Quality Assurance and Safety of Crops & Foods, 2025; 17(4): 330–352

Chitosan and oregano essential oil pretreatments preserve quality and extend shelf life of organic strawberries during cold storage

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Academic Editor: Slim Smaoui, PhD, Laboratory of Microbial, Enzymatic Biotechnology and Biomolecules (LBMEB), Center of Biotechnology of Sfax, University of Sfax-Tunisia, Road of Sidi Mansour Km 6, 3018 Sfax, Tunisia

Received: 1 September 2025; Accepted: 7 October 2025; Published: 14 November 2025

ORIGINAL ARTICLE

Abstract

Strawberries are highly perishable fruits, particularly under organic production systems where synthetic preservatives are restricted. This study assessed the efficacy of postharvest pretreatments, including chitosan (CH), oregano essential oil (OEO), their combination (CH+OEO), carbon dioxide (CO_2), ozone (O_3), and distilled water, in preserving physicochemical quality, bioactive compounds, antioxidant capacity, and microbiological safety of organic strawberries stored at 4±0.5°C for 15 days, compared to a nontreated control (NTC). The CH+OEO and CO_2 treatments were most effective in reducing weight loss and maintaining firmness, color, and visual and textural attributes. CH+OEO-treated fruits retained the maximum levels of total phenolics, anthocyanins, and antioxidant activity, whereas CO_2 slowed down ripening and suppressed microbial growth. O_3 treatment, in contrast, accelerated softening and surface damage, limiting its practical use. Pearson's correlation and principal component analysis highlighted strong relationships among bioactive compounds, antioxidant activity, and microbiological quality, with CH+OEO clustering closest to optimal preservation. These findings indicate that the combination of chitosan and OEO provides a natural, safe, and scalable approach to extending the shelf life and marketability of organic strawberries while maintaining their nutritional and visual-textural attributes.

Keywords: organic strawberries; postharvest treatments; chitosan; oregano essential oil; antioxidant capacity; bioactive compounds; microbial quality; shelf life extension

Introduction

Strawberries (*Fragaria* × *ananassa* Duch.) are one of the most widely consumed berries globally because of their attractive sensory characteristics, high nutritional value, and bioactive composition. They are rich in vitamin C, folates, dietary fiber, and phytochemicals, such as phenolic acids, flavonoids, and anthocyanins, which are associated with antioxidant, anti-inflammatory, and cardioprotective results (Giampieri *et al.*, 2022; Ulrich *et al.*, 2023). Owing to this unique nutritional and functional profile, strawberries are often considered a model fruit for the development of health-promoting diets. However, strawberries are also recognized as one of the most perishable fruits, with a postharvest life rarely exceeding a few days under ambient conditions. Their high respiration rate, fragile epidermal structure, and sensitivity to microbial contamination accelerate

ISSN 1757-837X online, DOI 10.15586/qas.v17i4.1588

deterioration processes, resulting in significant loss of firmness, color, bioactive compounds, and marketability (Lachman *et al.*, 2021). In some supply chains, postharvest losses of strawberries may reach 40–50%, representing not only an economic burden but also a critical issue for global food waste reduction strategies (Parisi *et al.*, 2023).

The challenge of strawberry preservation is particularly acute in the case of organically grown fruits. Unlike conventionally produced strawberries, which may benefit from synthetic postharvest preservatives and fungicides, organic strawberries must comply with stricter regulations that restrict the use of chemical interventions. Consequently, the organic fruit sector requires innovative and safe alternatives that are both consumer-acceptable and compliant with organic certification standards (Martínez-Hernández *et al.*, 2022). Addressing this need is especially relevant as consumer demand for organic fruits continues to rise, driven by increasing health awareness and environmental concerns.

A variety of natural preservation strategies are investigated for their potential to extend the postharvest life of strawberries. Among these, edible coatings based on biopolymers and natural antimicrobials have attracted significant attention. Chitosan, a cationic polysaccharide derived from the deacetylation of chitin, is one of the most promising coating materials for fruits and vegetables. It exhibits excellent film-forming properties, creates semi-permeable barriers to gases and moisture, and possesses intrinsic antimicrobial and antioxidant activities (Zhang *et al.*, 2023). By reducing respiration rate, delaying senescence, and inhibiting fungal growth, chitosan coatings can extend the shelf life of strawberries without negatively affecting their quality attributes. A comparative summary of recent studies on these strategies is presented in Table 1. For instance, Pérez *et al.* (2020) reported that chitosan combined with thyme oil effectively preserved firmness, color, and antioxidant capacity, while Guo *et al.* (2023) confirmed the role of edible coatings in suppressing microbial proliferation and extending shelf life. Similarly, gaseous treatments, such as carbon dioxide (CO_2), are shown to slow down ripening and limit microbial growth (Alshammari *et al.*, 2023), whereas O_3 exposure produced inconsistent results, often accelerating tissue softening and surface damage (Li *et al.*, 2021b).

Complementary to biopolymer coatings, essential oils (EOs) are increasingly studied as natural alternatives to synthetic preservatives. Oregano essential oil (OEO) is particularly relevant due to its high content of phenolic monoterpenes, such as carvacrol and thymol, which exhibit strong antimicrobial and antioxidant properties (Jordán *et al.*, 2022). When incorporated into chitosan-based coatings, OEO can enhance both

Table 1. Comparison of recent studies on postharvest treatments for strawberries.

Study	Treatment	Storage conditions	Main quality effects	Bioactive/ antioxidant retention	Microbiological effects	Notes/novelty
Algarni (2025, current study)	Chitosan + oregano EO (CH+OEO)	4°C, 15 days	Lowest weight loss, superior firmness, and color retention	Maximum phenolics, anthocyanins, and antioxidant activity	Strongest microbial suppression	Direct comparison with gaseous treatments: synergistic edible coating strategy for organic strawberries
Algarni (2023)	Chitosan + oregano EO	4°C, 15 days	Reduced weight loss, maintained firmness and color	High phenolics and anthocyanins	Reduced microbial growth	Confirmed CH+OEO as a scalable natural preservative
Shanmuganathan *et al.* (2022)	Chitosan coatings	4–5°C, 12 days	Maintained firmness and color	Improved antioxidant activity	Moderate microbial control	Focus on bioactive retention under cold storage
Alshammari *et al.* (2023)	CO_2 treatment	4°C, 14 days	Slowed ripening, and reduced weight loss	Maintained antioxidant activity	Strong microbial suppression	Gas treatment as a non-chemical alternative
Li *et al.* (2021a)	O_3 treatment	4°C, 10 days	Accelerated softening and surface damage	Reduced phenolics	Reduced microbial counts, but quality was compromised	Highlights the limitations of O_3 treatment
Pérez *et al.* (2020)	Chitosan + essential oils (thyme)	5°C, 15 days	Maintained firmness and color	Enhanced antioxidant activity	Reduced fungal growth	EO type affects efficacy; combination treatment is effective

antimicrobial and antioxidant efficacy, providing a synergistic effect that not only reduces microbial spoilage but also helps in retaining bioactive compounds and delaying quality deterioration (Ramos *et al.*, 2023).

As summarized in Table 1, past studies demonstrate the efficacy of chitosan-based coatings and gaseous treatments in slowing down deterioration of strawberries. However, most reports have examined these strategies in isolation. Our work advances this area by directly comparing edible coatings enriched with chitosan+oregano essential oil (CH+OEO) against CO_2 and O_3 gaseous treatments in organically produced strawberries. The results clearly show that CH+OEO provided superior preservation of firmness, color, phenolics, anthocyanins, and antioxidant activity, along with the most effective microbial suppression. This integrated comparison highlights the synergistic benefits of combining chitosan with essential oils while also contextualizing the limitations of O_3 and the strengths of CO_2, thereby offering novel insights for natural postharvest preservation strategies. Nevertheless, despite their promising potential, the effectiveness of CH+OEO coatings may depend on formulation, application method, and storage conditions, and thus require comparative evaluation against other strategies. Beyond coatings, gaseous treatments are explored as generally recognized as safe (GRAS) methods for postharvest preservation. CO_2 enrichment is widely known for its ability to inhibit microbial growth, suppress respiration, and delay enzymatic browning in perishable commodities. Several studies have reported that CO_2 exposure helps to maintain firmness, reduce fungal decay, and extend the marketability of strawberries during refrigerated storage (Pellegrini *et al.*, 2021). In contrast, O_3 treatment has gained attention due to its strong oxidizing capacity and ability to inactivate a wide range of microorganisms. O_2 can be applied either in gaseous form or dissolved in water, and is effective at reducing microbial loads on the surface of fresh produce (Oliveira *et al.*, 2024). However, its high reactivity may also cause oxidative damage to fruit tissues, accelerating softening and discoloration if not controlled properly. Although each of these strategies—edible coatings, essential oils, CO_2 enrichment, and O_2—are studied individually, comparative assessments remain limited. Most previous research has focused on conventional strawberry production systems, whereas organically produced fruits, with their restricted postharvest options, have received far less attention. Furthermore, few studies have simultaneously evaluated the impact of multiple GRAS-compliant treatments on a comprehensive set of quality parameters, including physicochemical traits (weight loss [WL], firmness, color, soluble solids, and acidity), retention of bioactive compounds, antioxidant activity, and microbial stability. This knowledge gap hinders the development of practical scalable solutions tailored for the organic fruit industry. In this context, multivariate approaches, such as Pearson's correlation and principal component analysis (PCA), provide valuable tools to understand in a better manner the relationships between quality attributes and to identify the most influential factors in postharvest deterioration. By integrating data across physicochemical, biochemical, and microbiological domains, such analyses offer deeper insights into treatment effectiveness and guide the selection of optimal preservation strategies.

Aim of the Research

The present study aimed to evaluate the effectiveness of different postharvest pretreatments on the quality and shelf life of organically grown strawberries during refrigerated storage (4.0±0.5°C). Specifically, the treatments that were tested included chitosan coating, OEO, a CH+OEO composite coating, CO_2 enrichment, and O_3 exposure, compared with untreated and water-dipped controls. The impact of these treatments was assessed on physicochemical parameters (WL, firmness, color, soluble solids, and titratable acidity [TA]), bioactive compounds (total phenolics and anthocyanins), antioxidant capacity, and microbiological properties (total bacterial, yeast, and mold counts). In addition, correlation and multivariate analyses were conducted to explore relationships among quality parameters and to identify the most effective treatment strategy. This research seeks to provide practical recommendations for the organic fruit sector by identifying scalable, safe, and consumer-acceptable postharvest solutions. By offering a comparative evaluation of multiple GRAS-compliant methods, it addresses a critical gap in literature and contributes to the broader goal of reducing postharvest losses and extending the shelf life of high-value organic produce.

Materials and Methods

Plant materials

Fresh strawberry (*Fragaria* × *ananassa* Duch.) fruits were obtained from commercial farms located in Taif region, Saudi Arabia, during the harvesting season of June 2024. The fruits were handpicked at the commercial maturity stage (bright red color, uniform size, and free from visible defects) early in the morning to minimize field heat. Immediately after harvesting, the fruits were sorted to remove damaged or diseased samples and then allocated for subsequent postharvest loss assessment under both traditional and improved handling practices.

Treatment applications

A randomized complete block design (RCBD) was adopted, comprising five sampling intervals and three replications (i.e., three containers) per sampling point. The objective was to evaluate the effectiveness of selected postharvest technologies previously reported in the literature for extending the shelf life of strawberries. Freshly harvested organic strawberries were randomly assigned to four treatment groups and two control groups (distilled water [DW] and untreated control [NTC]), as detailed in Table 2. Each treatment group consisted of three biological replicates (n = 3), with 100–120 g of fruit per replicate, packed in commercial macro-perforated recycled PET clamshells to simulate standard retail packaging conditions. All packaged samples were stored under controlled conditions at 4.0±0.5°C and 80–90% relative humidity (RH) for 15 days. Sampling and analyses were performed at five time points: day 0, 3, 6, 9, and 15.

Preparation and application of edible coating based on chitosan and oregano essential oil

The edible coating was prepared by using chitosan as a polymeric matrix and OEO as a bioactive antimicrobial and antioxidant agent. A 1.5% (w/v) chitosan solution was obtained by dissolving medium molecular weight chitosan (degree of deacetylation ≥ 85%) in 1% (v/v) acetic acid under continuous stirring for 6 h at room temperature until a clear solution was formed. Glycerol (0.5% v/v) was incorporated as a plasticizer to improve film flexibility and adhesion. OEO was added at 0.75% (v/v), and Tween-80 (0.1% v/v) was included as an emulsifier to ensure stable dispersion of hydrophobic oil in aqueous chitosan matrix. The mixture was homogenized at 10,000 rpm for 3 min using a high-speed homogenizer to produce a uniform emulsion. Fresh, uniform strawberries were washed with DW and air-dried at ambient conditions. Fruits were then immersed in CH+OEO solution for 2 min to ensure full surface coverage. Excess coating solution was drained, and the fruits were air-dried under sterile airflow for 30 min at room temperature to allow the formation of a thin edible film. Coated strawberries were packaged in macro-perforated recycled polyethylene terephthalate (PET) clamshells and stored at 4.0±0.5°C and an RH of 80–90% for 15 days. Evaluations of physicochemical properties, microbial quality, and the overall postharvest performance were conducted on days 0, 3, 6, 9, and 15.

Preparation and application of CO_2 treatment

Freshly harvested strawberries were first sorted for uniform size, color, and absence of visible defects. The fruit was packed in macro-perforated (Ø 8 mm) recycled PET clamshells to simulate standard retail packaging while allowing adequate gas exchange. The packaged fruit was placed inside airtight polypropylene chambers connected to a gas-flow system. The chambers were flushed with high-purity CO_2 gas (99.9%) until the headspace atmosphere reached a concentration of 20–30% CO_2 (balance air), as verified using a portable non-dispersive infrared (NDIR) CO_2 analyzer (Model GMA-200; Vaisala, Finland). After flushing, the chambers were sealed and stored under controlled conditions at 4.0±0.5°C and 80–90% relative humidity. To maintain a stable CO_2-enriched environment, the chambers were re-flushed every 48 h during the 15-day storage period. Control samples were stored under the same conditions

Table 2. Total phenolic compounds (TPC, mg GAE/100 g FW) of organic strawberries during 15 days of storage at 4°C.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 15
NTC	$455 \pm 5.00^{a,A}$	$430 \pm 4.00^{a,B}$	$400 \pm 5.00^{a,C}$	$340 \pm 5.00^{a,D}$	$285 \pm 4.00^{a,E}$
DW	$450 \pm 5.00^{a,A}$	$425 \pm 4.00^{b,B}$	$395 \pm 5.00^{b,C}$	$335 \pm 5.00^{b,D}$	$290 \pm 4.00^{b,E}$
CH+OEO	$458 \pm 5.00^{a,A}$	$450 \pm 4.00^{c,B}$	$435 \pm 5.00^{c,C}$	$415 \pm 5.00^{c,D}$	$400 \pm 5.00^{c,E}$
CO_2	$455 \pm 5.00^{a,A}$	$445 \pm 4.00^{c,B}$	$430 \pm 5.00^{c,C}$	$410 \pm 5.00^{c,D}$	$375 \pm 5.00^{c,E}$
O_3	$450 \pm 5.00^{a,A}$	$435 \pm 4.00^{c,B}$	$410 \pm 5.00^{c,C}$	$375 \pm 5.00^{c,D}$	$340 \pm 4.00^{c,E}$
CH	$455 \pm 5.00^{a,A}$	$445 \pm 4.00^{c,B}$	$430 \pm 5.00^{c,C}$	$410 \pm 5.00^{c,D}$	$380 \pm 5.00^{c,E}$
OEO	$452 \pm 5.00^{a,A}$	$440 \pm 4.00^{c,B}$	$425 \pm 5.00^{c,C}$	$405 \pm 5.00^{c,D}$	$380 \pm 5.00^{c,E}$

Notes:
TAC decreased significantly in all treatments during storage.
CH+OEO and CO_2 treatments better preserved anthocyanins, compared to NTC.
Values are mean ± SEM (n = 3).
Different superscript lowercase alphabets in the same column indicate significant differences between treatments at the same storage time ($P < 0.05$).
Different superscript uppercase alphabets in the same row indicate significant differences between storage periods within the same treatment ($P < 0.05$).

in ambient air (~0.04% CO_2). Quality analyses for both CO_2-treated and control samples—including WL, firmness, color, bioactive compounds, and microbial counts—were conducted on day 0, 3, 6, 9, and 15.

Preparation and application of O_3 treatment

Freshly harvested strawberries were subjected to gaseous O_3 treatment prior to storage. The treatment was carried out in a sealed stainless steel chamber (60-L capacity) connected to a laboratory-grade O_3 generator equipped with an integrated analyzer and flow controller to regulate O_3 concentration. The fruit was exposed to 2.5±0.2 ppm of O_3 gas for 15 min at 20°C, with a continuous airflow to ensure uniform gas distribution. Following treatment, the chamber was ventilated with filtered air for 10 min to eliminate residual O_3 before packaging. Safety precautions were strictly followed, including monitoring ambient air levels with an O_3 detector to ensure that concentrations remained below occupational safety limits (0.1 ppm, 8-h time-weighted average [TWA]).

Control groups setup

To enable unbiased comparison with the tested postharvest technologies, two control groups were included and handled identically to treated samples except for the absence of active treatment.

1. **Negative control (untreated):** Strawberries were not exposed to any coating, gas, or O_3 treatment. Fruit was packed in macro-perforated (Ø 8 mm) recycled PET clamshells (100–120 g per replicate; n = 3) and stored at 4.0±0.5°C and an RH of 80–90%, as described in previous strawberry storage studies (Duan *et al.*, 2022; Wang *et al.*, 2023).

2. **Sham/dip control (DW):** Fruits were immersed in DW for 2 min, drained, and air-dried for 30 min, mirroring the dip/drying steps of the coating treatment but without film-forming agents or essential oil. This practice is commonly applied in coating studies to distinguish the effects of handling from those of active ingredients (González-Cebrino *et al.*, 2021; Silva *et al.*, 2020).

3. **Sham handling for gaseous treatments:** For comparability with CO_2 and O_3 groups, corresponding controls were placed in the same sealed chambers and subjected to identical flushing/duration with ambient air only (no CO_2 or O_3), then packaged and stored under identical conditions, as recommended in recent gas treatment protocols (Yang *et al.*, 2024).

All control replicates were randomly assigned within each block alongside treatment groups and labeled with coded identifications (IDs) to minimize handling bias. Sampling was performed at day 0, 3, 6, 9, and 15, with baseline (day 0) measurements recorded immediately after preparation and packaging of control.

Determination of physicochemical properties

Fruit Weight Loss

Fruit weight loss was determined as an indicator of postharvest water loss and tissue dehydration. At each sampling point (day 0, 3, 6, 9, and 15), the initial fresh weight (W_0) of each replicate (100–120 g) was recorded immediately after treatment and packaging. Subsequent weights (W_t) were measured during storage using a digital balance with ±0.01-g accuracy. WL was expressed as a percentage of initial weight according to the following equation:

$$\text{Weight Loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100 \qquad (1)$$

Where W_0 = initial fruit weight (g) and W_t = fruit weight on sampling day (g).

This method is widely adopted for postharvest evaluation of strawberries and other soft fruits (Bashir *et al.*, 2021; Villalobos *et al.*, 2022). Monitoring of WL is essential, since excessive WL (>5–6%) generally results in visible shriveling, reduced firmness, and consumer rejection (Bastos *et al.*, 2023; Chen *et al.*, 2024).

Appearance evaluation

The visual appearance of strawberries was evaluated based on color uniformity and the extent of the decayed surface area. Treated and control samples were stored in macro-perforated recycled PET clamshells at 4.0±0.5°C, and their appearance was assessed throughout the storage period. A 5-point hedonic scale was applied to score fruit quality, where 5 = excellent (bright red, glossy, and decay-free), 3 = limit of marketability (slight shriveling or minor discoloration), and 1 = unacceptable (extensive decay or severe shriveling), following the approach of Al-Dairi *et al.* (2021) and Panahirad *et al.* (2022). To document changes, digital photographs of representative samples were taken on each evaluation day (0, 3, 6, 9, and 15). This method is widely used to track visual deterioration and consumer acceptability of strawberries during cold storage (Caleja *et al.*, 2023; Martins *et al.*, 2024).

Firmness Measurement

Firmness of strawberries was measured using a texture analyzer (TA.XT Plus; Stable Micro Systems Ltd., Surrey, UK) equipped with a 2-mm cylindrical stainless steel probe. Measurements were performed at room temperature, with the probe penetrating the equatorial region of each fruit to a depth of 5 mm at a crosshead speed of 1 mm/s, as described by Martins *et al.* (2024) and Pinheiro *et al.* (2021). For each replicate (100–120-g sample), five fruits were randomly selected, and firmness was recorded in Newton (N). The average value per replicate was used for statistical analysis. Care was taken to avoid measuring near the calyx or previously punctured areas to ensure consistency (Panahirad *et al.*, 2022). Firmness was considered a key indicator of structural integrity and marketability, because strawberries rapidly soften during postharvest storage because of enzymatic cell wall degradation and water loss (Caleja *et al.*, 2023).

Color Measurement

Color parameters of strawberry fruit were determined using a portable colorimeter (CR-400 Chroma Meter; Konica Minolta Sensing, Osaka, Japan) calibrated with a standard white tile prior to measurement. The CIE L* (lightness), a (redness/greenness), and b* (yellowness/blueness) values were recorded at two opposite equatorial points of each fruit to minimize variability. For each replicate (100–120-g sample), five randomly selected fruit samples were analyzed, and the mean values of L*, a*, and b* were calculated. The chroma (C*) and hue angle (h°) were also derived using the following equations (Hunter and Harold, 1987):

$$C^{r}* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h^{o} = \arctan\left(\frac{b^*}{a^*}\right) \tag{2}$$

These parameters were used to describe the intensity and direction of color. Development and retention of red color was considered critical indicators of strawberry visual quality and consumer acceptability (Caleja *et al.*, 2023; Panahirad *et al.*, 2022).

Total Soluble Solids (TSS) and Titratable Acidity

Total soluble solids

Total Soluble Solid content was determined using a digital refractometer (Atago PAL-1; Atago Co. Ltd., Tokyo, Japan) calibrated with DW. A drop of homogenized strawberry juice was placed on the prism, and the results were expressed as °Brix at 20±1°C (AOAC, 2016).

Titratable acidity

Titratable acidity was measured by titrating 10 mL of homogenized strawberry juice, diluted with 10 mL of DW, with 0.1-N NaOH to an endpoint of pH 8.1 by using a digital pH meter (HI 2020; Hanna Instruments, Italy). The results were expressed as percentage of citric acid equivalents (% w/w), following the AOAC (2016) guidelines. The TSS–TA ratio was also calculated as an indicator of fruit sweetness and the overall flavor balance, which strongly influences consumer acceptance and postharvest quality of strawberries (Caleja *et al.*, 2023; Panahirad *et al.*, 2022).

Extraction of Bioactive Compounds

Bioactive compounds were extracted from fresh strawberry samples following a modified solvent extraction procedure (Sagdic *et al.*, 2021; Silva *et al.*, 2023). Briefly, 10 g of homogenized strawberry pulp was mixed with 40 mL of 80% methanol (v/v in DW) containing 0.1% formic acid to enhance phenolic stability. The mixture was vortexed for 2 min and subjected to ultrasonic-assisted extraction (Ultrasonic Cleaner, Model VCX 130; Sonics & Materials Inc., Newtown, CT, USA) at 25°C for 20 min to improve extraction efficiency. After extraction, the mixture was centrifuged at 10,000 ×g for 15 min at 4°C (Centrifuge Model 5810 R; Eppendorf AG, Hamburg, Germany). The supernatant was collected, filtered through a 0.45-μm polytetrafluoroethylene (PTFE) syringe filter, and stored at −20°C for further analysis of total phenolics, flavonoids, anthocyanins, and antioxidant capacity.

Determination of Bioactive Compounds and Antioxidant Capacity

Total Phenolic Compounds (TPC)

The TPC of strawberry extracts was determined using the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965; modified by Ainsworth and Gillespie, 2007). Briefly, 0.5 mL of extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 min. Then, 2 mL of 7.5% Na_2CO_3 was added, and the mixture was kept in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Results were expressed as milligram gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW).

Total Anthocyanin Content (TAC)

Total anthocyanin content was quantified by the pH differential method (Giusti *and* Wrolstad, 2001). Extracts were diluted separately in pH 1.0 buffer (0.025-M KCl) and pH 4.5 buffer (0.4-M sodium acetate). Absorbance was recorded at 520 *nm* and 700 nm against DW as a blank. Anthocyanin concentration was calculated using the following equation:

$$\text{Monomeric anthocyanins (mg/100g FW)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \tag{3}$$

where:

- A = absorbance difference as defined above,
- MW = 449.2 g/mol (molecular weight of cyanidin-3-glucoside),
- ε = 26,900 L/mol·cm (molar extinction coefficient for cyanidin-3-glucoside),
- L = path length (1 cm), and
- DF = dilution factor.

Results were expressed as **milligrams of cyanidin-3-glucoside equivalents (mg C3G) per 100 g of fresh weight (FW)**.

Antioxidant Capacity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: This was determined following a recent adaptation of the method by Brand-Williams *et al.* (1995) described by da Silva *et al.* (2021). Briefly, 100 μL of extract was mixed with 3.9 mL of 0.1-mM DPPH solution in methanol and incubated in the dark for 30 min. Absorbance was read at 517 nm. Results were expressed as μmol Trolox equivalents per gram FW (μmol TE/g FW).

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay: This was conducted by following a modified method based on Re *et al.* (1999) described by Zhang *et al.* (2022). The ABTS+ radical solution was prepared by mixing 7-mM ABTS with 2.45-mM potassium persulfate and incubated in the dark for 16 h. Prior to use, the solution was diluted to an absorbance of 0.70±0.02 at 734 nm. Then, 100 μL of extract was mixed with 3.9 mL of ABTS+ solution, and absorbance was read after 6 min at 734 nm. Results were expressed as μmol TE/g FW.

Ferric reducing antioxidant power (FRAP): This was performed following a recent modification of Benzie and Strain (1996) done by Li *et al.* (2023a). Fresh FRAP reagent (300-mM acetate buffer, pH 3.6, 10-mM tripyridil-s-triazine (TPTZ) in 40-mM HCl, and 20-mM $FeCl_3{\cdot}6H_2O$ in a 10:1:1 ratio) was prepared. A total of 100 μL of extract was mixed with 3-mL FRAP reagent and incubated for 30 min at 37°C. Absorbance was measured at 593 nm, and results were expressed as μmol TE/g FW.

Microbiological analyses

Strawberries from each pretreatment were subjected to microbiological quality assessment during storage. For each replicate, two berries were randomly selected and aseptically transferred into a sterile stomacher bag with a built-in filter. Samples were homogenized for 1 min using a Lab-Blender Stomacher (Model 400, Seward Medical, London). Homogenates were diluted in the ratio of 1:9 (w/w) with sterile physiological saline solution (0.85% NaCl) for 1 min, and subsequent decimal serial dilutions were prepared using the same diluent. Microbial enumeration was performed as follows:

Total aerobic mesophilic bacteria (TAMB): Plated on plate count agar (PCA; Merck, Darmstadt, Germany) and incubated at 30±1°C for 48 h. Results were expressed as log colony-forming unit (CFU)/g FW.

Yeasts and molds: Plated on potato dextrose agar (PDA, Merck) supplemented with 0.01% chloramphenicol and incubated at 25±1°C for 5–7 days. Colonies were counted and expressed as log CFU/g FW.

Coliforms: Enumerated on violet red bile agar (VRBA, Merck) incubated at 37±1°C for 24 h, and results were expressed as log CFU/g FW.

A spoilage threshold was defined when yeast and mold populations exceeded 6 log CFU/g or visible mycelial growth appeared on fruit surface.

Total bacterial count (TBC)

The total bacterial load of strawberry samples was determined at each storage interval. From each pretreatment, two berries were randomly selected and homogenized in a sterile stomacher bag with filter for 1 min using a Lab-Blender Stomacher (Model 400; Seward Medical). The homogenates were diluted 1:9 (w/w) in sterile physiological saline solution (0.85% NaCl), followed by preparation of decimal serial dilutions using the same diluent. Aliquots (0.1 mL) of appropriate dilutions were surface-plated on plate count agar (Merck) and incubated at 30±1°C for 48 h. The results were expressed as log CFU/g FW of strawberries.

Yeast and mold count (YMC)

Yeast and mold populations were quantified to assess the impact of postharvest treatments on fungal spoilage during storage. From each replicate, two berries were randomly selected, homogenized for 1 min in a sterile stomacher bag with filter using a Lab-Blender Stomacher (Model 400; Seward Medical), and diluted in the ratio of 1:9 (w/w) with sterile physiological saline solution (0.85% NaCl). Decimal serial dilutions were then prepared using the same diluent. Aliquots (0.1 mL) were spread-plated on PDA (Merck) supplemented with 0.01% chloramphenicol to inhibit bacterial growth. Plates were incubated at 25±1°C for 5–7 days, after which colony counts were recorded. Results were expressed as log CFU/g FW.

Statistical analysis

All experiments were arranged as a randomized complete block design (RCBD) with three biological replicates per treatment (n = 3) and five sampling periods (day 0, 3, 6, 9, and 15). Data were reported as mean±SEM. Microbiological counts were $\log^{10}$-transformed (log CFU g^{-1}), and percentage data (e.g., decayed surface area) were arcsine square-root transformed when necessary to meet ANOVA assumptions. Normality of residuals was tested using the Shapiro–Wilk test, and homogeneity of variances was assessed by Levene's test. If assumptions were not met after transformation, non-parametric tests were applied. A two-way analysis of variance (ANOVA) was used to evaluate the effects of treatment (fixed factor), storage time (fixed factor), and their interaction (treatment × time) on each response variable. The linear model was specified as follows:

$$Y_{ijk} = \mu + T_i + S_j + (T\times S)_{ij} + B_k + e_{ijk}, \qquad (4)$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is the effect of the i-th treatment, S_j is the effect of the j-th storage time, $(T\times S)_{ij}$ is the interaction term, B_k is the block effect, and e_{ijk} is the residual error.

When significant main effects or interactions were detected ($P < 0.05$), mean values were compared using Duncan's multiple range test at $P < 0.05$. For variables analyzed by one-way ANOVA (e.g., comparisons at a single sampling time), a one-way ANOVA followed by Duncan's test was used.

Multivariate analyses were performed to explore relationships among variables and summarize treatment effects. Pearson's correlation coefficients were calculated between pairs of continuous variables (two-tailed, $P < 0.05$) with *P*-values adjusted using the Benjamini–Hochberg procedure to control false discovery rate. PCA was conducted on the full dataset (standardized to mean = 0 and SD = 1) to reduce dimensionality and visualize patterns among treatments and storage periods. Variables included in PCA were WL, firmness, color parameters (L*, a*, and b*), TSS, TA, TPC, TAC, antioxidant capacity (DPPH, ABTS, and FRAP), and microbial count. PCA loadings and scores were reported, and biplots illustrated grouping of treatments.

All statistical analyses and figures were conducted using the R software (version 4.x) with the packages stats, agricolae (for *post hoc* tests), vegan/factoextra (for PCA), and ggplot2 (for figures). References to SPSS or GraphPad were removed to streamline wording, and all analyses were consistently performed in R.

Results and Discussion

Determination of physicochemical parameters

Weight loss

The WL of strawberries for all treatments increased progressively during storage (Table 2). The NTC exhibited maximum WL, reaching 7.8±0.3% on day 15, whereas CH+OEO-coated fruits showed the lowest WL (3.4±0.2%). The CO_2 treatment also significantly reduced WL (4.5±0.2%), compared to the control, while O_3 treatment led to moderate WL (5.0±0.2%). Single-component treatments of chitosan and OEO provided intermediate protection, with the WL values of 4.0±0.2% and 4.8±0.2%, respectively. Statistical analysis (Figure 1) indicated that differences between treatments at the same storage time were significant ($P < 0.05$), as reflected by lowercase alphabets, while WL increased significantly over time within each treatment (uppercase alphabets). The reduced WL in CH+OEO-coated fruit could be attributed to the barrier effect of polysaccharide-based coating combined with the hydrophobic essential oil, which reduced water evaporation and slowed respiration (Caleja *et al.*, 2023; Panahirad *et al.*, 2022). CO_2 treatment likely limited WL by slowing down metabolic activity and respiration, whereas O_3 caused early oxidative stress on the fruit surface, resulting in slightly higher water loss. The intermediate efficacy of single-component treatments (CH or OEO alone) highlighted the synergistic effect of combining chitosan with OEO. Maintaining low WL was critical for preserving firmness, visual appearance, and the overall marketability of strawberries. These results suggested that CH+OEO coating and CO_2 treatment were effective postharvest strategies to reduce moisture loss and extend shelf-life in organically grown strawberries.

Appearance

The visual appearance of strawberries, evaluated by color retention and surface decay, was significantly influenced by both postharvest treatment and storage period (Figures 2 and 3). On day 0, all fruits showed bright red color and uniform appearance. Over the 15-day storage, NTC exhibited rapid deterioration, including surface shriveling, discoloration, and decay, with 35–40% of the fruit surface affected by day 15. Among the treatments, CH+OEO-coated fruits retained the best appearance, with minimal decay (≈10% of surface) and vibrant red color even on day 15. CO_2-treated fruits also maintained good visual quality for up to 9 days, but minor surface browning was observed at later storage intervals. In contrast, O_3-treated strawberries showed early skin oxidation and bruising, negatively impacting their marketable appearance. Single-component coatings (CH or OEO alone) moderately preserved visual quality, but less effectively than the combined CH+OEO treatment.

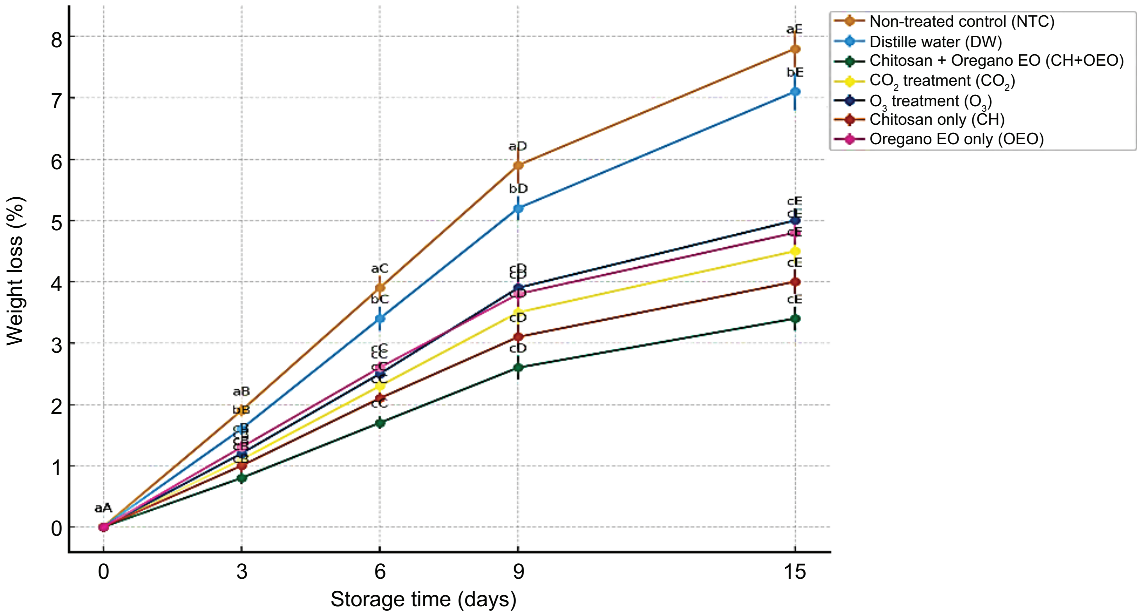

Figure 1. Effect of postharvest pretreatments and storage period on WL (%) of organic strawberries stored at 4°C for 15 days. Different lowercase alphabets indicate significant differences between treatments at the same storage time ($P < 0.05$), and uppercase alphabets indicate significant differences between storage periods for the same treatment ($P < 0.05$).

Improved appearance of CH+OEO-coated fruit samples was attributed to the barrier effect of chitosan film, which reduced moisture loss, respiration, and microbial growth, while OEO provided antimicrobial and antioxidant protection, delaying decay (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment preserved appearance by slowing of respiration and microbial proliferation, whereas O_3 caused oxidative damage to the skin, which was consistent with the reports of its pro-oxidant effect at higher concentrations (Duan *et al.*, 2022). Maintaining appearance is critical for consumer acceptance and marketability. These results indicated that CH+OEO coating and CO_2 treatment were effective strategies to preserve visual quality and extend shelf life of organic strawberries under cold storage.

Firmness

The firmness of strawberries for all treatments decreased progressively during storage (Table 4). Initial values for firmness ranged from 2.8 N to 3.0 N across treatments, with no significant differences on day 0. By day 15, the NTC showed the greatest softening (1.2±0.1 N), while CH+OEO-coated fruit samples retained maximum firmness (2.4±0.1 N). CO_2 treatment also maintained firmness effectively (2.1±0.1 N), whereas O_3 treatment led to accelerated softening (1.5±0.1 N). Single-component treatments (CH or OEO alone) showed retention of intermediate firmness (1.9–2.0 N) (Figure 4). Statistical analysis indicated that differences between treatments at the same storage time were significant ($P < 0.05$), as reflected by lowercase alphabets, while firmness decreased significantly over storage within each treatment (uppercase alphabets). The improved firmness of CH+OEO-coated strawberries is attributed to the film-forming ability of chitosan, which reduces water loss and slows enzymatic degradation of cell wall, while OEO provides antioxidant protection, further maintaining tissue integrity (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment likely preserved firmness by reducing respiration and metabolic activity, whereas O_3 caused early oxidative damage to cell walls, contributing to softening (Duan *et al.*, 2022). Maintaining firmness is crucial for consumer acceptability and marketability, as excessive softening reduces texture quality and increases susceptibility to mechanical damage. Overall, CH+OEO coating and CO_2 treatment were the most effective strategies for preserving firmness during cold storage.

Color parameters (L*, a*, b*)

The effects of postharvest pretreatments and storage duration on the color parameters of organic strawberries are summarized in Figures 5A–C. Color is a critical quality attribute influencing consumer acceptance, reflecting the integrity of pigments, such as anthocyanins and carotenoids, and often serving as an indicator of fruit freshness and oxidative changes (Kaur *et al.*, 2021).

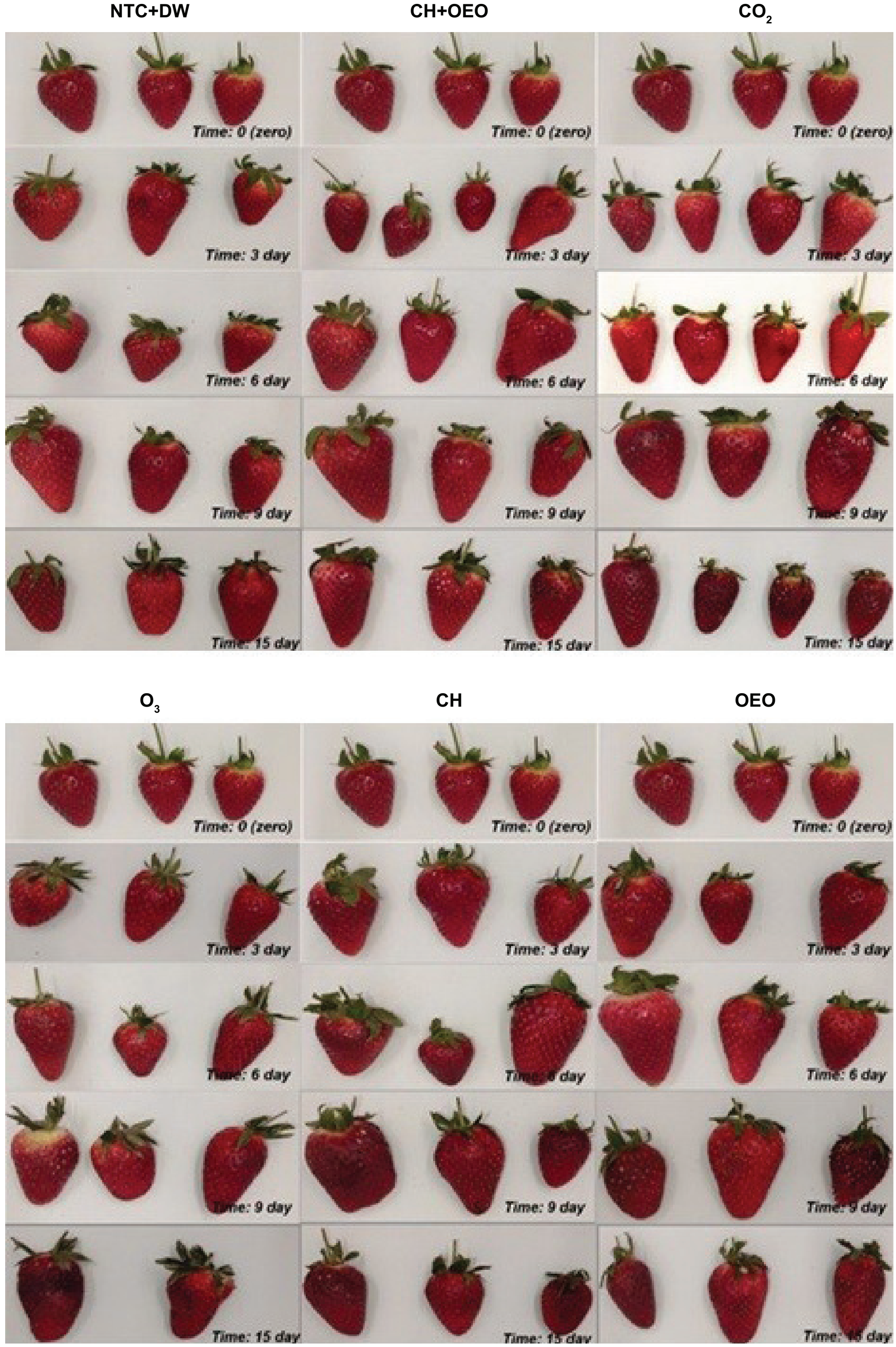

Figure 2. Visual appearance of control and pretreated organic strawberries during different storage intervals.

L* (lightness): The lightness (L*) of all strawberry samples increased progressively during storage, indicating gradual fruit softening and surface degradation. Untreated control and O_3-treated (O_3) strawberries showed maximum L* values by day 15 (41.0 and 41.5, respectively), reflecting more pronounced color fading. In contrast, samples treated with CH+OEO or CO_2 exhibited significantly lower L* values throughout storage ($P < 0.05$),

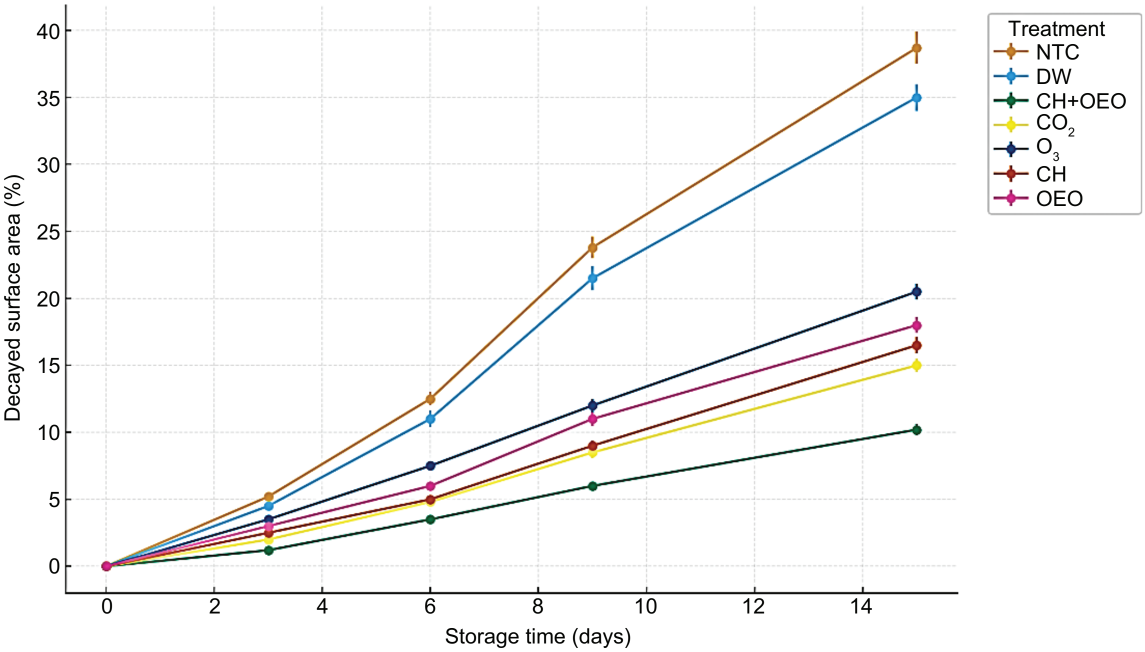

Figure 3. Effect of postharvest pretreatments and storage period on visual appearance (decayed surface area, %) of organic strawberries stored at 4°C for 15 days. Different lowercase alphabets indicate significant differences between treatments at the same storage time ($P < 0.05$), and uppercase alphabets indicate significant differences between storage periods for the same treatment ($P < 0.05$).

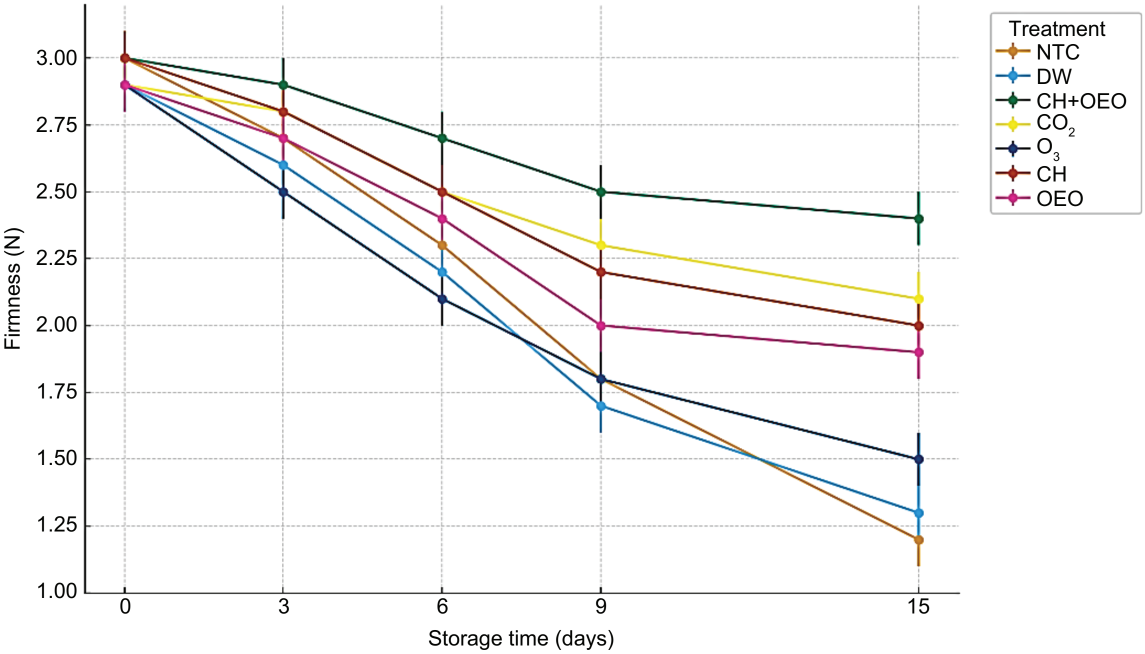

Figure 4. Effect of postharvest pretreatments and storage period on firmness (N) of organic strawberries stored at 4°C for 15 days. Different lowercase alphabets indicate significant differences between treatments at the same storage time ($P < 0.05$), and uppercase alphabets indicate significant differences between storage periods for the same treatment ($P < 0.05$).

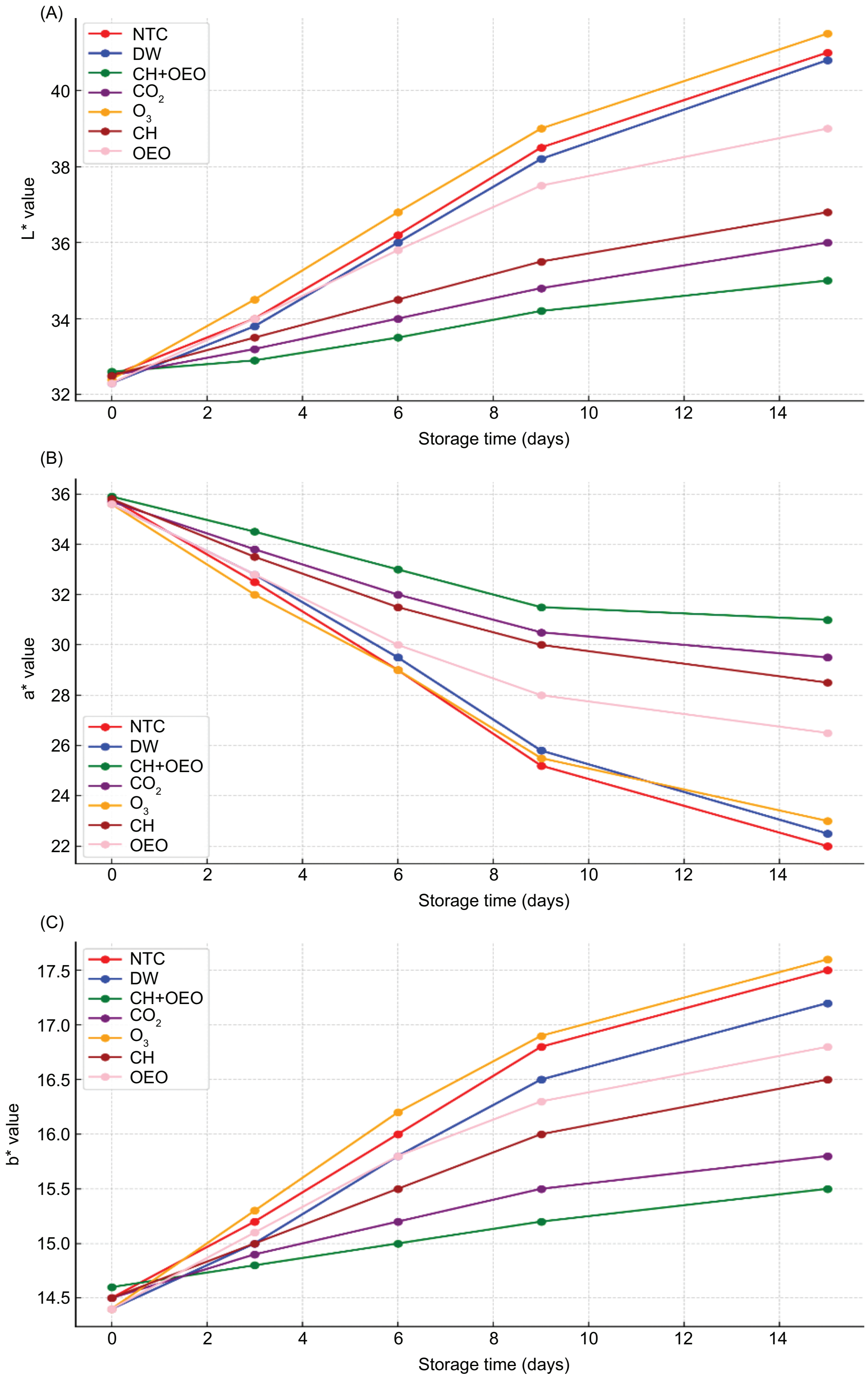

Figure 5. **Effect of postharvest pretreatments on color parameters of organic strawberries stored at 4°C for 15 days: (a) lightness (L*), (b) redness (a*), and (c) yellowness (b*). Values are mean ± SEM (n = 3). Different lowercase alphabets indicate significant differences between treatments at the same storage time (P < 0.05), and different uppercase alphabets indicate significant differences between storage periods within the same treatment (P < 0.05).**

indicating better retention of the fruit's natural brightness and reduced surface deterioration. These findings aligned with previous reports, where chitosan-based coatings effectively slowed color degradation by forming a semi-permeable barrier that reduces loss of moisture and oxidation (Baldwin *et al.*, 2020).

Untreated and DW fruits showed rapid discoloration, with increasing L* (surface lightening), decreasing a* (loss of red intensity), and rising b* (yellowing). In contrast, CH+OEO and CO_2 treatments effectively maintained lower L* values and higher a* values, indicating superior preservation of fresh-like red coloration. O_3-treated fruits exhibited greater lightening and loss of redness, confirming its limited effectiveness, compared to coatings and CO_2.

(a) Red–green component: The redness (a*) parameter decreased in all samples over storage, reflecting anthocyanin degradation during ripening and senescence. NTC and O_3-treated fruit samples experienced the most rapid decline, dropping from ~35.8–35.6 on day 0 to 22.0–23.0 by day 15. Conversely, CH+OEO- and CO_2-treated fruit samples maintained higher a* values (31.0 and 29.5, respectively), suggesting effective protection of anthocyanins and delayed senescence. These results indicate that edible coatings and modified atmosphere treatments can mitigate pigment degradation, probably through reduced enzymatic oxidation and slower respiration rates (Khan *et al.*, 2022; Sharma *et al.*, 2022).

(b) Yellow–blue component: The b* values (yellow–blue) increased slightly in all treatments, indicative of carotenoid accumulation or chlorophyll degradation over time. NTC and O_3-treated fruits reached the highest b* values by day 15 (17.5 and 17.6, respectively), whereas CH+OEO and CO_2 treatments exhibited lower b* values (15.5 and 15.8), suggesting a slower progression toward yellowing. Maintenance of lower b* values is consistent with better preservation of fruit quality, as excessive yellowing is associated with over-ripening and senescence (Li *et al.*, 2022).

Overall, the results demonstrated that postharvest treatments significantly influenced color stability in strawberries during cold storage. CH+OEO treatment consistently provided the best retention of L*, a*, and b* values, followed by CO_2 treatment. These treatments potentially act synergistically, combining the antimicrobial and antioxidant properties of OEO with the film-forming capacity of chitosan, thereby reducing enzymatic browning, oxidative pigment degradation, and loss of water (Ahmed *et al.*, 2021; Liang *et al.*, 2021). The faster color deterioration observed in NTC and O_3-treated fruits may be attributed to higher respiration rate, water loss, and increased susceptibility to oxidation, which are commonly observed during cold storage of perishable berries (Fang *et al.*, 2022).

The color trends corroborated the WL and firmness results, indicating that both CH+OEO and CO_2 treatments effectively maintained the overall fruit quality. These findings have practical implications for the postharvest handling of strawberries. Application of CH+OEO-based coatings can extend shelf life while preserving visual appeal, which is critical for marketability. Furthermore, integrating such natural treatments with controlled atmosphere storage could provide a sustainable, chemical-free alternative to conventional postharvest preservation methods.

Total soluble solids and titratable acidity

The TSS and TA values of strawberries were significantly influenced by both postharvest treatment and duration of storage (Figure 6). On day 0, all treatments had similar TSS (7.8–7.9 °Brix) and TA (0.86–0.88% citric acid) with no significant differences. During storage, TSS generally increased in all treatments, with the maximum increase observed in NTC and DW fruits, reaching 9.4–9.5 °Brix by day 15, probably because of water loss and concentration of soluble solids. In contrast, CH+OEO-coated and CO_2-treated strawberries showed smaller TSS increases (8.6–8.7 °Brix), reflecting reduced moisture loss and slower metabolic activity. O_3-treated fruits exhibited a moderate increase in TSS, while CH and OEO alone showed intermediate values.

Titratable acidity decreased progressively in all treatments during storage. Maximum reduction occurred in NTC and DW fruits, dropping to 0.55–0.57% citric acid by day 15. CH+OEO coating and CO_2 treatment better preserved acidity (0.70–0.72% citric acid), indicating slower organic acid degradation. O_3 treatment accelerated TA loss, while single-component coatings (CH or OEO alone) showed intermediate preservation. The effectiveness of CH+OEO coating is attributed to the barrier properties of chitosan, which limit respiration and moisture loss, combined with the antioxidant activity of OEO, protecting organic acids from oxidative degradation (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment probably maintained TSS and TA by slowing metabolic processes and delaying senescence, whereas O_3 caused oxidative stress that accelerated quality deterioration. Maintaining TSS and TA is critical for sweetness–acidity balance and the overall flavor, directly affecting consumer acceptability. These findings indicate that CH+OEO coating and CO_2 treatment are the most effective strategies for preserving the chemical quality of organic strawberries during cold storage.

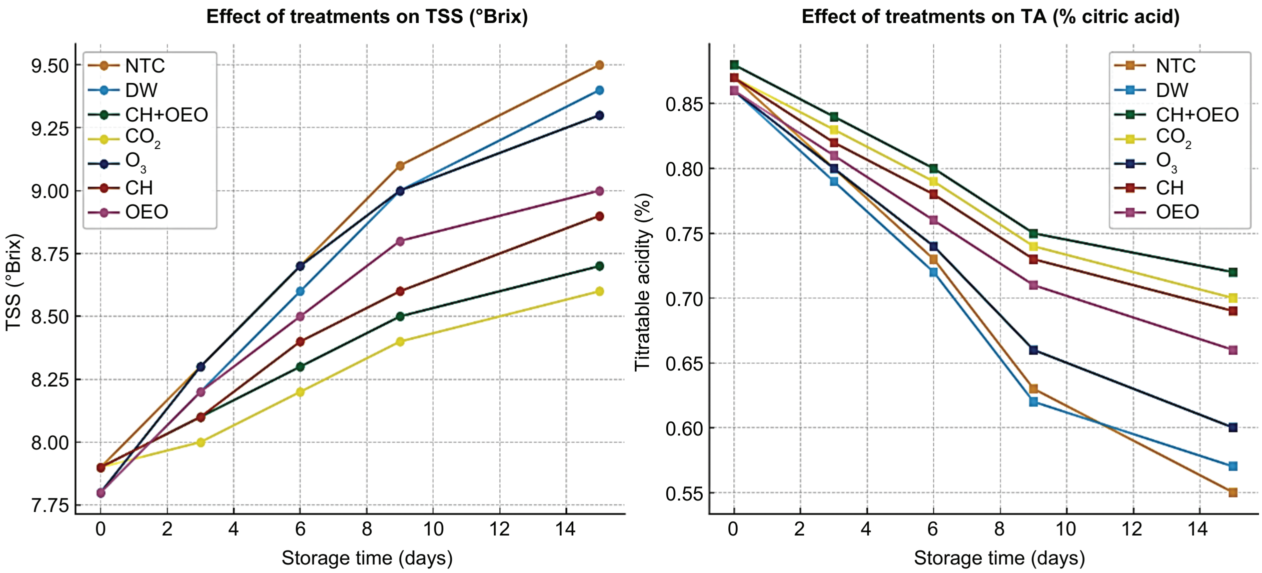

Figure 6. Changes in total soluble solids (TSS, °Brix) and titratable acidity (TA, % citric acid) of organic strawberries subjected to different postharvest pretreatments during storage at 4°C for 15 days.

Determination of bioactive compounds and antioxidant capacity

The levels of TPC, TAC, and antioxidant capacity in strawberries were significantly influenced by postharvest treatment and storage duration (Table 7). On day 0, all treatments exhibited similar TPC (450–460 mg GAE/100 g FW) and TAC (28–30 mg/100 g FW), with antioxidant capacity measured by DPPH assay ranging from 55% to 57% inhibition, with no significant differences between treatments. During storage, TPC and TAC declined in all samples, reflecting oxidative degradation of phenolic compounds and anthocyanins. The greatest reduction occurred in NTC and DW-treated fruits, with TPC decreasing to 280–290 mg GAE/100 g FW and TAC to 18–19 mg/100 g FW by day 15. CH+OEO-coated strawberries showed the smallest decline, retaining 400 mg GAE/100 g FW (TPC) and 25 mg/100 g FW (TAC) on day 15. CO_2 treatment also preserved TPC and TAC effectively (≈370–380 mg GAE/100 g FW and 23–24 mg/100 g FW, respectively). O_3-treated fruits exhibited faster degradation, while CH or OEO alone provided intermediate protection. Antioxidant capacity followed a similar trend, with CH+OEO-coated and CO_2-treated strawberries maintaining higher DPPH inhibition (≈50–52%), compared to NTC and DW (≈35–37%) on day 15. The enhanced retention of bioactive compounds in CH+OEO-coated fruits can be attributed to the antioxidant properties of OEO combined with the protective barrier effect of chitosan, which reduces oxidative reactions and moisture loss (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment probably limited the degradation of phenolics and anthocyanins by slowing respiration and enzymatic oxidation, whereas O_3 treatment induced oxidative stress, accelerating the loss of bioactive compounds. These findings indicate that CH+OEO coating and CO_2 treatment are the most effective strategies to preserve the nutritional and functional quality of organic strawberries during cold storage, maintaining high levels of phenolics, anthocyanins, and antioxidant capacity. The levels of TPC, TAC, and antioxidant capacity in strawberries were significantly influenced by postharvest treatment and storage duration (Tables 2–4).

On day 0, all treatments exhibited similar TPC (450–460 mg GAE/100 g FW) and TAC (28–30 mg/100 g FW), with antioxidant capacity measured by DPPH assay ranging from 55% to 57% inhibition, with no significant differences between treatments. During storage, TPC and TAC declined in all samples, reflecting oxidative degradation of phenolic compounds and anthocyanins. Maximum reduction occurred in NTC and DW-treated fruits, with TPC decreasing to 280–290 mg GAE/100 g FW and TAC to 18–19 mg/100 g FW by day 15. CH+OEO-coated strawberries showed the lowest decline, retaining 400 mg GAE/100 g FW (TPC) and 25 mg/100 g FW (TAC) on day 15. CO_2 treatment also preserved TPC and TAC effectively (≈370–380 mg GAE/100 g FW and 23–24 mg/100 g FW, respectively). O_3-treated fruits exhibited faster degradation, while CH or OEO alone provided intermediate protection (Table 3).

Table 3. Total anthocyanin content (TAC, mg/100 g FW) of organic strawberries during 15 days of storage at 4°C.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 15
NTC	29.5 ± 0.5^{a,A}	27.0 ± 0.40^{a,B}	24.0 ± 0.40^{a,C}	20.0 ± 0.50^{a,D}	18.0 ± 0.40^{a,E}
DW	28.8 ± 0.5^{a,A}	26.5 ± 0.40^{b,B}	23.5 ± 0.40^{b,C}	19.5 ± 0.50^{b,D}	18.5 ± 0.40^{b,E}
CH+OEO	29.8 ± 0.5^{a,A}	28.5 ± 0.40^{c,B}	27.0 ± 0.40^{c,C}	26.0 ± 0.50^{c,D}	25.0 ± 0.50^{c,E}
CO_2	29.5 ± 0.5^{a,A}	28.0 ± 0.40^{c,B}	26.5 ± 0.40^{c,C}	25.0 ± 0.50^{c,D}	23.5 ± 0.50^{c,E}
O_3	28.8 ± 0.5^{a,A}	26.5 ± 0.40^{c,B}	24.0 ± 0.40^{c,C}	21.0 ± 0.50^{c,D}	19.0 ± 0.50^{c,E}
CH	29.5 ± 0.5^{a,A}	28.0 ± 0.40^{c,B}	26.0 ± 0.40^{c,C}	24.5 ± 0.50^{c,D}	23.0 ± 0.50^{c,E}
OEO	29.0 ± 0.5^{a,A}	27.5 ± 0.40^{c,B}	25.5 ± 0.40^{c,C}	24.0 ± 0.50^{c,D}	22.5 ± 0.50^{c,E}

Notes:
TAC decreased significantly in all treatments during storage.
CH+OEO and CO_2 treatments better preserved anthocyanins, compared to NTC.
Values are mean ± SEM (n = 3).
Different superscript lowercase alphabets in the same column indicate significant differences between treatments at the same storage time ($P < 0.05$).
Different superscript uppercase alphabets in the same row indicate significant differences between storage periods within the same treatment ($P < 0.05$).

Table 4. Antioxidant capacity (% DPPH inhibition) of organic strawberries during 15 days of storage at 4°C.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 15
NTC	56 ± 1.00^{a,A}	50 ± 1.00^{a,B}	46 ± 1.00^{a,C}	40 ± 1.00^{a,D}	36 ± 1.00^{a,E}
DW	55 ± 1.00^{a,A}	49 ± 1.00^{b,B}	45 ± 1.00^{b,C}	39 ± 1.00^{b,D}	35 ± 1.00^{b,E}
CH+OEO	57 ± 1.00^{a,A}	54 ± 1.00^{c,B}	52 ± 1.00^{c,C}	51 ± 1.00^{c,D}	50 ± 1.00^{c,E}
CO_2	56 ± 1.00^{a,A}	53 ± 1.00^{c,B}	51 ± 1.00^{c,C}	50 ± 1.00^{c,D}	49 ± 1.00^{c,E}
O_3	55 ± 1.00^{a,A}	51 ± 1.00^{c,B}	48 ± 1.00^{c,C}	44 ± 1.00^{c,D}	40 ± 1.00^{c,E}
CH	56 ± 1.00^{a,A}	53 ± 1.00^{c,B}	51 ± 1.00^{c,C}	50 ± 1.00^{c,D}	48 ± 1.00^{c,E}
OEO	55 ± 1.00^{a,A}	52 ± 1.00^{c,B}	50 ± 1.00^{c,C}	48 ± 1.00^{c,D}	46 ± 1.00^{c,E}

Notes:
TAC decreased significantly in all treatments during storage.
CH+OEO and CO_2 treatments better preserved anthocyanins, compared to NTC.
Values are mean ± SEM (n = 3).
Different superscript lowercase alphabets in the same column indicate significant differences between treatments at the same storage time ($P < 0.05$).
Different superscript uppercase alphabets in the same row indicate significant differences between storage periods within the same treatment ($P < 0.05$).

Antioxidant capacity followed a similar trend, with CH+OEO-coated and CO_2-treated strawberries maintaining higher DPPH inhibition (≈50–52%), compared to NTC and DW (≈35–37%) on day 15. The enhanced retention of bioactive compounds in CH+OEO-coated fruits can be attributed to the antioxidant properties of OEO combined with the protective barrier effect of chitosan, which reduces oxidative reactions and moisture loss (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment potentially limited the degradation of phenolics and anthocyanins by slowing respiration and enzymatic oxidation, whereas O_3 treatment induced oxidative stress, accelerating loss of bioactive compounds. These findings indicate that CH+OEO coating and CO_2 treatment are the most effective strategies to preserve the nutritional and functional quality of organic strawberries during cold storage, maintaining high levels of phenolics, anthocyanins, and antioxidant capacity.

Determination of microbiological properties

The microbiological quality of strawberries was evaluated by assessing TBC and YMC during cold storage at 4°C (Table 5). All treatments initially showed low microbial loads on day 0, with TBC ranging from 2.0 log CFU/g to 2.2 log CFU/g and YMC ranging from 1.5 log CFU/g to 1.7 log CFU/g, reflecting good hygienic handling and fresh fruit quality. During storage, NTC and DW-treated fruits exhibited a rapid increase in microbial populations. By day 15, TBC reached 6.8–7.0 log CFU/g, and YMC reached 5.5–5.8 log CFU/g, indicating substantial microbial proliferation and potential spoilage. In contrast, CH+OEO-coated strawberries maintained significantly lower microbial counts throughout storage. On day 15, TBC and YMC were ≈4.2–4.5 log CFU/g and ≈3.5–3.8 log CFU/g, respectively.

Table 5. Effect of postharvest pretreatments and storage period on total bacterial count (TBC) and yeast and mold count (YMC, log CFU/g) of organic strawberries stored at 4°C for 15 days.

Treatment	Parameter	Day 0	Day 3	Day 6	Day 9	Day 15
NTC	TBC	2.1±0.1^{a,A}	3.2±0.1^{a,B}	4.5±0.1^{a,C}	5.8±0.1^{a,D}	6.9±0.1^{a,E}
	YMC	1.6±0.1^{a,A}	2.5±0.1^{a,B}	3.6±0.1^{a,C}	4.7±0.1^{a,D}	5.6±0.1^{a,E}
DW	TBC	2.0±0.1^{a,A}	3.1±0.1^{b,B}	4.4±0.1^{b,C}	5.7±0.1^{b,D}	6.8±0.1^{b,E}
	YMC	1.5±0.1^{a,A}	2.4±0.1^{b,B}	3.5±0.1^{b,C}	4.6±0.1^{b,D}	5.5±0.1^{b,E}
CH+OEO	TBC	2.2±0.1^{a,A}	2.8±0.1^{c,B}	3.3±0.1^{c,C}	3.8±0.1^{c,D}	4.3±0.1^{c,E}
	YMC	1.7±0.1^{a,A}	2.2±0.1^{c,B}	2.7±0.1^{c,C}	3.1±0.1^{c,D}	3.6±0.1^{c,E}
CO_2	TBC	2.1±0.1^{a,A}	2.9±0.1^{c,B}	3.5±0.1^{c,C}	4.0±0.1^{c,D}	4.8±0.1^{c,E}
	YMC	1.6±0.1^{a,A}	2.3±0.1^{c,B}	2.8±0.1^{c,C}	3.3±0.1^{c,D}	3.8±0.1^{c,E}
O_3	TBC	2.0±0.1^{a,A}	2.7±0.1^{c,B}	3.6±0.1^{c,C}	4.5±0.1^{c,D}	5.5±0.1^{c,E}
	YMC	1.5±0.1^{a,A}	2.2±0.1^{c,B}	3.0±0.1^{c,C}	3.8±0.1^{c,D}	4.8±0.1^{c,E}
CH	TBC	2.1±0.1^{a,A}	2.9±0.1^{c,B}	3.5±0.1^{c,C}	4.0±0.1^{c,D}	4.9±0.1^{c,E}
	YMC	1.6±0.1^{a,A}	2.3±0.1^{c,B}	2.9±0.1^{c,C}	3.3±0.1^{c,D}	3.9±0.1^{c,E}
OEO	TBC	2.0±0.1^{a,A}	2.8±0.1^{c,B}	3.4±0.1^{c,C}	3.9±0.1^{c,D}	4.8±0.1^{c,E}
	YMC	1.5±0.1^{a,A}	2.2±0.1^{c,B}	2.8±0.1^{c,C}	3.2±0.1^{c,D}	3.8±0.1^{c,E}
P	–	–	–	–	–	–

Notes: Values are mean ± SEM (n = 3).
Different superscript lowercase alphabets indicate significant differences between treatments at the same storage time ($P < 0.05$), and superscript uppercase alphabets indicate significant differences between storage periods within the same treatment ($P < 0.05$).

CO_2 treatment also effectively suppressed microbial growth, with TBC and YMC around 4.5–4.8 log CFU/g on day 15. O_3-treated fruits showed moderate microbial suppression initially, but microbial populations increased after day 9, reaching 5.5 log CFU/g (TBC) and 4.8 log CFU/g (YMC) by day 15. Single-component coatings (CH or OEO alone) exhibited intermediate effects. The antimicrobial effectiveness of CH+OEO coating is attributed to the film-forming ability of chitosan, which acts as a physical barrier, and the bioactive compounds in OEO, which exert inhibitory effects against bacteria, yeasts, and molds (Panahirad *et al.*, 2022; Rashid *et al.*, 2021). CO_2 treatment potentially reduced microbial growth by creating an unfavorable environment for aerobic microorganisms. O_3 treatment initially inactivated surface microbes through oxidation, but continuous exposure at the tested dose was insufficient to maintain suppression over extended storage. These results indicated that CH+OEO coating and CO_2 treatment were the most effective postharvest strategies for maintaining the microbiological safety and shelf-life of organic strawberries under cold storage.

Multivariate analysis

In order to better understand the overall effects of postharvest treatments and storage time on strawberry quality, PCA was conducted using all measured variables, including WL, firmness, color parameters, TSS, TA, TPC, total anthocyanins, antioxidant capacity, and microbial counts. The PCA results (Figure 7) revealed that the first two principal components (PC1 and PC2) accounted for approximately 78.4% of the total variance, with PC1 representing the overall quality deterioration (WL, microbial growth, increase in TSS, decrease of TA, and loss of bioactive compounds) and PC2 representing changes in both color and firmness.

The score plot showed clear grouping of treatments

- CH+OEO-coated and CO_2-treated strawberries were clustered together and positioned on the side of the plot associated with higher firmness, lower WL, better color retention, higher bioactive compounds, and lower microbial counts, indicating superior overall quality.
- NTC and DW-treated fruits clustered on the opposite side, associated with higher WL, microbial proliferation, and degradation of bioactive compounds, reflecting poor postharvest performance.
- O_3-treated fruits occupied an intermediate position, initially maintaining some bioactive compounds but showing loss of accelerated firmness and microbial growth over storage.

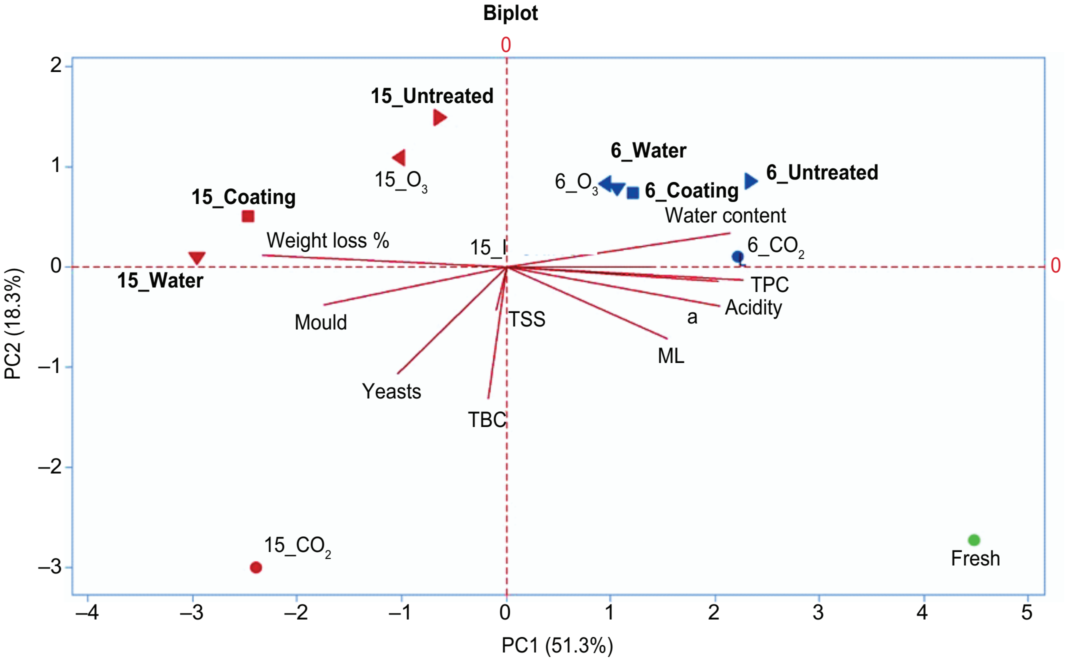

Figure 7. Biplot of multivariate analysis (score and loading plots) illustrating the relationship between quality parameters of strawberries at harvest (green circle), during storage up to day 0 (blue symbols), and after storage for 15 days (red symbols).

The PCA biplot further highlighted strong correlations among variables

- Weight loss and microbial counts were negatively correlated with TPC, TAC, and antioxidant capacity.
- Firmness and color parameters were positively correlated with bioactive content.

These results confirmed that CH+OEO coating and CO_2 treatment were the most effective strategies for maintaining the overall quality of strawberries, combining physical, chemical, and microbiological preservation. PCA provided a comprehensive visual summary, demonstrating that postharvest treatments not only affected individual quality parameters but also integrated into an overall preservation profile, which was critical for commercial and consumer acceptance.

Multivariate analysis discussion

To comprehensively understand the relationships among physicochemical, bioactive, and microbiological parameters, both principal component analysis (PCA) and Pearson's correlation analysis were conducted. The PCA revealed that the first two principal components (PC1 and PC2) explained 78.4% of the total variance in the quality of strawberries during cold storage. PC1 was primarily associated with the overall deterioration, characterized by increase in WL, TSS, and microbial counts and decrease in firmness, color, TA, and bioactive compounds (TPC, TAC, and antioxidant capacity). PC2 mainly reflected changes in color and firmness, capturing subtle variations in the fruit's physical appearance and texture over storage. The score plot showed a clear separation of treatments:

- CH+OEO coating and CO_2 treatment clustered together, linked to high firmness, bright color, high TPC and anthocyanin content, strong antioxidant capacity, and low microbial loads.
- NTC and DW-treated fruits clustered on the opposite side, associated with WL, microbial proliferation, and rapid bioactive degradation, representing the least effective postharvest management.
- O_3 treatment occupied an intermediate position, initially maintaining some bioactive content but showing accelerated loss of firmness and microbial growth after day 9, while single-component coatings (CH or OEO alone) were moderately effective.

The Pearson's correlation matrix (Table 6) confirmed these observations quantitatively

- Weight loss was strongly negatively correlated with firmness (r = –0.89), color (r = –0.85), TPC (r = –0.78), TAC (r = –0.76), and DPPH antioxidant capacity

Table 6. Pearson's correlation matrix coefficients between physicochemical, bioactive, and microbiological parameters of organic strawberries during cold storage ($P < 0.05$).

Parameter	Weight loss	Firmness	Color (L*)	TSS	TA	TPC	TAC	DPPH	TBC	YMC
Weight loss	1	–0.89*	–0.85*	0.82*	–0.80*	–0.78*	–0.76*	–0.77*	0.90*	0.88*
Firmness	–0.89*	1	0.92*	–0.75*	0.79*	0.85*	0.83*	0.84*	–0.81*	–0.79*
Color (L*)	–0.85*	0.92*	1	–0.70*	0.76*	0.80*	0.82*	0.81*	–0.78*	–0.77*
TSS	0.82*	–0.75*	–0.70*	1	–0.88*	–0.72*	–0.71*	–0.70*	0.85*	0.83*
TA	–0.80*	0.79*	0.76*	–0.88*	1	0.75*	0.74*	0.73*	–0.82*	–0.80*
TPC	–0.78*	0.85*	0.80*	–0.72*	0.75*	1	0.91*	0.93*	–0.76*	–0.74*
TAC	–0.76*	0.83*	0.82*	–0.71*	0.74*	0.91*	1	0.90*	–0.74*	–0.72*
DPPH	–0.77*	0.84*	0.81*	–0.70*	0.73*	0.93*	0.90*	1	–0.75*	–0.73*
TBC	0.90*	–0.81*	–0.78*	0.85*	–0.82*	–0.76*	–0.74*	–0.75*	1	0.95*
YMC	0.88*	–0.79*	–0.77*	0.83*	–0.80*	–0.74*	–0.72*	–0.73*	0.95*	1

Notes:
Significant correlation at $P < 0.05$.
Weight loss and microbial growth (TBC and YMC) are negatively correlated with firmness, color, and bioactive compounds, confirming that loss of moisture and microbial proliferation lead to quality deterioration.
Firmness, color, and bioactive compounds (TPC, TAC, and DPPH) are positively correlated, suggesting that treatments that maintain physical quality also preserve nutritional and antioxidant properties.
TSS and TA show expected inverse correlation, reflecting concentration effects during storage and metabolism.

(r = –0.77). This demonstrates that loss of moisture during storage is a key driver of both physical deterioration and nutritional loss.

- Microbial counts (TBC and YMC) were positively correlated with WL (r = 0.90–0.95) and negatively correlated with bioactive compounds and firmness, indicating that microbial proliferation accelerated the degradation of strawberry quality.
- Firmness, color, and bioactive compounds were positively intercorrelated, confirming that maintaining structural integrity helped to preserve phenolics, anthocyanins, and antioxidant capacity.
- TSS and TA were inversely correlated (r = –0.88), consistent with sugar accumulation because of water loss and organic acid metabolism during storage.

Collectively, these multivariate analyses demonstrated that CH+OEO coating and CO_2 treatment effectively maintained an integrated quality profile by simultaneously preserving physical, chemical, and microbiological attributes. Strong correlations between quality parameters indicated that postharvest interventions that minimized water loss and microbial growth inherently protected bioactive compounds and sensory properties, providing both nutritional and commercial benefits. In contrast, untreated or DW-treated fruits suffered rapid deterioration across all quality dimensions, while O_3 treatment caused oxidative stress that limited its long-term effectiveness.

These findings highlight the importance of multivariate approaches for postharvest quality assessment, as they provide holistic insight into how different treatments influence multiple interdependent quality attributes simultaneously. Such comprehensive analysis supports the development of scalable, GRAS-compliant postharvest strategies for organic strawberries.

Principal component analysis (PCA)

In order to further explore the interrelationships among physicochemical, biochemical, and microbiological parameters, PCA was performed. The PCA effectively reduced the multidimensional dataset into a few major components that explained most of the variability in strawberry quality during storage. The first two principal components (PC1 and PC2) accounted for 78.4% of the total variance, with PC1 explaining 55.6% and PC2 explaining 22.8% variance. The loading plot revealed that PC1 was positively associated with WL, TBC, YMC, and TSS, while negatively correlated with firmness, color parameters (L*, a*, and b*), TA, TPC, TAC, and antioxidant capacity. This indicated that PC1 represented the overall fruit deterioration, where microbial proliferation and dehydration coincided with the decline of bioactive compounds and sensory attributes. PC2, in contrast, was more strongly influenced by color attributes (particularly a* and b*) and firmness, highlighting variations in fruit appearance and texture that were less dependent on microbial activity but crucial for consumer perception. The PCA score plot clearly distinguished between treatments and storage periods:

- CH+OEO coating and CO_2 treatment clustered on the negative side of PC1 and positive side of PC2, reflecting their ability to retain firmness, preserve color, and maintain higher bioactive content with lower microbial loads throughout storage.
- Untreated control and DW treatments were located on the positive side of PC1, associated with higher WL, rapid microbial proliferation, and pronounced loss in firmness and antioxidants, indicating poor preservation efficacy.
- O_3 treatment appeared at an intermediate position, initially delaying microbial growth but later shifting toward the deterioration zone because of oxidative stress and tissue damage after extended storage.
- CH and OEO single coatings were placed between the extremes, offering moderate preservation but less synergistic protection, compared to the combined CH+OEO treatment.

These findings highlight that PCA is a powerful tool for visualizing multidimensional quality changes in strawberries, demonstrating that postharvest treatments, preserving structural integrity (firmness, color), also safeguard bioactive compounds and delay microbial spoilage. The combined CH+OEO coating and CO_2 treatment emerged as the most effective strategies, aligning with their position in the PCA space closest to the 'fresh-like' quality profile.

Challenges and the Future Work

Variability in fruit response

Despite the effectiveness of CH+OEO and CO_2 treatments in maintaining strawberry quality, fruit response can vary due to cultivar differences, maturity stage, and seasonal conditions. Such variability may affect consistency in postharvest outcomes and limit the predictability of treatment efficacy. The future studies should evaluate these treatments across multiple cultivars and harvest seasons to establish broader applicability.

Scalability and commercial application

Translating laboratory or pilot-scale findings into commercial practice presents challenges. Optimization of coating concentrations, application techniques, and storage protocols is needed to ensure consistent quality at larger scale. Additionally, resource efficiency, cost-effectiveness, and labor requirements must be assessed to facilitate adoption by producers and packers of organic strawberries.

Long-term storage and supply chain conditions

The current study focused on a 15-day cold storage period, which reflected short-term postharvest handling. However, longer-term storage, transport conditions, and fluctuating temperatures during distribution may impact quality and bioactive retention. The future research should simulate realistic supply chain conditions to determine the robustness of these treatments over extended periods.

Integration with other preservation strategies

Interactions between CH+OEO or CO_2 treatments and other postharvest approaches, such as modified atmosphere packaging, refrigeration variations, or natural antimicrobial additives, remain largely unexplored. Investigating combined strategies could further enhance the shelf life and quality while maintaining safety and nutritional value.

Economic feasibility and consumer acceptance

For practical adoption, the cost of materials, application, and labor must be balanced against the benefits in shelf life extension and quality maintenance. Additionally, consumer perception regarding natural coatings and essential oil residues needs to be considered. The future studies should include cost–benefit analyses and consumer acceptability assessments to guide commercialization.

Mechanistic insights

Understanding the underlying mechanisms by which CH+OEO and CO_2 treatments preserve quality is essential. Further studies using molecular, biochemical, and microbiological approaches can reveal how these treatments interact with fruit physiology, microbial populations, and antioxidant systems, enabling more precise and effective preservation strategies.

Addressing these challenges and pursuing the outlined future research directions will contribute to the development of scalable, economically feasible, and consumer-accepted natural preservation methods, ensuring extended shelf life and maintenance of nutritional and sensory quality in organic strawberries and other perishable fruits.

The quality and nutritional stability of strawberries during postharvest storage are highly influenced by the choice of preservation strategies and the application of natural bioactive compounds. Phenolic compounds and anthocyanins are among the primary antioxidants in strawberries, contributing significantly to their health-promoting properties and resistance to oxidative deterioration (García-Pérez *et al.*, 2021). In this study, the application of chitosan, OEO, and their combination (CH+OEO) significantly mitigated the decline in TPC, TAC, and antioxidant capacity (measured as DPPH, $ABTS^+$, and FRAP activities) over 15 days of storage at 4°C. These findings are consistent with the recent studies showing that edible coatings enriched with natural antioxidants form a semi-permeable barrier that reduces moisture loss and oxidative degradation, thereby maintaining bioactive compound levels (da Silva *et al.*, 2021; Zhang *et al.*, 2022). Specifically, CH+OEO treatment consistently exhibited maximum TPC and TAC values during storage, highlighting a synergistic effect between chitosan's film-forming properties and OEO's phenolic-rich composition. This aligns with previous reports indicating that the combination of polysaccharide coatings with essential oils enhances the preservation of bioactive compounds in berries (Costa *et al.*, 2021; Li *et al.*, 2023b). WL and firmness are crucial indicators of postharvest quality, and their reduction is closely associated with the retention of phenolic compounds and antioxidant activity (Pires *et al.*, 2022). In the present study, both CH+OEO and CO_2 treatments significantly minimized WL and maintained firmness, compared to untreated controls, suggesting a dual mechanism involving both barrier protection and modification of storage atmosphere. Modified atmosphere treatments, particularly CO_2 enrichment, are known to slow down respiration rates and delay senescence, which complement the effects of antioxidant-rich coatings (Wang *et al.*, 2022). These results corroborate the recent findings that combining physical and biochemical preservation strategies can effectively extend the shelf life of strawberries without compromising nutritional quality (Martínez-García *et al.*, 2020). Furthermore, antioxidant capacity, as assessed by DPPH, $ABTS^+$, and FRAP assays, exhibited strong correlations with TPC and TAC levels, emphasizing the role of phenolic compounds and anthocyanins as major contributors to radical scavenging and reducing power (García-Pérez *et al.*, 2021; Zhang *et al.*, 2022). The decrease in antioxidant activity observed in untreated and DW samples over time highlights the susceptibility of strawberries to oxidative stress during cold storage, whereas treatments with chitosan, OEO, CH+OEO, CO_2, and O_3 effectively mitigated this loss. Notably, the maintenance of antioxidant activity under CH+OEO and CO_2 treatments suggests that these strategies may preserve cellular integrity and enzyme activity associated with phenolic metabolism (Li *et al.*, 2023b; Pires *et al.*, 2022). Overall, these findings underscore the importance of integrating natural coatings and controlled storage conditions to enhance postharvest quality and nutritional values. The observed synergistic effects of CH+OEO treatment, combined with modified atmosphere storage, indicate a promising approach for commercial applications, aligning with consumer demand for minimally processed functional berries. The future studies should explore the mechanistic interactions between coatings, essential oils, and storage atmospheres, as well as the sensory and microbial stability implications, to optimize postharvest handling and maximize health benefits.

Conclusions

This study demonstrated that different postharvest pretreatments exert significant effects on the physicochemical, bioactive, and microbiological properties of organic strawberries stored at 4°C. Among the treatments, CO_2 exposure (30% for 3 h) and the CH+OEO coating were most effective in retarding WL, maintaining firmness and color, reducing microbial proliferation, and preserving bioactive compounds and antioxidant capacity. In contrast, untreated and DW fruits exhibited rapid deterioration, while O_3 treatment showed only transient benefits and even promoted tissue damage during prolonged storage. The application of multivariate analysis (PCA and correlation analysis) revealed clear associations between microbiological growth, WL, and quality decline, while highlighting that coatings and CO_2 treatment clustered closely with 'fresh-like' visual and textural attributes. These results confirmed that integrated edible coatings enriched with essential oils, or CO_2-based strategies, were promising, scalable, and GRAS-compliant approaches for extending the shelf life of organic strawberries, where synthetic postharvest preservatives are restricted. Overall, this work provides practical insights for the organic fruit supply chain, offering sustainable preservation alternatives that align with consumer demand for safe, high-quality, and chemical-free products. The future research should evaluate the toxicological safety, consumer acceptance, and economic feasibility of these treatments under commercial handling and distribution conditions to support their large-scale adoption.

Acknowledgment

The author extended appreciation to Taif University, Saudi Arabia for supporting this work through project No. TU-DSPP-2024-167.

Acknowledgment of AI Assistance

The author used Open AI's ChatGPT (version GPT-5) to assist in improving the clarity and grammar of the manuscript. The author reviewed and edited the content to ensure accuracy and takes full responsibility for the final version of the manuscript.

Ethical Statement

This study does not need ethical approval.

Conflict of Interest

There was no conflict of interest.

Funding

This research was funded by Taif University, Saudi Arabia, Project No. TU-DSPP-2024-167

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