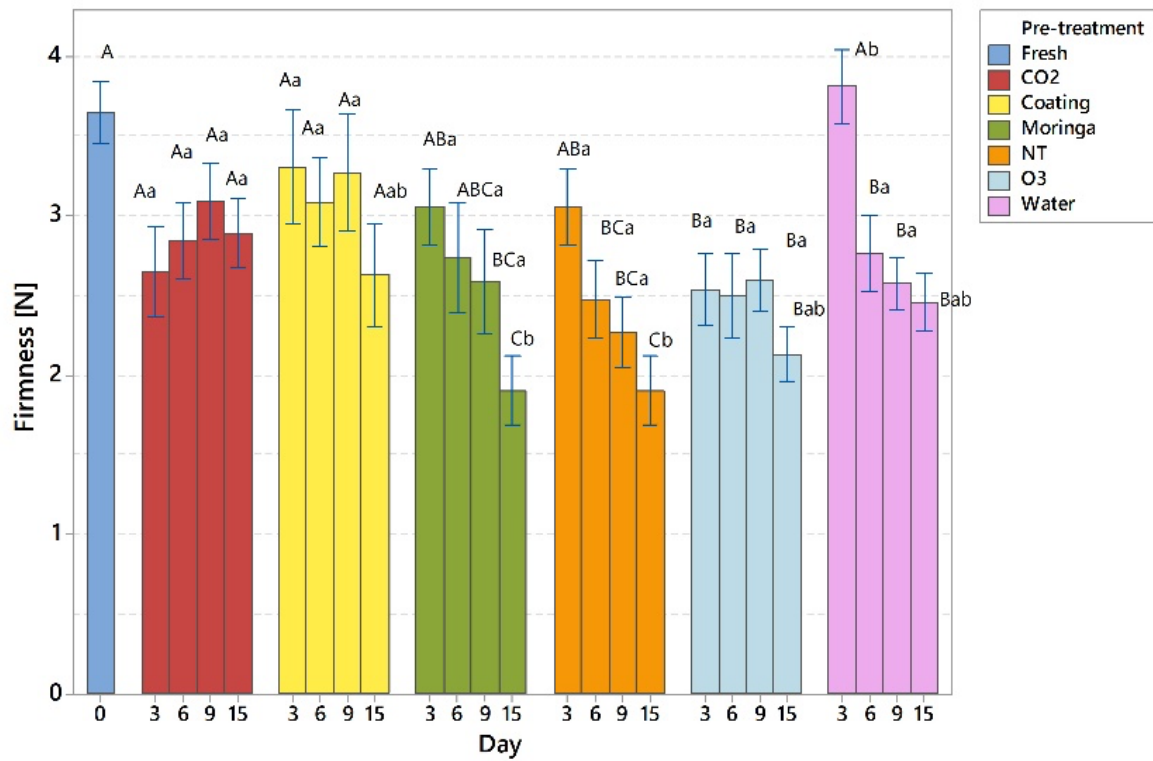
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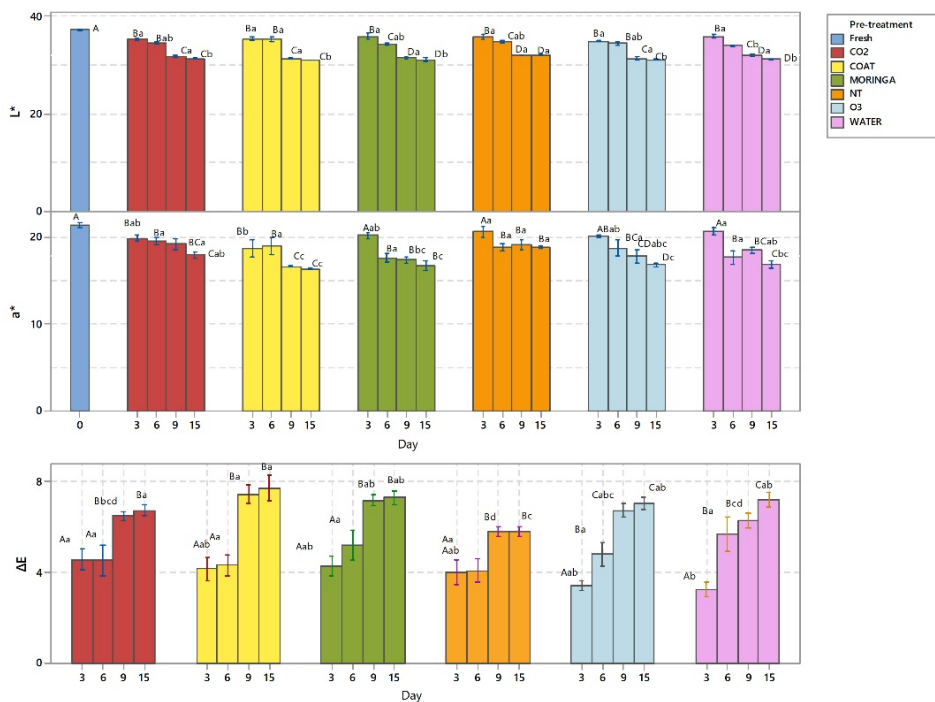
**Figure 1.** Effect of postharvest pre-treatments and storage period on weight loss of organic strawberries at 4°C; bars do not share the same letter are significantly different at  $p < 0.05$ ; different uppercase letters indicate a significant difference between storage time for each treatment; different lowercase letters indicate a significant difference between treatments at the same storage time.



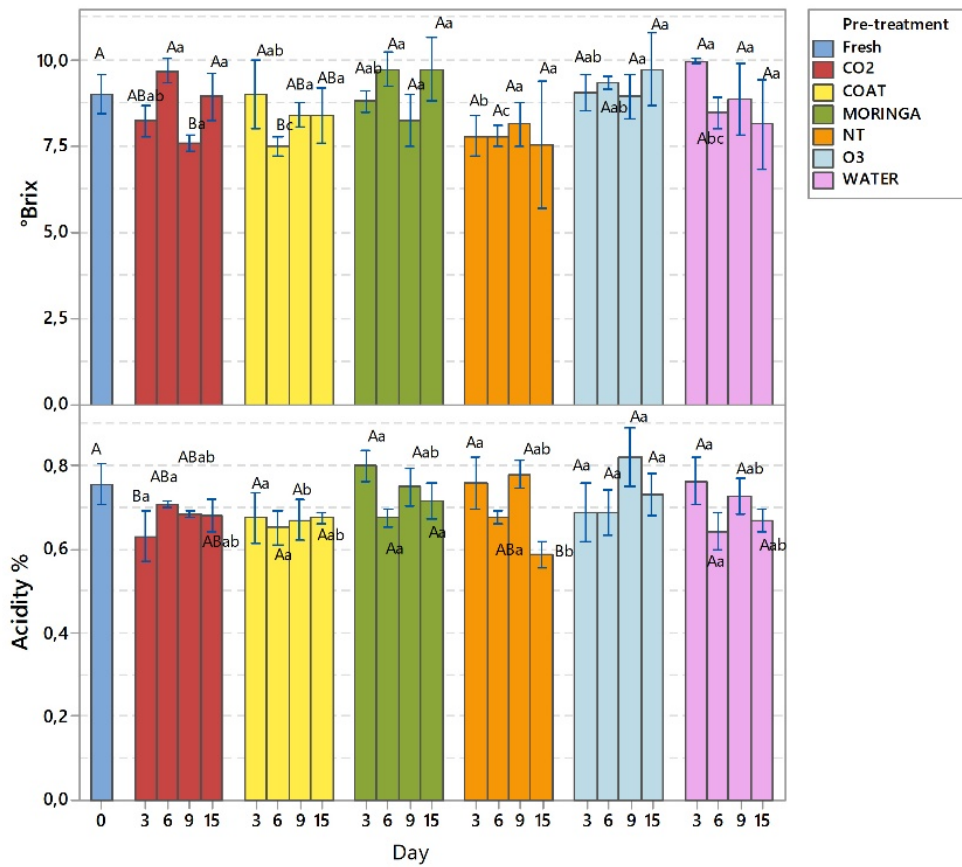
**Figure 2.** Pictures were taken at every sampling period of the non-treated (control) and pre-treated organic strawberries.



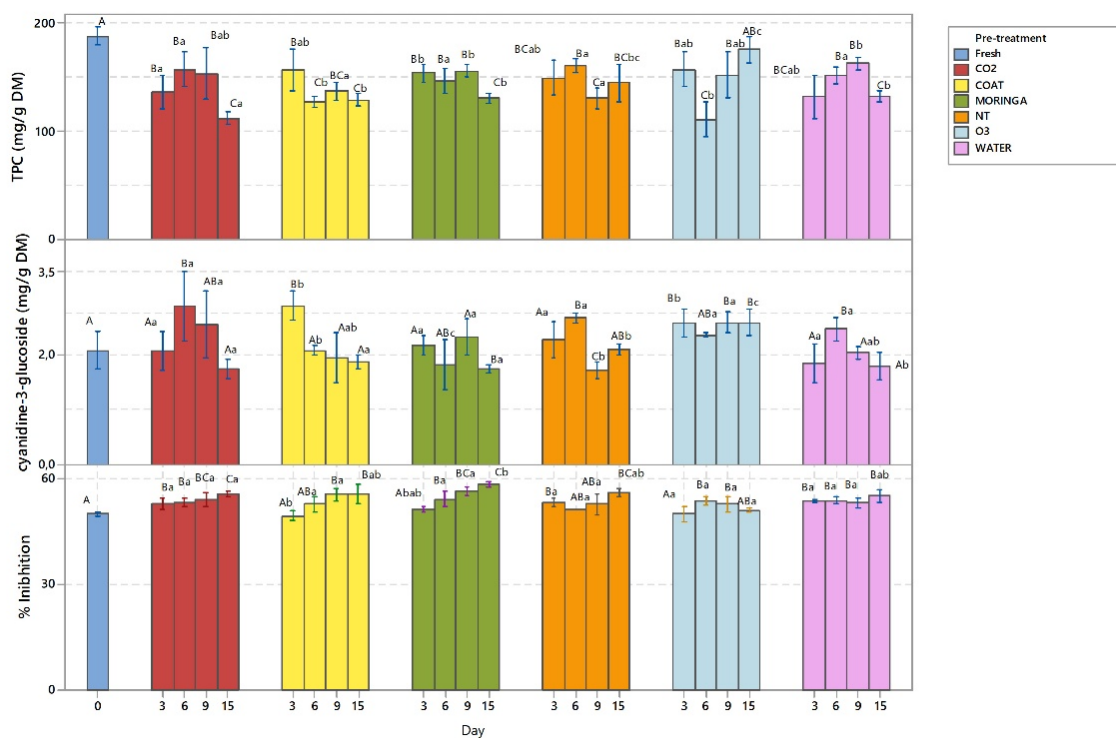
**Figure 3.** Effect of postharvest pre-treatments and storage period on firmness [N] of organic strawberry fruit at 4°C; bars do not share the same letter are significantly different at  $p < 0.05$ ; different uppercase letters indicate a significant difference between storage times for each treatment; different lowercase letters indicate a significant difference between treatments at the same storage time.



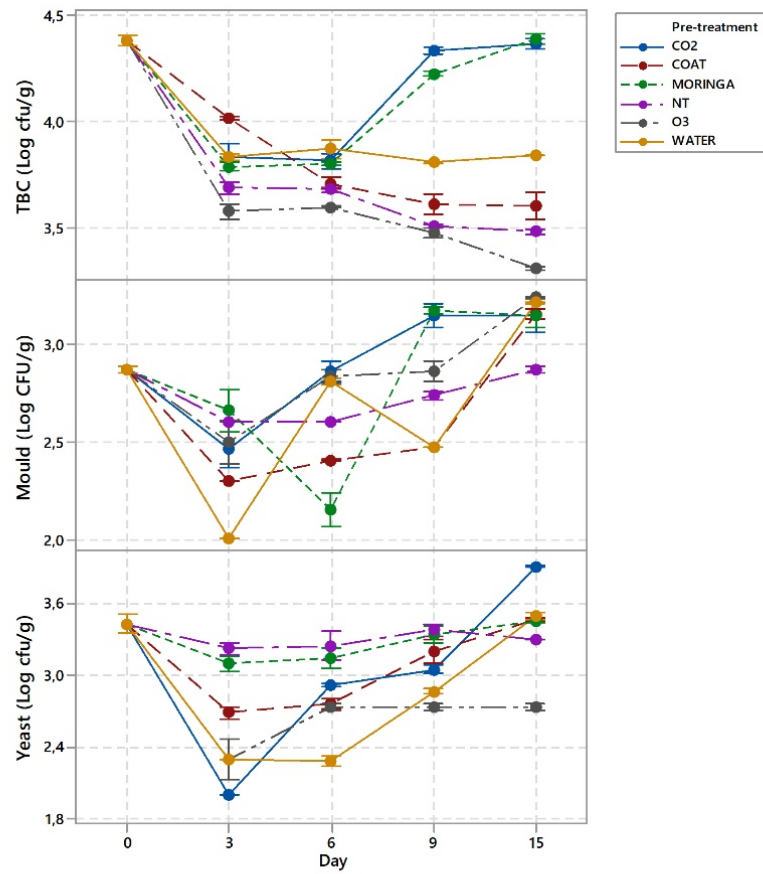
**Figure 4.** Effect of postharvest pre-treatments and storage period on L\*, a\*, and ΔE color parameters of organic strawberry fruit at 4°C; bars do not share the same letter are significantly different at  $p < 0.05$ ; different uppercase letters indicate a significant difference between storage times for each treatment, different lowercase letters indicate a significant difference between treatments at the same storage time.



**Figure 5.** Effect of postharvest pre-treatments and storage period on TSS (°Brix) and TA (%) of organic strawberry fruit at 4°C; Bars do not share the same letter are significantly different at  $p < 0.05$ ; different uppercase letters indicate a significant difference between storage times for each treatment; different lowercase letters indicate a significant difference between treatments at the same storage time.

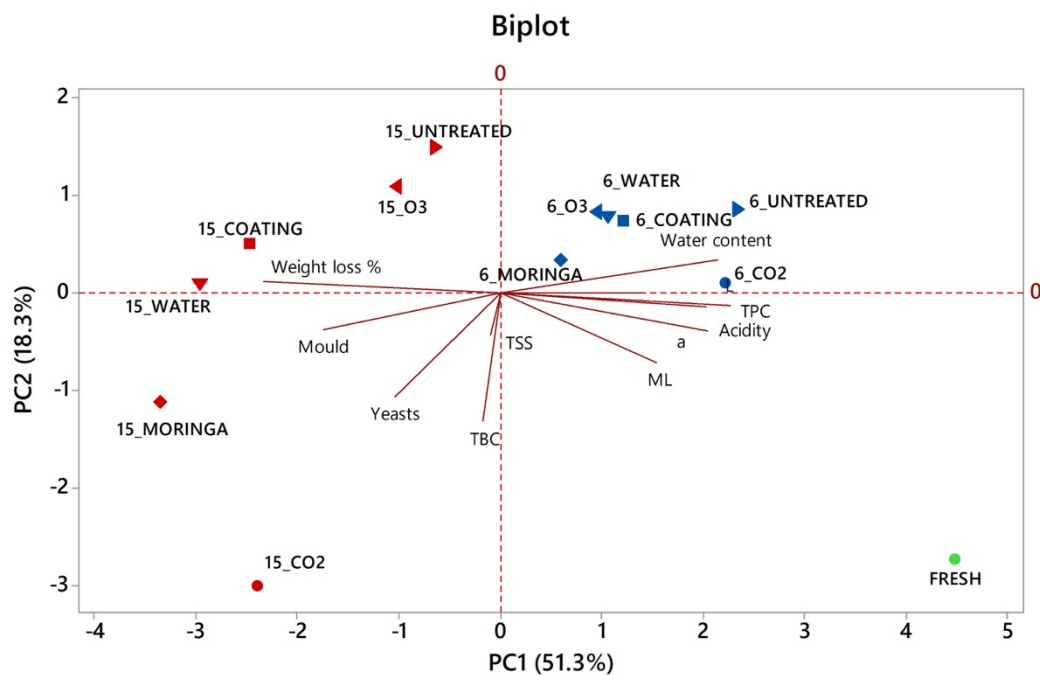


**Figure 6.** Effect of postharvest pre-treatments and storage period on TPC, cyanidine-3-glucoside, and DPPH of organic strawberry fruit at 4°C. Results are reported per gram of dry matter (DM). Bars do not share the same letter are significantly different at  $p < 0.05$ ; different uppercase letters indicate a significant difference between storage times for each treatment; different lowercase letters indicate a significant difference between treatments at the same storage time.



**Figure 7.** Evolution of TBC, yeast, and mould in organic strawberries during storage at 4°C.





**Figure 8.** Biplot graph (score plot + loading plot) of multivariate statistical processing obtained from the relationship matrix with the quality parameters of the products stored up to 6 (blue symbols) and 15 days (red symbols). The product at harvesting is the green circle (fresh strawberries).

**Table 1.** The experimental treatments were applied to organic strawberries.

Code	Treatment
CO <sub>2</sub>	30% CO <sub>2</sub> – 70% N <sub>2</sub> (3 h at 4°C)
O <sub>3</sub>	5ppm ozone (30 min at 4°C)
Coating	2% Sodium Alginate – 2% Na <sub>2</sub> CO <sub>3</sub>
Mle – coating	2% Sodium Alginate - 10% Moringa leaf extract
Water	Distilled water
NT	Non-treated

**Table 2.** Biplot coordinates of the samples and Euclidean distance with the fresh strawberries (FP).

Product	PC1	PC2	Euclidean distance with FP
Fresh	4,48	-2,73	-
6_CO <sub>2</sub>	2,21	0,1	3,63
6_O <sub>3</sub>	0,95	0,82	5,01
6_COAT	1,21	0,74	4,77
6_MORINGA	0,59	0,34	4,96
6_NT	2,33	0,85	4,18
6_WATER	1,05	0,79	4,91
15_CO <sub>2</sub>	-0,64	1,48	6,63
15_O <sub>3</sub>	-1,01	1,09	6,69
15_COAT	-2,47	0,51	7,67
15_MORINGA	-3,35	-1,11	8,00
15_NT	-2,39	-3	6,88
15_WATER	-2,96	0,09	7,96