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*Original Research*

# Cold Plasma Treatment: A Novel Approach for Microbial Inactivation and Shelf-Life Enhancement of Strawberries

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**Abstract**

**Background**: Strawberries is a fruit rich in various nutrients. Their thin skin structure and high respiratory activity make them susceptible to microbial contamination and rapid spoilage. Traditional preservation techniques still have limitations. Therefore, this study aims to investigate the effect of plasma-processed air (PPA) on strawberry preservation. **Methods**: The efficacy of plasma-processed air generated via sliding arc discharge, with treatment times of 15 s, 30 s, and 60 s, was compared to untreated controls in terms of microbial suppression and quality preservation of fresh strawberries during cold storage. **Results**: PPA treatment significantly reduced surface microbial colonies, with a 60 s exposure achieving the lowest colony count (1.18 log colony-forming units (CFU)/g), representing approximately a 95% reduction compared to control (2.49 log CFU/g). While PPA exposure maintained nutritional parameters (total soluble solids (TSS), titratable acid (TA), pH, ascorbic acid (ASA)) at levels not significantly different from those of the untreated fruit ($p > 0.05$), PPA exposure effectively mitigated quality deterioration. PPA-treated strawberries were firmer, showed better color retention and less weight loss, maintained cell membrane integrity, and had lower ethylene production ($p < 0.05$). Notably, shelf-life extension was dose-dependent, reaching 15, 18, and 16 days at 4 °C for the treated strawberries (15 s, 30 s, and 60 s treatments, respectively) compared to 9 days for the untreated control fruit. **Conclusion**: PPA can effectively extend strawberry shelf-life by maintaining fruit quality and inactivating microorganisms. Furthermore, this continuous industrialized green treatment technology could ultimately be used as a standard and applied linearly during the cold chain transportation of fresh produce.

**Keywords:** plasma-activated gas treatment; strawberry preservation; shelf-life extension; colony inactivation

## 1. Introduction

Strawberry is a highly perishable fruit prized for their distinctive aroma, polyphenol-rich composition, and proven health benefits in chronic disease prevention [1–3]. However, their thin epidermal structure and high respiratory activity render them vulnerable to mechanical injury, microbial colonization (particularly *Botrytis cinerea*), and rapid postharvest deterioration [4]. While conventional preservation methods including thermal processing [5], edible coatings [6], and ozone treatment demonstrate partial efficacy [7], practical limitations persist regarding chemical residues, energy consumption, and operational complexity.

Emerging as a breakthrough in non-thermal food processing, low-temperature plasma technology has revolutionized food industry through its unique combination of microbial inactivation and quality preservation mechanisms and has been widely applied across multiple areas [8]. This technology generates reactive oxygen/nitrogen species (ROS/RNS), ultraviolet photons, and charged particles through gas ionization, which synergistically disrupt microbial cellular components while maintaining the bioactive compounds of the fruit to achieve a bactericidal effect [9–11]. Notably, plasma-activated water (PAW) applications can extend shelf-life of various produce including apple [12] and blueberry [13], yet this technology faces some challenges due to real-time production costs and inconsistent sterilization efficacy [14].

The present study introduces an innovative: plasma-processed air (PPA) technology under ambient instead of inert gases atmosphere. This approach significantly reduces operational costs while enabling continuous processing which brings a critical advantage for commercial scalability. Building upon recent advancements in dielectric barrier discharge systems, our investigation focuses on PPA's dual capacity to suppress microbial proliferation (particularly total viable counts and Botrytis infection) and modulate physiological responses in stored strawberries. Through comprehensive evaluation of physicochemical parameters (texture, color stability, weight loss), biochemical markers (ethylene biosynthesis, membrane permeability), and nutritional retention (ascorbic acid), this work addresses a critical research gap in plasma application protocols for soft fruits. The findings hold significant implications for optimizing postharvest strategies, particularly in addressing the 20–35% annual loss of strawberry production attributed to inadequate preservation methods.

The implementation of active gas sterilization technology, characterized by short contact time and no chemical residues, without the use of any chemical agents, combined with modified atmosphere packaging (MAP) immediately after sterilization, eliminates the risk of secondary contamination and enables continuous online processing. This avoids the drawbacks of other non-thermal disinfection methods, such as the potential health hazards of ultraviolet (UV) disinfection and the limitations of pulsed electromagnetic field technology due to medium restrictions. The research apparatus uses electricity as the sole consumable energy source, with air as the medium, eliminating other energy consumption and significantly reducing costs. By establishing correlations between plasma treatment parameters and fruit quality indicators, our study provides actionable insights for implementing PPA technology in commercial cold chain systems.

## 2. Materials and Methods

### *2.1 Plant Material and Reagents*

Strawberry cv. Dandong (n = 576 fruit), were purchased from Beijing Agricultural Products Market, Beijing, China; LB Nutrient Agar, Beijing Aoboxing Biotechnology Co., Ltd., Beijing, China; Sodium Chloride (analytically pure), Phenolphthalein (98% mass fraction), Shanghai Aladdin Biochemical Science and Technology Co., Ltd., Shanghai, China; Sodium hydroxide standard solution, Shandong Keyuan Biochemistry Co., Ltd., Heze, Shandong, China; Potassium iodide (analytically pure), Tianjin Juhengda Chemical Co., Ltd., Tianjin, China; soluble starch (analytically pure), Tianjin Fuchen Chemical Reagent Co., Ltd., Tianjin, China.

### *2.2 Cold Atmospheric Plasma*

The experiment employed the apparatus shown in Fig. 1. PPA was generated by an arc discharge system (Lab-V1 plasma preservation device), which ignited an air flow between two-point electrodes (spacing: 3 cm) via a 20–40 kV high-voltage electric field to form a plasma discharge. Using air as the gas source, the cold plasma device was activated to produce atmospheric pressure cold plasma (CAPP). The generated CAPP was introduced into a sealed chamber via a vacuum circulation pump (flow rate: 40 L/min), thereby producing plasma-activated gas (PPA). The discharge power was set at 220 W, with the high-voltage power supply operating at a center frequency of 50 kHz and a discharge volume of 30 $dm^3$. Strawberries were processed in batches within the chamber for treatment durations of 15 seconds, 30 seconds, and 60 seconds. After preheating is complete, sample processing begins. Energy consumption during the experiment is the product of the equipment power and the processing time.

### *2.3 Experimental Setup*

Uniform strawberry samples with consistent size, color, and weight, and free of mechanical damage were selected. Remove the pedicel and place the fruit in a refrigerated device (model: YC-300L) at a constant temperature of 4 °C for 4–6 hours to achieve thermal equilibrium.

Strawberries were randomly assigned to three experimental treated and one untreated experimental groups, PPA15, PPA30, PPA60 and CK, respectively (15 s PPA treatment = PPA15; 30 s PPA treatment = PPA30; 60 s PPA treatment = PPA60; untreated control = CK). Samples were packed in polypropylene trays (Model: TQBC-0775; dimensions: 222 × 132 × 40 mm; Sealed Air Co., Ltd., Shanghai, China) containing six fruit each. Each treatment group was triplicated (3 trays containing 6 six strawberries each per treatment).

Fig. 1 shows the atmospheric plasma processing equipment, comprised by a plasma generator, a conveyor belt, a laminated machine, and a heat sealer. After the air was broken down in the discharge ceramic tube, plasma active substances were generated, and used to treat the strawberries in the chamber. Each batch of strawberries (6 fruit each) was processed at a time. After processing, all samples were immediately transferred to the conveyor belt and heat sealed with food-grade polyethylene (PE) film to complete the entire sterilization and packaging process, and stored in a crisper under controlled temperature conditions (4 °C ± 2 °C). Quality assessment was carried out every 3 days throughout the storage period.

### *2.4 Quality Analyses*

#### 2.4.1 Determination of Respiration Rate

The Check point headspace MAP gas analyser (Shanghai AMETEK Trading Co., Ltd., Shanghai, China) was used to measure the levels of oxygen and carbon dioxide in the packages. The air indicator was calibrated and a sealed adhesive strip was attached to the top, middle and bottom of each package. One sample was taken from each strip and the average of the three data points was taken and the final value calculated.

#### 2.4.2 Determination of Ethylene Content

The ethylene content (ppm) of the packages was measured using a gas chromatograph (Model GC7860, Shanghai Youke Instrumentation Co., Ltd., Shanghai, China), with samples taken from the top, middle and bottom positions of each package, and the average of the three data points was calculated as the final value.

#### 2.4.3 Determination of Cell Membrane Permeability

The relative conductivity method was used to measure cell membrane permeability. Damage to the cell membrane under abiotic stress leads to electrolyte leakage, and changes in membrane permeability are quantified by measuring changes in solution conductivity.

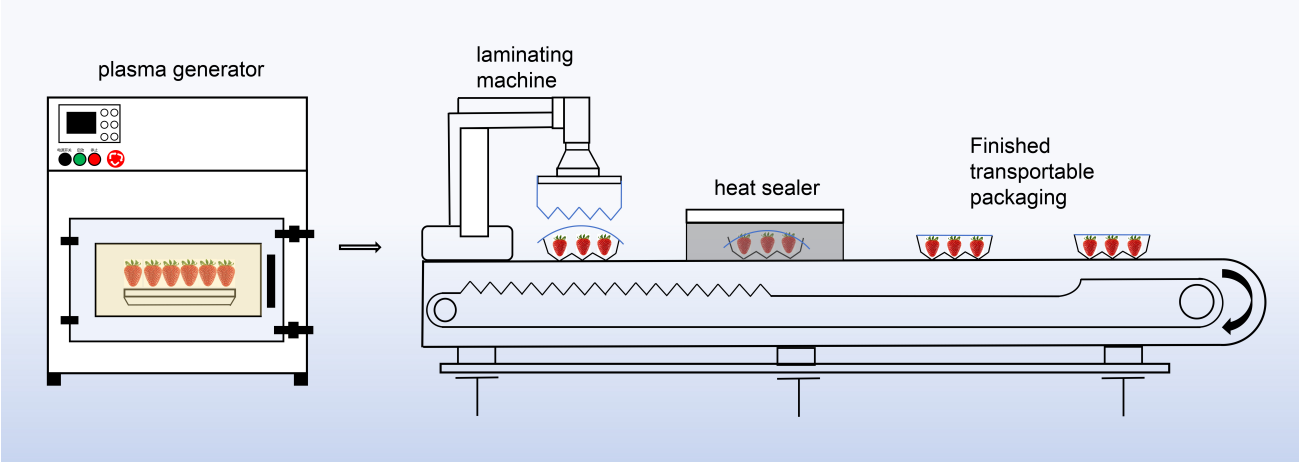


**Fig. 1. Atmospheric plasma equipment and flow chart used for processing strawberries.**

Strawberry tissue (2 g) was mixed with 20 mL deionized water inside a beaker. Then, the strawberry-deionized water mixture was let to rest at constant room temperature for 1 h to determine the initial conductivity ($L_0$) measured using a conductivity meter (AZ-8362, Hengxin Industrial Co., Ltd., Taichung, China). Afterward, the beaker containing the mixture was transferred to a water bath at 100 °C for 15 min. After that, it was left to cool at room temperature and conductivity measured again ($L_1$). Relative conductivity was calculated using the following formula:

$$\text{Relative electrical conductivity } (\%) = \frac{L_0}{L_1} \times 100 \quad (1)$$

2.4.4 Determination of Weight Loss

Based on the weight changes during storage, the weight loss rate a calculated using the weighing method described by Wang *et al.* (2019) [15]. The weight was measured using an electronic balance (model: MTB5000D, Shenzhen Meifu Electronics Co., Ltd., Shenzhen, Guangdong, China), and the calculated formula was as follows:

$$\text{Weight loss } (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\% \quad (2)$$

2.4.5 Determination of Firmness

A G-type fruit hardness tester (Yueqing Aidebao Technology Co., Ltd., Wenzhou, Zhejiang, China) was used to test the hardness of strawberries according to the method described by Yang *et al.* (2023) [16]. After zeroing the device, the center of the strawberry was selected, and the reading was taken when the penetration depth reached the instrument scale (unit: kg/cm). The average hardness of six strawberries in each tray was taken as the final result for each treatment.

2.4.6 Determination of Surface Color

Using a DS-200 colour difference meter (Zhejiang Caipu Technology Co., Ltd., Hangzhou, Zhejiang, China), the CIE Lab method was used [17]. After calibrating the instrument, each group (6 strawberries) was sampled and tested. The sampling location was the equatorial part of the strawberry. Each strawberry was sampled three times at equidistant positions, and the L*, a*, and b* values were measured separately. The average value of each group of strawberries was taken as the final value. The formula for calculating color difference is as follows:

$$\Delta E = \sqrt{(\Delta L^*)^{\wedge 2} + (\Delta a^*)^{\wedge 2} + (\Delta b^*)^{\wedge 2}} \quad (3)$$

where ΔL*, Δa* and Δb* denote the luminance difference, red-green difference, and yellow-blue difference.

2.4.7 Determination of Total Soluble Solids (TSS)

Mash the strawberries and filter them. The total soluble solids content was measured using a PAL-$\alpha$ refractometer (Guangzhou Aito Scientific Instrument Co., Ltd., Guangzhou, Guangdong, China) according to the method described by Yi *et al.* (2022) [18]. The average of three measurements was taken as the final value, TSS data were expressed as percentage (%).

2.4.8 Determination of Titratable Acid (TA)

The titratable acid content was determined using the sodium hydroxide solution titration method [19]. Based on the acid-base neutralization reaction, the acidic substances in the sample were titrated with a standard sodium hydroxide solution, and the endpoint is determined by the color change of the indicator. The total acid content was calculated based on the volume of sodium hydroxide consumed.

The formula for calculating the titratable acid contents as follows (units: %):

$$TA = \frac{\omega_1 \times 0.632\%}{\omega_0} \quad (4)$$

2.4.9 Determination of Ascorbic Acid (ASA)

Potassium iodate titration was used to determine the ascorbic acid content in strawberries [19]. After grinding the strawberry juice, take 1.0 g of juice, recorded as $\omega_0$, add 0.5 g of starch aqueous indicator, dilute using 5.0 g of water, titrate with potassium iodide solution until the sample solution turns brown (no colour fading within 30 s), recorded as $\omega_1$, and the formula for calculating ascorbic acid content is as follows (units: mg/100 g):

$$ASA = \frac{\omega_1 \times 152.5}{\omega_0} \text{mg}/100\ \text{g} \quad (5)$$

2.4.10 Determination of pH

The pH value of strawberry juice was measured using a FE28 pH (Mettler-Toledo Technology Co., Ltd., Shanghai, China) meter. The average value of three repeated measurements was used.

2.4.11 Determination of Colonies

Total colony counts on strawberry surfaces were determined according to GB 4789.2-2022. A 10 g fruit sample was homogenized using sterile physiological saline. Dilutions were prepared at different concentrations (–1 to –3). One milliliter of each dilution was transferred to plate count agar (PCA) medium cooled to 46 °C and mixed. The mixtures were cooled to room temperature under a laminar flow hood until the agar solidified. The plates were incubated at 36 °C ± 1 °C for 48 hours. After incubation, dilutions with colony counts within the countable range were selected. The surface colony count was tallied, and the corresponding log value was calculated based on the dilution factor and count results.

2.4.12 Determination of Decay Rate

Unpackaged strawberries were assessed by observing signs of decay on the surface of the fruit, characterized by softening of the injured area and the appearance of brown spots [20], and the total amount of decay was recorded. The decay rate was calculated using the following formula:

$$\text{Decay rate}\ (\%) = \frac{\text{number of decayed fruits}}{\text{total number of fruits}} \times 100 \quad (6)$$

2.4.13 Sensory Evaluation

Following the method described by Fu *et al.* (2024) [21], each group of strawberries was evaluated for color, aroma, softness, and appearance-related factors (Table 1). Under constant environmental conditions (temperature 21 °C, relative humidity 60%, daylight, air circulation), three independently conducted assessments were performed by three uniformly trained evaluators. The final score was determined as the average of all individual scores.

*2.5 Statistical Analysis*

The data collected was analyzed by one-way ANOVA to determine the significant differences between treatments (SPSS 20.0, IBM, New York, USA). The least significant difference a $p < 0.05$ was also used, and the data were expressed as mean and standard-deviation. Furthermore, plots were analyzed using Origin 2014 (OriginLab Corporation, Massachusetts, USA), with the different letters (a, b, c) within each graph representing significant differences between treatments at each storage time.

## 3. Results and Discussion

*3.1 Effect of PPA Treatment on Strawberry Quality Characteristics Before and After Treatment*

Table 2 shows quality attributes of PPA-treated and untreated strawberries.

For the weight loss of strawberries at moment zero, the treatment group showed a slight reduction of less than 0.1% compared to the control sample, indicating that the PPA had a negligible effect on this quality parameter. The results of this study are in agreement with the results of Rana *et al.*, 2020 [22], who studied whole fresh strawberries using in-package air discharges to generate plasma, as well as Giannoglou *et al.*, 2021 [23] who showed that plasma treatment of strawberries did not significantly change their weight loss percentage score.

The color (L* and a*) of the PPA-treated strawberries was retained, and hardness showed only minor changes, none of which were significant ($p > 0.05$) compared to the control, as confirmed by sensory evaluation on day 0. Additionally, TSS, TA, pH, and ASA content remained stable across treatment conditions ($p > 0.05$), indicating that PPA treatment did not affect strawberry nutrient composition. These findings align with previous studies [22], which reported no significant impact of plasma treatment on strawberry flavor or color. Furthermore, no significant difference in respiratory rate was observed between the treatment group and the control group ($p > 0.05$), which is consistent with the findings of Zhang *et al.*, 2023 [24] that plasma treatment initially causes only minor changes in the respiratory rate of blueberries.

It is worth noting that, compared with the control group, the total number of bacterial colonies on strawberries significantly decreased after PPA treatment ($p < 0.05$), and the reduction in bacterial colony counts on the strawberry surface became more pronounced as treatment time increased (Fig. 2). The total number of bacterial colonies on the surface of strawberries in the control group was 2.49 log CFU/g. After PPA treatment for 15 s, 30 s, and 60 s, the total number of bacterial colonies was 2.04 log CFU/g, 1.47

**Table 1. Sensory evaluation criteria of strawberry.**

| Value of a score | Colour | Fruit morphology | Odour |
|---|---|---|---|
| 9∼10 | Bright red, shiny | No deformation | Intense fruity aroma |
| 6∼8 | Dark red, slightly dull | Slight atrophy | Weak aroma and no odour |
| 3∼5 | Fuchsia, dull | Significantly shrivelled | No detectable aroma |
| 0∼2 | Brown, extremely dull | Extremely shrivelled mould | Off-odors present |

**Table 2. Quality parameters of strawberries under different processing conditions (before preservation).**

| Parameter of analysis | Type of processing | | | |
|---|---|---|---|---|
| | CK | PPA15 | PPA30 | PPA60 |
| Weight loss (%) | $0.000 \pm 0.000^{a}$ | $0.024 \pm 0.002^{a}$ | $0.021 \pm 0.006^{a}$ | $0.020 \pm 0.004^{a}$ |
| L* | $31.27 \pm 1.07^{a}$ | $31.25 \pm 0.52^{a}$ | $31.39 \pm 0.88^{a}$ | $31.07 \pm 0.57^{a}$ |
| a* | $35.65 \pm 1.19^{a}$ | $36.28 \pm 1.03^{ab}$ | $35.45 \pm 0.75^{a}$ | $35.22 \pm 1.03^{ab}$ |
| Firmness (N) | $1.48 \pm 0.10^{a}$ | $1.47 \pm 0.34^{a}$ | $1.43 \pm 0.05^{a}$ | $1.49 \pm 0.05^{a}$ |
| TSS (%) | $11.17 \pm 0.23^{a}$ | $11.23 \pm 0.03^{a}$ | $11.60 \pm 0.20^{a}$ | $11.42 \pm 0.02^{a}$ |
| TA (%) | $0.80 \pm 0.13^{a}$ | $0.77 \pm 0.09^{a}$ | $0.83 \pm 0.06^{a}$ | $0.84 \pm 0.14^{a}$ |
| ASA (%) | $37.36 \pm 1.17^{a}$ | $35.97 \pm 1.18^{a}$ | $39.54 \pm 0.55^{a}$ | $37.61 \pm 0.35^{a}$ |
| pH | $3.44 \pm 0.04^{a}$ | $3.44 \pm 0.02^{a}$ | $3.45 \pm 0.04^{a}$ | $3.43 \pm 0.04^{a}$ |
| $O_2$ (%) | $19.87 \pm 0.04^{a}$ | $19.88 \pm 0.02^{a}$ | $19.87 \pm 0.10^{a}$ | $19.88 \pm 0.04^{a}$ |
| $CO_2$ (%) | $0.00 \pm 0.01^{a}$ | $0.00 \pm 0.02^{a}$ | $0.00 \pm 0.01^{a}$ | $0.00 \pm 0.01^{a}$ |
| Colony number (log CFU/g) | $2.49 \pm 0.08^{a}$ | $2.04 \pm 0.11^{b}$ | $1.47 \pm 0.09^{c}$ | $1.18 \pm 0.09^{d}$ |

*Different superscript letters ($^{a}$, $^{b}$, $^{c}$ and $^{d}$) represent significant differences in the data (same row); the same letter is a non-significant difference ($p > 0.05$), and a different letter is a significant difference ($p < 0.05$). CK, control; PPA, plasma-processed air; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s; TSS, total soluble solids; TA, titratable acid; ASA, ascorbic acid.

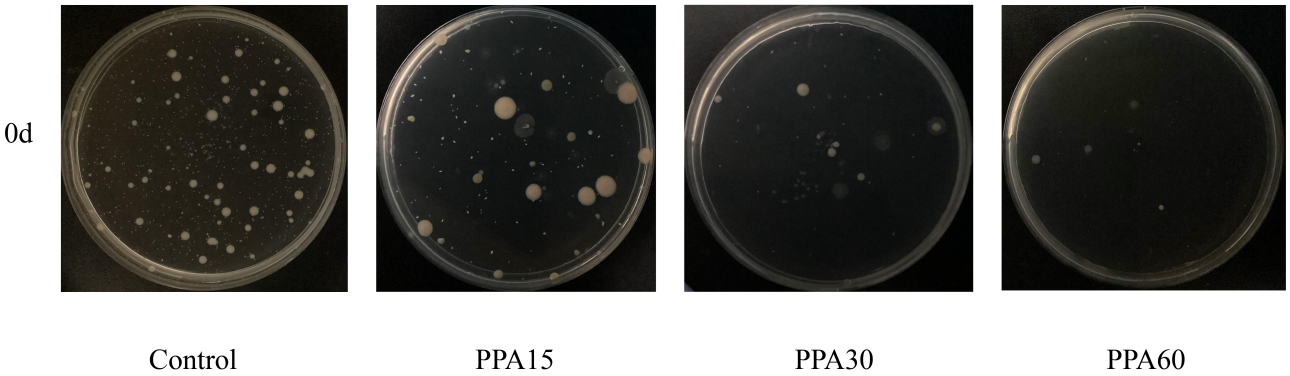


**Fig. 2. Number of strawberry surface colonies under different treatment conditions (before preservation).**

log CFU/g, and 1.18 log CFU/g, respectively, representing reductions of 64.52%, 90.45%, and 95.10% compared to the untreated control. The 60 s PPA treatment demonstrated significant sterilization efficacy, achieving a reduction of 1.31 log levels.

As expected, the reactive oxygen and nitrogen species generated by surface discharge diffuse into the fruit, exerting a sterilizing effect on the strawberries and reducing the microbial content on their surfaces. The sterilization efficacy of PPA treatment increases with longer treatment times, as the active components within PPA come into more prolonged and thorough contact with the strawberry surfaces. This confirms that PPA, after diffusing to the surface of strawberries in the treatment chamber, can effectively inactivate microorganisms on the surface of strawberries [25], and its inactivation effect is proportional to the treatment time.

### *3.2 Effect of PPA Treatment on Strawberry Quality During Cold Storage*

Moisture loss in strawberries, resulting from ripening, respiration, and transpiration processes, leads to diminished surface gloss, wilting, and shriveling. Weight loss rate serves as a critical indicator for evaluating the efficacy of postharvest preservation treatments [26]. The weight loss rate exhibited a progressive increase with prolonged

storage duration (Fig. 3). One-way ANOVA revealed statistically significant differences ($p < 0.05$) between PPA-treated groups and the control during the initial storage phase. Notably, the PPA30 treatment demonstrated exceptional efficacy, achieving a 0.93% weight loss rate by day 3 (1.68%, 1.72%, and 1.2% for CK, PPA15, and PPA60, respectively). Compared to the control group, this treatment effectively reduced the rate of weight loss. No statistically significant differences ($p > 0.05$) were detected between treated and control groups in the later storage stages. Weight loss rate of the PPA30-treated was slightly lower but there was no significant difference compared to the PPA15 or PPA60. Compared to the control group (CK), its weight loss rate was lower. This observation can be attributed to the metabolic activity-induced wilting and water transpiration occurring predominantly in the early storage phase [4], suggesting that PPA treatment exerts inhibitory effects on strawberry metabolic processes. During the advanced storage phase, the progression of fruit decay resulted in reduced moisture loss, leading to a stabilization of weight loss rates.

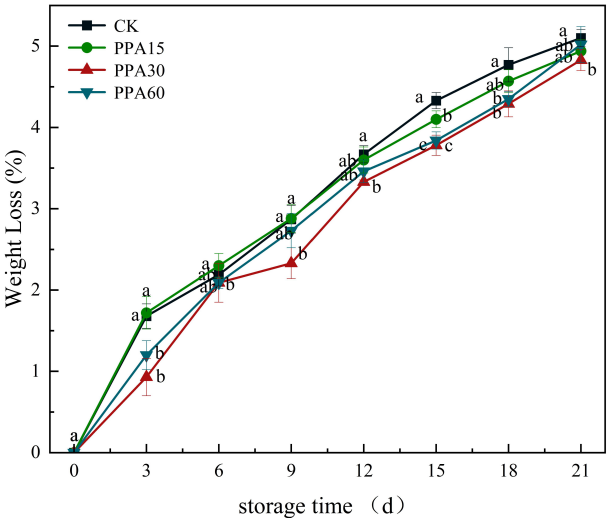


**Fig. 3. Effects of different PPA treatments on weight loss of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

Firmness represents a critical quality attribute in strawberries, serving as a key indicator of both ripeness and postharvest storage quality [27]. All treatments exhibited a biphasic firmness pattern during storage, characterized by initial increase followed by gradual decline (Fig. 4). This phenomenon may be attributed to incomplete maturity at harvest, with subsequent postharvest ripening during storage, accompanied by progressive strengthening of cell wall integrity and pectin cross-linking [28], as well as moisture loss-induced tissue densification leading to increased firmness. Prolonged storage resulted in significant softening, likely due to enzymatic degradation of cell wall components, and based on the morphological characteristics of the decayed area (such as softening of the flesh) and the extent of decay, it can be inferred that this may be related to microbial activity.

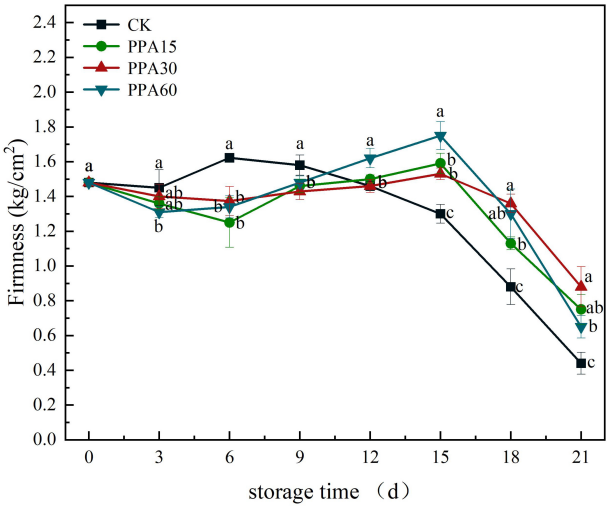


**Fig. 4. Effects of different PPA treatments on firmness of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

PPA-treated strawberries showed the highest stability in firmness ($p < 0.05$). An increase in the initial firmness was observed in strawberries, regardless of the treatment (0–6 days for control, 0–15 days for PPA-treated), attributable to ongoing ripening processes and moisture loss, suggesting that PPA treatment effectively delays strawberry softening. Among treatments, PPA30 demonstrated optimal efficacy in hardness preservation, with only an 8% decrease before softening began on the fifth day (CK, PPA15, and PPA60 showed decreases of 12.16%, 23.64%, and 12.16%, respectively). During late storage, the PPA-treated group maintained superior firmness, potentially due to suppressed pectin methylesterase (PME) activity. PME, abundantly present in plant cell walls, catalyzes pectin demethylation leading to cell wall loosening and consequent firmness reduction [27]. This finding aligns with Giannoglou *et al*., 2021 [23], who reported that plasma treatment had a significant effect on PME activity inhibition.

The color parameters L*, a*, b*, and ΔE were used to evaluate color changes in stored strawberries showing a gradual decrease during storage, while the color difference value ΔE gradually increased (Fig. 5). The redness value a*

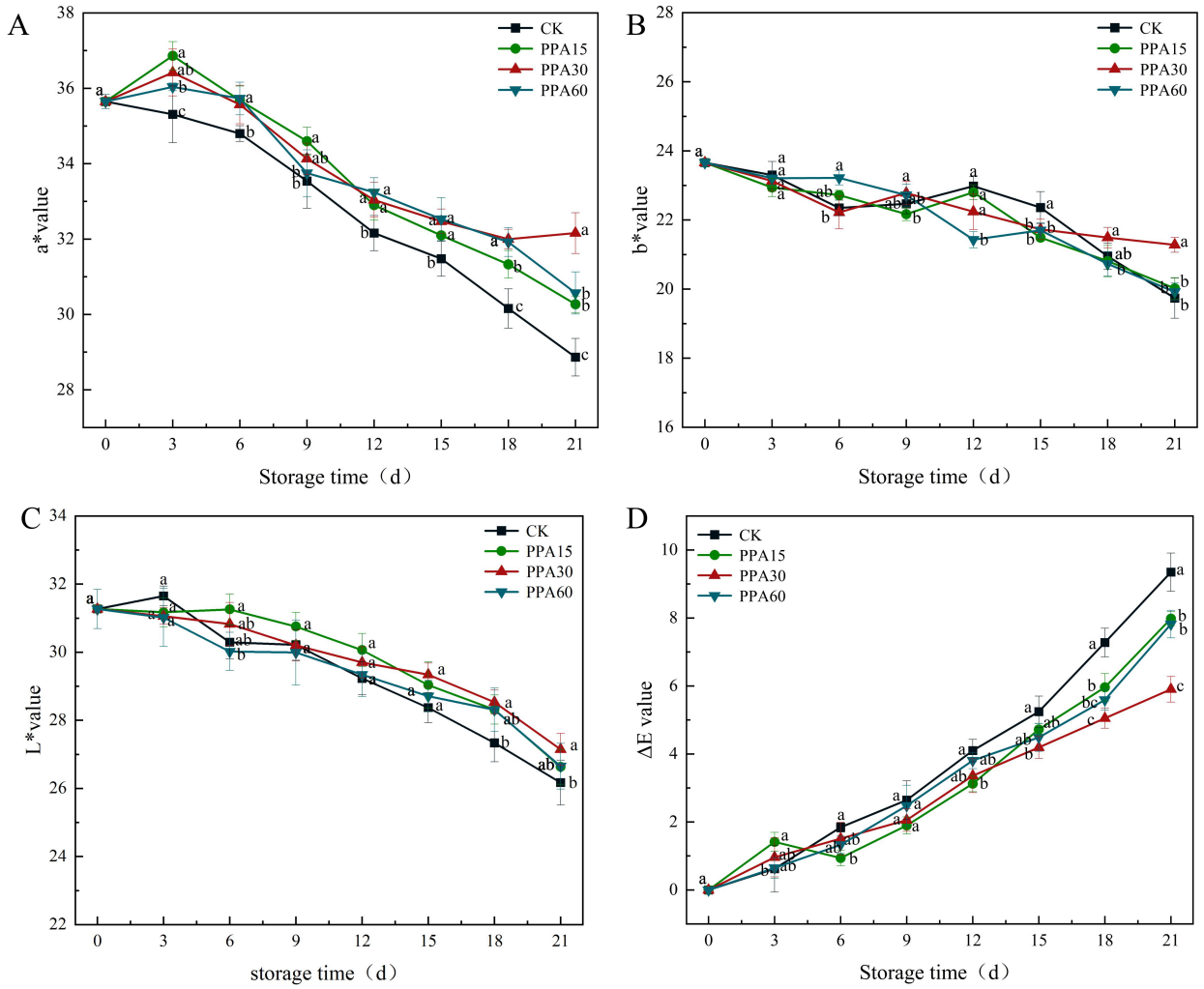


**Fig. 5. Effect of PPA treatments on a* (A), b* (B), L* (C) and ΔE (D) values of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

on the surface of strawberries is crucial for market and sensory acceptance. Compared to the control, the a* value of PPA-treated samples showed a slower decrease ($p < 0.05$). This result is consistent with the study by Hou *et al*., 2019 [29], which reported that plasma treatment improves the color stability of blueberry. After harvest, surface brightness L* values of strawberry changed due to physiological activities, fruit ripening, enzymatic oxidation, and anthocyanin synthesis [4]. Although the L* values of the PPA-treated strawberries were slightly higher than those of the control group, suggesting that the inhibitory effect of metabolic processes on surface reflectance is limited, this difference was not statistical significance. These results are consistent with the findings of Misra *et al*. (2014) [30], who showed that atmospheric cold plasma treatment had a limited effect on strawberry surface color. The b* value remained relatively stable throughout storage, while the color difference ΔE between treatments exhibited significant variability ($p > 0.05$), indicating that plasma gas treatment plays a crucial role in preserving strawberry surface color.

Total soluble solids (TSS) content, a key biochemical indicator for assessing strawberry sugar content [31], exhibited a continuous decline in both control and treated fruits during storage (Fig. 6A), with no significant difference observed between treatments and control throughout. This reduction can be attributed to the combined effects of respiratory metabolism and microbial activity [25], where soluble carbohydrates are metabolized to sustain cellular functions while microbial proliferation concurrently consumes available nutrients. Notably, the PPA30 treatment demonstrated superior efficacy in TSS preservation, suggesting an optimal treatment for maintaining fruit quality within the tested concentration range. These findings corroborate previous reports on low-temperature plasma applications in strawberry preservation [25], supporting the potential of plasma-based technologies in postharvest management.

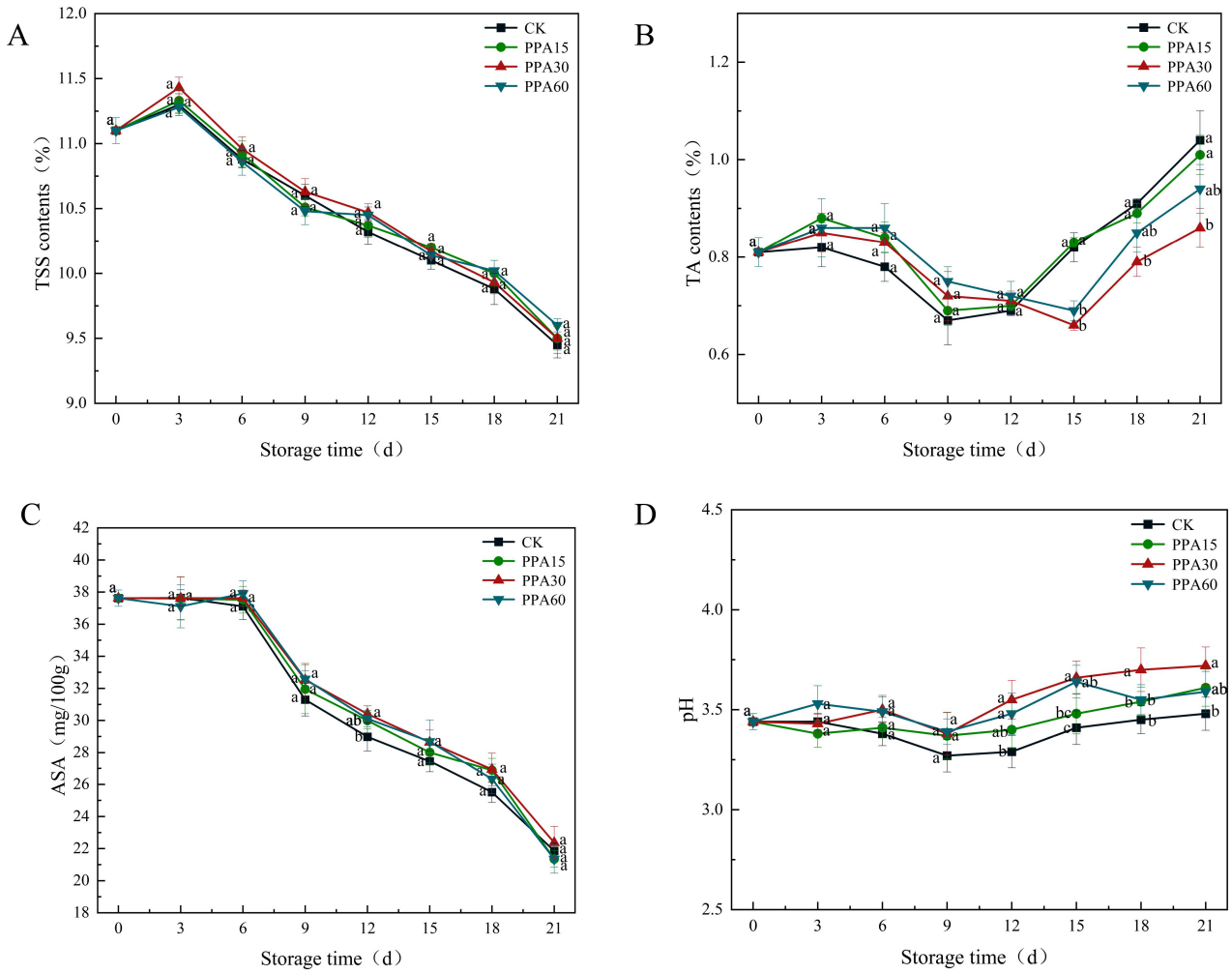


**Fig. 6. Effect of different PPA treatments on TSS (A), TA (B), ASA (C) and pH (D) of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

The titratable acidity (TA) content, a key biochemical indicator of sugar-acid balance and sensory quality in strawberries, exhibited a characteristic biphasic pattern during storage (Fig. 6B), with an initial decline (0–12 days) followed by a subsequent increase. This dynamic profile primarily reflects metabolic regulation, particularly through respiratory pathways [32]. The initial TA decrease corresponds to normal physiological maturation, where organic acids are preferentially metabolized as respiratory substrates. PPA treatment had limited impact on TA dynamics, with no statistically significant differences observed between the 0–12 day treatment groups and the control group ($p > 0.05$), indicating minimal interference with primary metabolic processes. The subsequent rise in TA is closely associated with accelerated fruit senescence and microbial proliferation, where hydrolytic enzymes from spoilage microorganisms degrade fruit components and produce acidic metabolites. Notably, after 12 days, total acid accumulation in PPA-treated samples was significantly lower than in the control group ($p < 0.05$), indicating its effective inhibition of microbial activity and maintenance of fruit quality. Among the treatments, PPA30 exhibited optimal performance by maintaining the most stable TA profile throughout storage, highlighting its superior efficacy in delaying senescence while preserving fruit biochemical composition, thereby substantiating the potential of PPA treatment as an effective postharvest intervention strategy for quality maintenance during extended storage periods.

The antioxidant properties of ASA, a water-soluble antioxidant with strong reducing properties, helps reducing oxidative damage while enhancing fruit resistance to abiotic and biotic stresses [26]. Our results showed a progressive decline in ASA content during storage across all treatments (Fig. 6C), attributable to the combined effects of exogenous oxidative stress, endogenous respiratory metabolism, and microbial growth. However, no significant differences

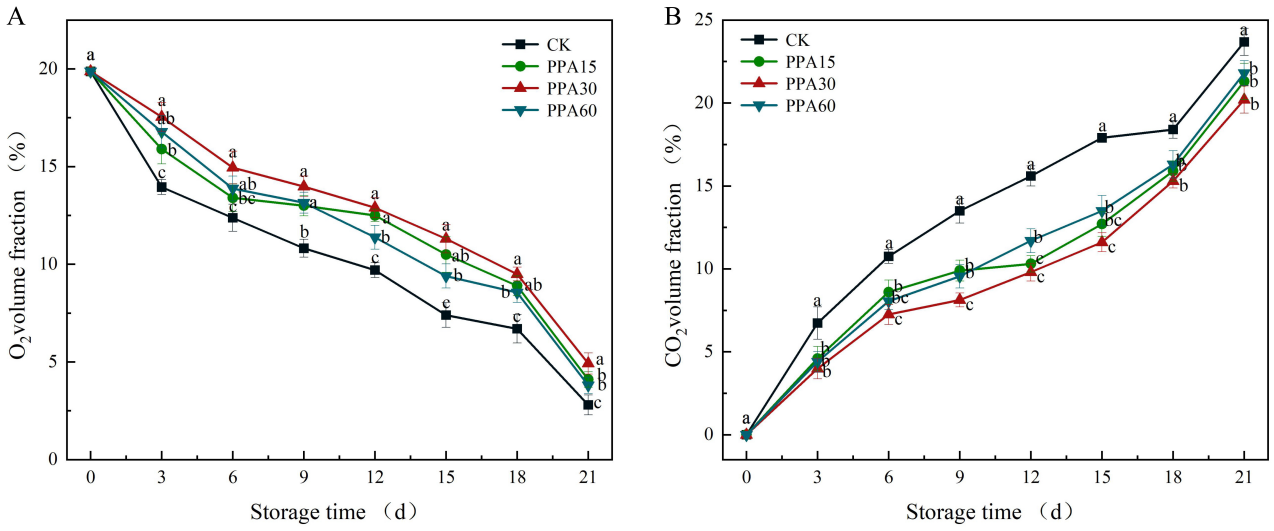


**Fig. 7. Effect of different PPA treatments on the $O_2$ (A) and $CO_2$ (B) levels inside packages of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

in ASA content were observed between treated and control strawberries throughout the entire storage period (0–21 days) ($p > 0.05$). This indicates that the PPA treatment had no significant effect on the ASA content of strawberry under the present experimental conditions, and did not significantly interfere with the ASA-related metabolic pathways or antioxidant system in strawberry, thus maintaining the relative stability of the ASA content. The observed stability implies that the applied PPA treatment preserved the suggests antioxidant capacity of strawberries without inducing substantial changes in their redox homeostasis.

The pH value can affect the balance of organic acids and sugars to maintaining the unique sweet and sour flavour of strawberries, and too high or too low of a value may lead to an imbalance in flavour, which has an important effect on storage quality of fruits and vegetables [33]. Results from our study showed constrained pH fluctuations within a relatively narrow range throughout storage (Fig. 6D), with the initial phase showing minimal divergence between treated and control fruit. However, prolonged storage resulted in systematically higher pH values in PPA-treated samples, potentially mediated through suppressed microbial proliferation and consequent reduction in acid generation on fruit surfaces, though statistical analysis indicated these differences were not significant ($p > 0.05$). These results suggest that while PPA treatment exhibits a directional influence on pH modulation through microbial suppression, the intervention does not fundamentally alter the intrinsic acid-base equilibrium of strawberry tissues, consistent with previous findings regarding plasma-based treatments [23]. Overall, the results suggest PPA treatment primarily affects secondary microbial-mediated acid production rather than inducing substantial modifications to the primary biochemical parameters governing strawberry pH characteristics.

Postharvest respiratory metabolism in strawberries, a critical physiological process for maintaining cellular functions [30], was significantly modulated by PPA treatment as evidenced by characteristic gas composition changes showing progressive $O_2$ depletion and $CO_2$ accumulation (Fig. 7). These results were consistent with established respiratory patterns in strawberry fruit [28]. Comparative gas analysis revealed significantly higher $O_2$ and lower $CO_2$ levels in PPA-treated samples compared to control ($p < 0.05$). The PPA30 treatment exhibited optimal respiratory suppression, achieving 40% inhibition of respiratory activity. In addition, PPA treatment showed antimicrobial effect, reducing the number of microorganisms on the surface of strawberries, decreasing the respiratory metabolic activity of microorganisms, and indirectly reducing the production of $O_2$ and the consumption of $CO_2$ inside the package. Similarly, Du *et al.* (2024) [34] also showed that plasma treatment inhibited wolfberries respiration during storage.

Ethylene, is a phytohormone that promotes the respiration of fruits and vegetables, and accelerates ripening and senescence, affecting the quality of fruits during storage [28]. During the storage period, the ethylene content of strawberries showed an increasing trend, regardless of the treatment (Fig. 8A). The PPA15 and PPA30 treated strawberries showed significantly lower ($p < 0.05$) ethylene content compared to the control group, with up to 15.9% of the ethylene content being inhibited from being released. The PPA60 treatment showed a higher peak of ethylene, which may be attributed to the prolonged PPA treatment, that might have triggered a stress the stress response of from

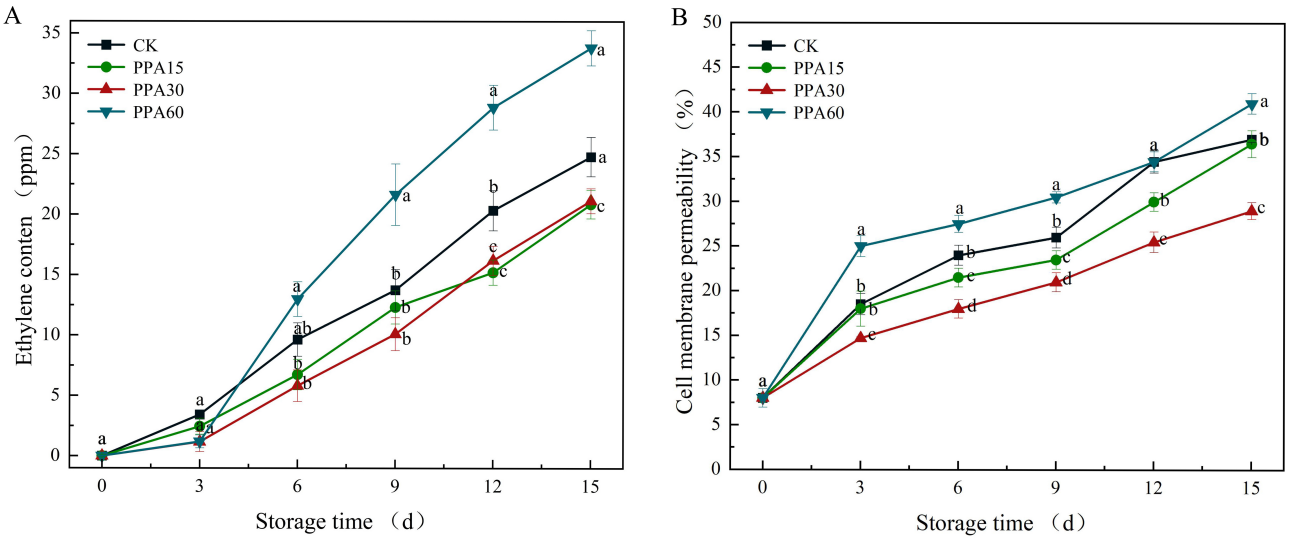


**Fig. 8. Effect of different PPA treatments on ethylene production (A) and cell membrane permeability (B) of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b, c and d" are used to indicate the differences between the averages of the samples on the days of measurement.

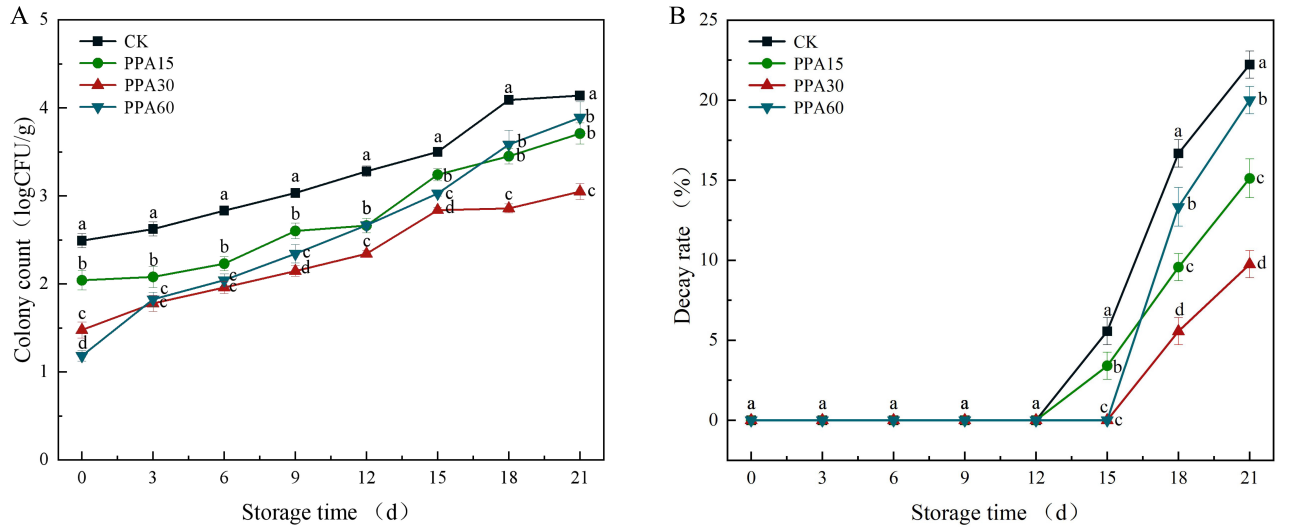


**Fig. 9. Effect of different PPA treatments on colony count (A) and decay rate (B) of strawberries during cold storage.** CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b, c and d" are used to indicate the differences between the averages of the samples on the days of measurement.

strawberries and thus leading to the synthesis of a higher amount of ethylene in response to the external stimuli. PPA delays strawberry ripening by reducing ethylene production through the suppression of respiration, metabolism, and other physiological activities. However, excessively prolonged PPA treatment may negatively impact the physiological state of the fruit.

Cell membrane permeability, which serves as a critical indicator of cellular integrity and senescence progression in fruit, exhibited a characteristic increase during storage (Fig. 8B), reflecting oxidative damage [35]. The integrity of the cell wall provides a stable environment for the selective permeability of the cell membrane. Therefore, our results suggest that the integrity of the cell wall of strawberries was disrupted during storage. Statistical analysis showed significant differences ($p < 0.05$) in membrane permeability between PPA-treated strawberries, with PPA15 and PPA30 treatments demonstrating reductions of 1.4% and 21.6%, respectively. These results that the plasma treatment plasma-induced microbial inactivation and subsequent attenuation of oxidative stress. Conversely, PPA60 treatment resulted in 10.8% higher membrane permeabil-

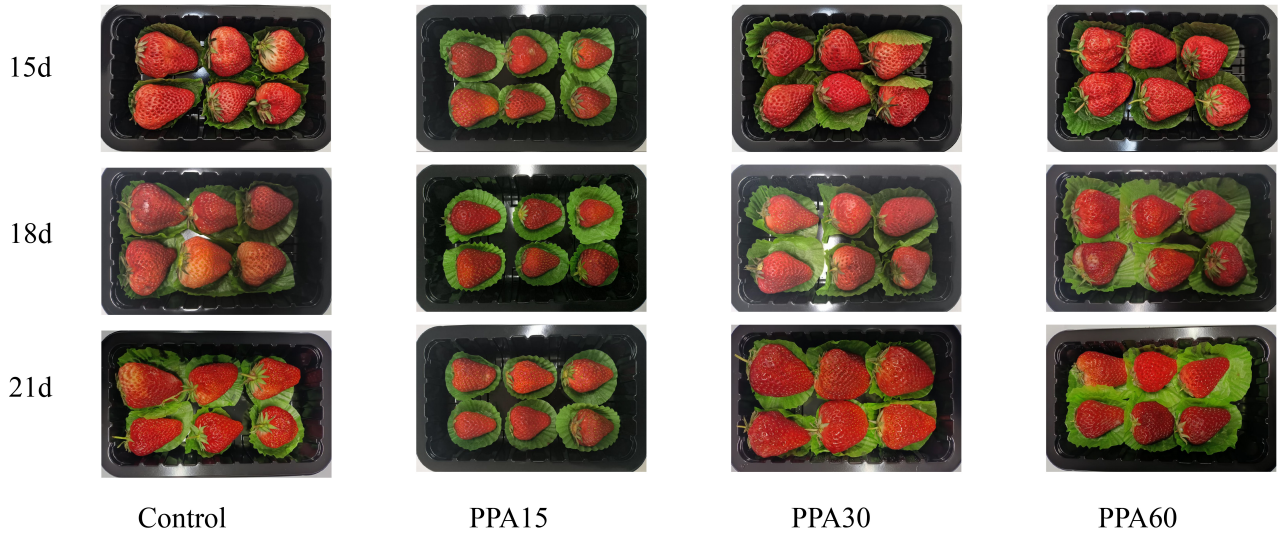


**Fig. 10. Strawberry samples rot with PPA treatment and storage time.**

ity than the control, revealing a dose-dependent duality of plasma effects. That is, while moderate exposure (15–30 s) seemed to preserve membrane integrity through microbial suppression, prolonged treatment (60 s) induced cellular damage through reactive oxygen and nitrogen species-mediated membrane peroxidation, consistent with previous reports of plasma-induced cellular damage at excessive exposure durations [36].

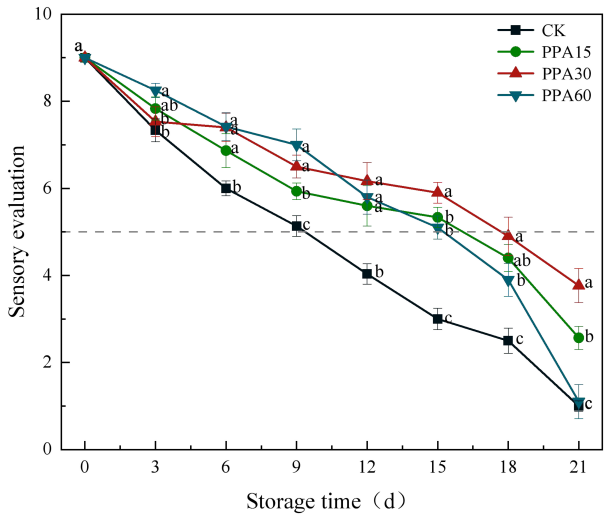


**Fig. 11. Effect of different PPA treatments on sensory quality of strawberries during cold storage.** Dash line (rating of 5) represents minimum quality acceptability. CK, control; PPA15, PPA treatment for 15 s; PPA30, PPA treatment for 30 s; PPA60, PPA treatment for 60 s. Different letters indicate significant differences ($p < 0.05$), "a, b and c" are used to indicate the differences between the averages of the samples on the days of measurement.

The microbial load on strawberry surfaces, is a critical food safety concern and primary contributor to postharvest decay [37]. *Botrytis cinerea*, also known as gray mold, one of the primary fungus causing strawberry spoilage, and responsible for most of postharvest losses. In this study, the number of colonies on the surface of strawberries showed a gradual increase during storage regardless of the treatment (Fig. 9). However, a significant growth suppressio was observed in PPA-treated fruit compared to the control ($p < 0.05$). The microbial inhibitory effect of PPA treatment was mainly attributed to reactive oxygen and nitrogen species (ROS/RNS) in non-thermal plasma, which disrupt microbial cell membrane integrity, leading to intracellular content leakage and suppressed proliferation [11,38].

PPA30 reduced the total colony count by 1.09 log units (91.85% lower than the control group) (Fig. 9A), with a final microbial load of 3.04 log CFU/g. This indicates effective growth inhibition, but it did not meet sterilization standards, as the reduction did not reach the 6-log threshold required for sterilization. Reactive oxygen species/reactive nitrogen species (ROS/RNS) primarily damage cell membranes rather than causing complete cell disintegration. As shown by Giannoglou *et al*., 2021 [23], total bacterial counts decreased by 0.6 log levels after cold plasma treatment. The mechanism of action of PPA aligns with microbial inhibition rather than sterilization, consistent with the plasma-induced cell membrane damage mechanism. Rana *et al*., 2020 [22] also confirming the effectiveness of PPA in controlling microbial growth.

The decay rate analysis revealed significant preservation effects of PPA treatment, with treated strawberries exhibiting delayed onset and reduced severity of decay compared to the control (Fig. 9B, Fig. 10). While decay initiated on day 15 in both control and PPA15 treated fruit, PPA30 and PPA60 treatments extended the decay-free period to

day 18, demonstrating temporal extension of fruit quality (Fig. 11). Quantitative assessment showed PPA30 achieved optimal efficacy with 56.1% decay reduction, surpassing PPA15 (32.0%) and PPA60 (10.0%), revealing a non-linear relationship between treatment duration and preservation outcomes. That is, insufficient exposure (PPA15) allowed for residual microbial proliferation, while excessive exposure time (PPA60) induced cellular damage compromising fruit defense mechanisms, as evidenced by accelerated late-stage decay. These findings align with established plasma research showing positive correlation between treatment duration and antimicrobial efficacy [39], while supporting observations of potential tissue damage from prolonged PPA exposure [36]. Results from our study highlight the critical importance of optimizing treatment parameters to balance immediate antimicrobial effects with preservation of host resistance mechanisms for maximal postharvest benefit.

Sensory evaluation, as a comprehensive assessment index, integrates a variety of sensory experiences to reveal the overall quality of fruits and vegetables during handling and storage, and has great significance to the commerciality of strawberries [40]. Sensory evaluation revealed a progressive deterioration in strawberry quality during storage (Fig. 11), with PPA-treated strawberries demonstrating significantly ($p < 0.05$) superior sensory attributes compared to untreated fruit. These results suggest that PPA treatment had a good effect on maintaining the quality characteristics of strawberries during storage. The shelf-life analysis revealed substantial extension in PPA-treated strawberries. With PPA30 treated fruit exhibiting optimal performance by doubling the storage duration (18 days) relative to the control (9 days), while PPA15 and PPA60 achieved intermediate preservation (15 and 16 days respectively). This preservation effect stems from dual mechanisms, that is, physiological regulation through suppression of respiratory metabolism and ethylene biosynthesis, coupled with antimicrobial action that collectively delay senescence and maintain organoleptic properties (Fig. 12) The non-linear response to treatment duration, characterized by maximal

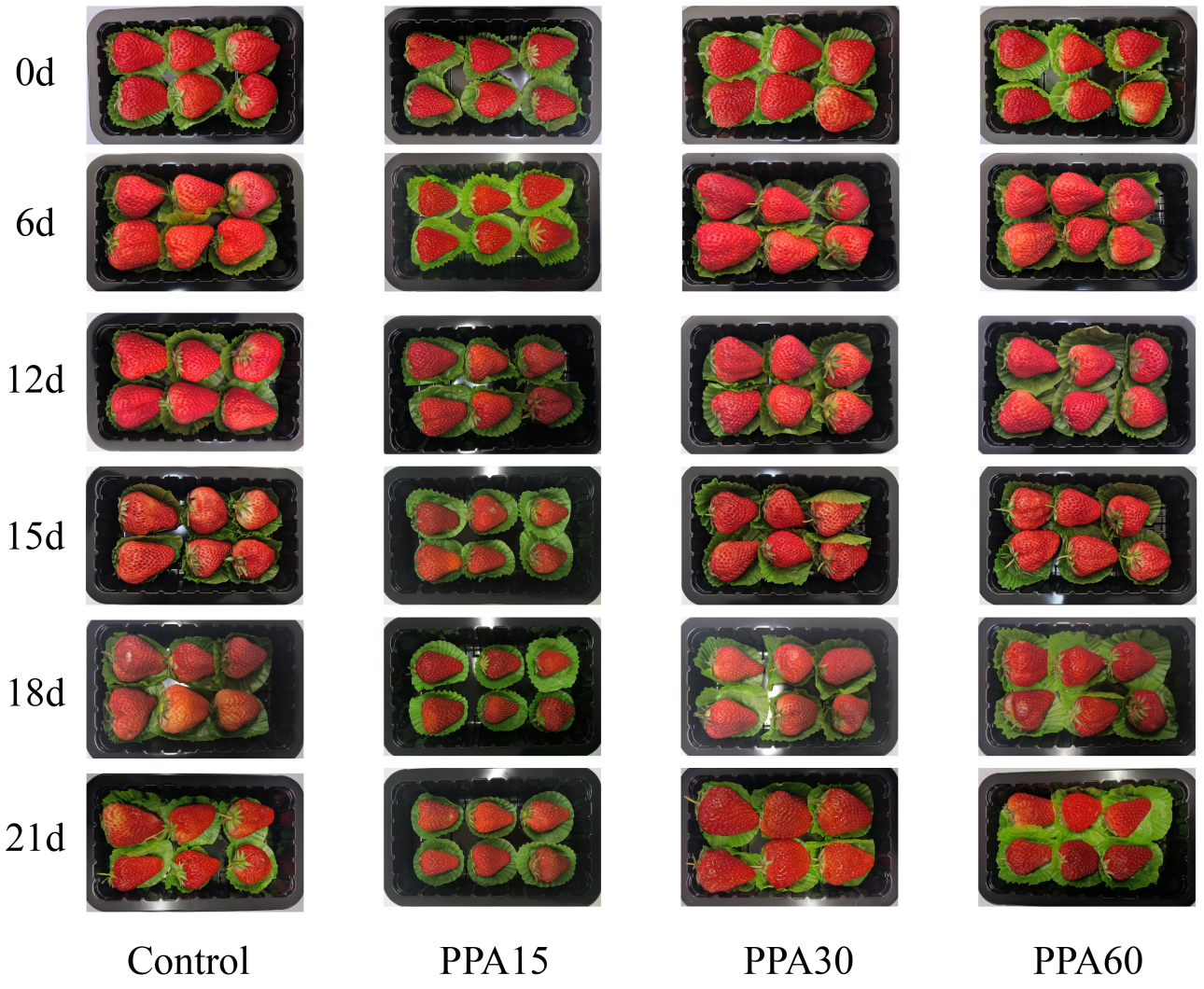


**Fig. 12. Effect of different treatments on strawberry storage periods.**

efficacy at intermediate exposure (PPA30), suggests an optimal balance between beneficial physiological modulation and potential oxidative stress, highlighting the importance of precise parameter optimization in plasma applications for postharvest management.

## 4. Conclusions

PPA treatments significantly inhibited microbial growth and extended the shelf life of strawberries ($p < 0.05$). The results showed that PPA30 (30 s exposure) achieved the best preservation effect. The colony counts in the PPA-treated fruit remained lower than those in the untreated strawberries throughout the storage period. Insufficient exposure (PPA15) caused residual microorganisms to proliferate, while excessive treatment (PPA60) caused cell damage, thereby weakening the fruit's defense mechanisms. That is, within a certain range, treatment time is positively correlated with antibacterial effect, but long-term exposure may cause tissue damage. This correlates with extended shelf life: 15 days (PPA15), 18 days (PPA30), and 16 days (PPA60) at 4 °C, establishing PPA30 as the most effective treatment. Compared to the control, treated strawberries maintained good firmness and weight without affecting cell membrane integrity or primary chemical components ($p > 0.05$). Notably, regulation of ethylene biosynthesis and color stability were observed, indicating that PPA might have a dual role in microbial control and delaying aging. As a non-thermal and energy-efficient technology, PPA may help achieving efficient microbial inactivation during transportation while preventing from secondary contamination. Combined with its energy efficiency and lack of thermal damage, it can maintain fruit quality parameters (including texture, surface color, and metabolic activity), making it particularly suitable for heat-sensitive agricultural products. This makes PPA a strong alternative to traditional preservation methods. These findings confirm the industrial application potential of PPA in fresh fruit preservation. In addition, the device is environmentally friendly and pollution-free, does not contain any chemical additives, and uses only electricity as its energy source, giving it a cost advantage. It can be used in combination with modified atmosphere packaging, confirming the industrial application potential of PPA in fresh fruit preservation. Future research should focus on optimizing operational procedures and further exploring the molecular-level interactions between plasma components and fruit tissue.

## Availability of Data and Materials

Data are available from the author on request.

## Author Contributions

CYW and YBF and LL designed the research study. CYW and YBZ and YQL and YMZ performed the research. CYW and JZS and YBZ analyzed the data. CYW wrote the manuscript. All authors have contributed to the editorial changes made to the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.


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Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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