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Original article

Effect of Plasma Activated Water on Microbiological Quality and Shelf-life of Strawberries

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Abstract

This study investigated the potential of plasma-activated water (PAW) as a non-thermal decontamination method for strawberries. PAW was produced by exposing potable water to dry air atmospheric plasma under various conditions (1 kVa, 10-40 min). Three PAW samples with different pH values (2.5, 2.75, and 3.0) were characterized by monitoring their pH, electrical conductivity, oxidation-reduction potential (ORP), and the concentrations of hydrogen peroxide, nitrite, and nitrate during 3 weeks of storage. The antimicrobial efficacy of PAW was evaluated against *Escherichia coli* ATCC 25922 and Botrytis cinerea in suspension. PAW with pH 2.5 exhibited the strongest antimicrobial activity, reducing *E. coli* and *B. cinerea* populations by up to 7 log CFU mL-1 and 5 log CFU mL-1, respectively, after 30 minutes of contact in suspension. The storage study demonstrated that PAW-treated strawberries maintained significantly lower microbial counts throughout 7 days of storage at 4 °C. On strawberry surfaces, a 15-minute treatment with PAW (pH 2.5) reduced *E. coli* and *B. cinerea* populations by 3.13 and 1.99 log CFU g-1, respectively. The total aerobic psychrophilic bacteria count in PAW-treated strawberries increased by only 0.8 log CFU g-1 during storage, compared to 1.4 log CFU g-1 in control samples. Importantly, PAW treatment did not adversely affect physical and chemical quality attributes of strawberries, including pH, ascorbic acid content, total antioxidant activity, anthocyanin content, and color parameters. These findings demonstrate that PAW is an effective, environmentally friendly alternative to conventional chemical sanitizers for reducing microbial contamination on strawberries while preserving their quality during refrigerated storage.

Keywords: Strawberry, Plasma Activated Water, Decontamination, *Botrytis Cinerea, Escherichia Coli,* Nutritional Quality.

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INTRODUCTION

Strawberries have become one of the important horticultural crops because of their unique sensory charactersitics and nutritional value, during the last decades. Strawberries are rich source of vitamin A (60 IU/100g), C (30-120 mg/100g), B_1, B_3, phenolic compunds and minerals. Several studies indicate that strawberries have beneficial health effects such as anti-imflammatory and anticancer effects because of their high content of bioactive compounds. (Rana et al., 2023; Sadowska et al., 2020). However, their soft structure make them easily subject to mechanical damage and prone to microbiological perishability poses significant challenges In case of mechanical damage and contamination the fruit's high moisture content create an ideal environment for pathogen growth. The perishability of the strawberries raises concerns about food safety and postharvest spoilage (Lafarga et al., 2019; Machado-Moreira et al., 2019).

Strawberries are generally accepted as safebecause of their low pH (3.2-4.2) regarding food borne pathogens However, numerous studies have linked strawberries to foodborne illnesses caused by pathogens such as *Staphylococcus aureus*, *Escherichia coli* 0157:H7, *Salmonella* spp. Fungal species especially *Botrytis cinerea* are important in fruit decay These microbial contaminants not only cause health risks but also contribute to important economic losses (Delbeke et al., 2015; Dziedzinska et al., 2018; Ozbudak et al., 2025; Yin et al., 2022).

Traditional decontamination methods for fruits often rely on chemical sanitizers like chlorinated water, chlorine dioxide, and organic acids. However, these traditional fruit decontamination treatments usually result in less than a 2-log reduction of microbial load. Besides, chemical sanitizers may produce harmful by-products that threaten human health and the environment (Lafarga et al., 2019; Wei et al., 2017). Heat treatments effectively lower microbial populations but tend to degrade both the sensory and nutritional attributes of fruit products (Polak et al., 2024). Due to these limitations, there is a need for developing novel efficient and environmentally friendly decontamination methods.

Plasma Activated Water (PAW) is a promising non-thermal food preservation method. PAW is generated by exposing water to cold plasma. During the PAW production reactive species such as hydrogen peroxide (H_2O_2) and reactive oxygen and nitrogen species (ozone, nitrites and nitrates) are formed. These reactive species are the main components responsible for PAW's antimicrobial effects. These species can interact with microbial cell components such as membranes, proteins, and DNA, leading to their inactivation (Ghimire et al., 2024; Wang et al., 2021). The antimicrobial effect of PAW may vary according to factors such as plasma exposure duration, final pH, concentration of the reactive species (Chiappim et al., 2021). Apart from microbial inactivation, PAW has been used for various applications, including enzyme inactivation, pesticide degradation, decomposition of organic compounds and toxins, enhancement of germination and plant growth (Brisset & Pawlat, 2016; Crema et al., 2020; Mogo et al., 2022; Sojithamporn et al., 2023; Stoleru et al., 2020; Xu et al., 2016).

Studies have shown that PAW treatment can effectively reduce microbial loads on fresh produce without significantly altering their nutritional and sensory qualities (Laurita et al., 2021; Xiong et al., 2024). Dolezalova and Lukes (2015) reported that direct interaction between plasma and liquid significantly alters and enhances the formation of reactive species and microbial inactivation mechanisms. In another study investigating the microbial inactivation mechanism of PAW, a 20-minute plasma treatment within the liquid phase resulted in a 5-log CFU/mL reduction in microbial load (Tian et al., 2015). In contrast, PAW produced by plasma application to the water surface only achieved a 1.2-log CFU/mL reduction. These findings indicate that PAW generated within the liquid phase exhibits significantly higher inactivation efficiency compared to PAW produced at the water surface (Lukes et al., 2012).

For strawberries specifically, PAW treatment has demonstrated potential in maintaining firmness, color, and overall quality during storage (Yang et al., 2023). Unlike conventional chemical sanitizers, PAW is environmentally friendly as it reverts to water without leaving harmful residues, aligning with consumer demand for safer and more sustainable food processing methods (Royintarat et al., 2020; Thirumdas et al., 2018). PAW could be a good alternative to direct atmospheric plasma treatment of foods. In contrast to direct cold atmospheric plasma treatments, PAW has distinct advantages due to its composition and fluidity, which allow reactive species to uniformly interact with microorganisms on irregular surfaces, resulting in enhanced antimicrobial efficacy. Its ease of use, absence of secondary pollution, and cost-effectiveness position PAW as a promising alternative to conventional disinfectants (Z. Xu et al., 2020). Recent studies have highlighted its potential not only in food preservation and surface decontamination but also in the sterilization of medical devices and treatment of wounds with bacterial infections (Laurita et al., 2021; D. Xu et al., 2020; Zhang et al., 2016).These varied applications and safety advantages underscore the relevance and novelty of investigating PAW as a practical, non-thermal solution for microbial control in fresh produce.

This study aimed to investigate PAW's effect on the microbiological quality of strawberries during storage. The specific aims were to (1) characterize the physicochemical properties of PAW at different production parameters, (2) assess the antimicrobial efficacy of PAW against common strawberry spoilage and pathogenic microorganisms, (3) determine the optimal PAW application conditions for strawberry decontamination, and (4) evaluate the impact of PAW treatment on the physical and chemical quality attributes of strawberries during storage.

MATERIALS And METHODS

PAW Production and Characterization

PAW was produced using an atmospheric dry air atmospheric pressure plasma jet system (Plasmatreat GmbH, Steinhagen, Germany), which comprised a 1 kVA plasma generator (FG5001), a

rotary plasma nozzle (RD2004), a high-voltage transformer, and a pressure supply control unit. Potable water (250 mL, initial pH 8.03) in a glass beaker exposed to plasma jet directed above the water surface for durations ranging from 10 to 40 minutes. The plasma jet to water level distance was 4 cm. To enhance the distribution of plasma-generated reactive species and improve the overall efficiency of the plasma treatment, a magnetic stir bar was placed inside the beaker containing the potable water and continuously rotated at 500 rpm using a magnetic stirrer (RCT Basic, IKA, Staufen, Germany) throughout the plasma exposure period. In addition, the water temperature was continuously monitored using a digital thermometer (30.1048, TFA Dostmann, Germany).

The quality parameters of the produced PAW samples, including pH, oxidation-reduction potential (ORP), electrical conductivity, and concentrations of hydrogen peroxide, nitrate, and nitrite, were measured immediately after production and monitored every week (1^{st}, 7^{th}, 14^{th}, and 21^{st} day) during a 3-week storage period at room temperature.

pH Measurement of PAW samples

The pH values of PAW samples were determined using a digital pH meter (SG-2 SevenGo, Mettler-Toledo, Switzerland). All measurements were performed at room temperature and the pH meter was calibrated with standard buffer solutions (pH 2.00, 4.01 and 7.00) before measurements.

Oxidation-Reduction Potential (ORP)

ORP measurements (mV) were performed using a pH meter (SG-2 SevenGo, Mettler-Toledo, Switzerland) equipped with an ORP electrode (51343200 ORP electrode, Mettler-Toledo, Switzerland) at room temperature. The meter was calibrated using a standard redox buffer (51350060 Redox buffer Mettler-Toledo, Switzerland).

Electrical Conductivity Measurement

Electrical conductivity of PAW samples were measured using a conductivity meter (HI 9812-s Hanna Instruments,).

Hydrogen Peroxide, Nitrate, and Nitrite Concentration Measurement

Concentrations of nitrate, and nitrite in PAW samples were determined using a water spectrophotometer (DR3900, Hach-Lange, Düsseldorf, Germany) using LCK340 Nitrate and LCK 341 nitrite test kits. Measurements were performed according to the manufacturer's instructions. Hydrogen peroxide concentration was measured by MQuant® Peroxide Test strips (Merck, Darmstadt, Germany).

Antimicrobial Efficacy Assessment of PAW

Test Microorganisms

The antimicrobial efficacy of PAW was evaluated against two test microorganisms: *Escherichia coli* ATCC 25922 (as a model for bacterial contamination) and *Botrytis cinerea* (as a model for fungal contamination). *B. cinerea* spore suspension was prepared by surface washing of a 5-day culture grown on Potato Dextrose Agar (PDA) at 25°C with 0.5% Tween 80 solution. The suspension was centrifuged at 7500 g for 10 minutes at 4°C, and the pellet was resuspended in 10 mL of 20% glycerol solution. The spore concentration was determined by plating serial dilutions on PDA using the spread plate method and counting colonies after 5 days of incubation at 25°C.

Evaluation of PAW's Antimicrobial Activity in Suspension

E. coli and *B. cinerea* spore suspension (approximately 7 log CFU/mL) were exposed to PAW samples with different pH values (2.5, 2.75, and 3.0) for various contact times (5, 15, and 30 minutes). After treatment, appropriate dilutions were prepared using Maximum Recovery Diluent, and viable counts were determined using the pour plate method with suitable media (TBX agar for *E. coli* and PDA for *B. cinerea*). The reduction in microbial load was calculated by subtracting the log CFU/mL after PAW treatment from the initial log CFU mL^{-1}.

Determination of Optimal PAW Application Time for Strawberries

Fresh strawberries were purchased from a local market in Edirne, Turkey. The fruits were washed with sterile distilled water and surface-inoculated with *E. coli* and *B. cinerea* cultures (approximately 5 log CFU g^{-1}).

For the spray inoculation of strawberries, the stock culture of *E. coli* was activated in tryptic soy broth at 37 °C for 24 h before the preparation of the inoculum. Cells in the subculture were separated by centrifugation (10,000 g, 10 min) (3 K30, Sigma, Osterode am Harz, Germany) at 4 °C. Pellets were washed and centrifuged twice in sterile physiological saline. E. coli cells were resuspended in physiological saline to obtain approxi-mately 9 log CFU mL−1. Before inoculation *E. coli* suspension and *B. cinerae* spore suspension were mixed in a spray bottle (0309-1100, Bürkle, Bad Bellingen, Germany). Strawberries were then sprayed with a spray bottle for inoculation. After inoculation, samples were drained for 1 h under at room temprature to remove excess inoculum.

After inoculation, the strawberries (200 g) were immersed in pH 2.5 PAW (500 mL) for different contact times (5, 15, and 30 minutes). Control samples were immersed in sterile water for the same durations. Following treatment, the microbial load on strawberry surfaces was enumerated to determine the most effective PAW application time.

PAW Treatment of Strawberries and Microbiological And Physicochemical Quality During Storage

For the PAW treatment 200 g strawbery samples were immersed in 500 mL for 15 min. For the control samples, 200 g strawberry samples were immersed in sterile distilled water for 15 min. After the tretment, excess water on the surface of the samples were removed with absorbent paper gently and the samples were air dried for 30 min at room temperature. After drying, the samples were packaged in polyethylene terephthalate (PET) boxes. All samples were stored at 4 °C for 7 days, and the following parameters were monitored at regular intervals (days 0, 2, 4, and 7):

Microbiological Analysis

Total Aerobic Psychrophilic Bacteria Count

Appropriate dilutions of strawberry samples were prepared and plated on Plate Count Agar. The plates were incubated at 4°C for 7 days, and the results were expressed as log CFU g^{-1}.

Total Mold and Yeast Count

Appropriate dilutions were plated on Potato Dextrose Agar and incubated at 30°C for 3-5 days. Results were expressed as log CFU g^{-1}.

Physicochemical Quality Assessment

pH Determination of Strawberries

To obtain the juice of strawberry samples, the samples were crushed in a homogenizer. Then, the pH of strawberry juice was measured using a pH meter (Mettler Toledo, Switzerland).

Surface Color Analysis

Color measurements of strawberries were performed using a colorimeter (CR-5, Konika-Minolta, Japan) according to the Commission Internationale de l'Eclairage L*a*b* (CIELAB) color scale. The total color difference (ΔE) between treated and control samples was calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$$

where ΔL*, Δa*, and Δb* represent the differences in L*, a*, and b* values between samples. Three different zones were measured for each strawberry (Duran et al., 2016).

Ascorbic Acid (Vitamin C) Analysis

Ascorbic acid content in strawberry samples was determined according to the method proposed by Giovanelli et al. (2002). Samples were homogenized with 1% metaphosphoric acid solution (10 mL per 1 g of sample) and centrifuged at 11,000 g for 10 minutes at 4°C. The supernatant was filtered through a 0.45 µm filter and analyzed using HPLC with the following conditions:

- Column: C-18 (250 × 4.6 mm ID, 5 µm)
- Flow rate: 0.5 mL/min
- Detection wavelength: 254 nm
- Mobile phase: Water pH 3 (adjusted with H_3PO_4)
- Column temperature: 35°C
- Injection volume: 20 µL

Ascorbic acid content was calculated using a standard curve and expressed as mg/kg fresh weight.

Total Antioxidant Activity Analysis

Antioxidant activity was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) method as described by Re et al. (1999). The decrease in absorbance at 734 nm was measured after mixing 30 µL of sample with 3 mL of ABTS radical solution for 6 minutes. Trolox calibration curve was used for quantification and the results were expressed as µmol Trolox/mL.

Total Anthocyanin Analysis

Total monomeric anthocyanin content was determined using the pH differential method as described by Fuleki and Francis (1968). Absorbance was measured at 516 nm and 700 nm in buffers at pH 1.0 and pH 4.5. The anthocyanin content was calculated using the following formula:

Total anthocyanin (mg/L) = (A × 1000 × MW × DF) / (ε × L)

where A = (A_{516} - A_{700}) pH 1.0 - (A_{516} - A_{700}) pH 4.5, MW = molecular weight of the predominant anthocyanin, DF = dilution factor, ε = molar absorptivity, and L = path length (1 cm).

Pelargonidin-3-Glucoside is the the main anthocyanin present in strawberry (MW: 434.4 g mol-1, ε=22 390 L mol^{-1} cm^{-1}) (Liu et al., 2024). The results were expressed per kg of fresh weight strawberries.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to determine significant differences between treatments, and Tukey's multiple range test was used to separate means at a 5% significance level ($p < 0.05$). Also, A paired t-test was conducted to evaluate the statistical difference between the two conditions. The assumption of normality was assessed using the Shapiro-Wilk test. If normality was satisfied ($p > 0.05$), a paired t-test was performed.

RESULTS and DISCUSSION

Production and Characterization of PAW

PAW Temperature

Temperature variations during PAW production were monitored using a thermometer. To prevent system overheating, the plasma device was operated for 10 minutes followed by a 10-minute rest period. The water used for PAW production was maintained at room temperature, with an initial temperature of 22.2 °C (Table 1). The PAW temperature increased progressively with exposure time, reaching 46.5 °C after 40 minutes of plasma treatment.

Table 1. Change in PAW temperature during production.

Measurement Time	PAW Temperature
0 min	22.2 ± 0.5 °C
10 min	31.0 ± 1.4 °C
20 min	38.8 ± 1.3 °C
30 min	44.2 ± 2.1 °C
40 min	46.5 ± 2.5 °C

The observed temperature increase during PAW production is consistent with findings from other studies. This thermal effect is attributed to the energy transfer from the plasma discharge to the water (Traylor et al., 2011). The temperature rise may influence the formation of reactive species in PAW and consequently its antimicrobial efficacy. The effectiveness of PAW decreases with increasing preparation temperature due to reduced reactive oxygen and nitrogen species stability and solubility, highlighting that higher temperatures negatively impact PAW quality (Zhou et al., 2024).

Physicochemical Properties of PAW During Storage

PAW samples with different pH values (2.5, 2.75, and 3.0) were produced by varying the plasma treatment time. PAW with pH 2.5, 2.75, and 3.0 were obtained after approximately 40, 30, and 20 minutes of plasma treatment, respectively. For subsequent production, rather than using fixed exposure times, plasma treatment was continued until the target pH was reached, as the time required to achieve a specific pH was found to depend on the initial water volume.

The physicochemical properties of PAW samples, including pH, electrical conductivity, oxidation-reduction potential (ORP), and concentrations of hydrogen peroxide, nitrite, and nitrate, were monitored during a three-week storage period (at 4 °C) (Table 2).

Table 2. Changes in PAW quality parameters during three weeks of storage.

Parameter	**PAW**	**Days**			
		1	**7**	**14**	**21**
pH	pH 2.50	2.32±0.05	2.34±0.09	2.24±0.12	2.26±0.03
	pH 2.75	2.50±0.06	2.51±0.04	2.41±0.06	2.41±0.14
	pH 3.00	2.68±0.07	2.67±0.08	2.62±0.11	2.56±0.07
Conductivity (µs cm^{-1})	pH 2.50	1580±10	880±23	930±9	900±14
	pH 2.75	1030±23	630±17	620±18	630±12
	pH 3.00	710±18	500±21	490±10	460±24
ORP (mV)	pH 2.50	609.3±2.7	607.5±5.9	613.2±1.9	611.5±2.2
	pH 2.75	597.5±3.4	597.5±8.1	600.9±1.5	593.9±1.6
	pH 3.00	586.4±4.2	586.7±1.6	590.0.±1.1	508.9±3.3
H_2O_2 (mg L^{-1})	pH 2.50	0.5±0.0	0.5±0.0	0.5±0.0	-
	pH 2.75	0.5±0.0	0.5±0.0	0.5±0.0	-
	pH 3.00	0.5±0.0	0.5±0.0	0.5±0.0	-
Nitrite (mg L^{-1})	pH 2.50	15.2±1.3	15.4±1.7	4.4±0.5	12.6±1.4
	pH 2.75	8.4±1.1	11.5±0.6	4.4±0.2	2.5±0.3
	pH 3.00	15.6±0.8	8.2±1.2	3.3±0.4	1.5±0.4
Nitrate (mg L^{-1})	pH 2.50	417±6.3	480±12.1	480±9.8	463±5.0
	pH 2.75	310±1.3	343±2.5	335±7.6	346±8.2
	pH 3.00	222±4.8	264±7.2	257±9.3	270±6.4

The pH values of all three PAW samples (initial pH 2.5, 2.75, and 3.0) exhibited slight but consistent decreases over the storage period. For instance, the pH 2.5 sample decreased from 2.32 to 2.26, while the pH 3.0 sample declined from 2.68 to 2.56. These minor reductions are in agreement with Zhang et al. (2024) who reported that PAW maintained relatively stable pH levels during 30-day storage under refrigerated conditions. At cold storage tempratures (4 °C) chemical reactions are slowed down helping to maintain the pH stable over time.

Electrical conductivity showed a more noticeable decline during the first week, particularly in the pH 2.5 sample, which dropped from 1580 to 880 µS/cm. After this initial decline, conductivity remained relatively stable. In contrast, Zhang et al. (2024) reported that conductivity remained nearly constant throughout their 30-day study. This discrepancy may be attributed to differences in plasma generation methods, gas composition, or initial water chemistry. The drop in conductivity observed in this study could reflect the gradual decomposition or transformation of ionic species generated during plasma activation.

The ORP values remained relatively stable over the entire storage period in all PAW samples, with only minor fluctuations (e.g., 609.3 to 611.5 mV for pH 2.5). This consistency suggests that the

oxidative potential of PAW is retained during storage, which is crucial for its sustained antimicrobial activity.

Hydrogen peroxide levels remained constant at 0.5 mg/L throughout the first 14 days, although data for day 21 were not available. The stability of H_2O_2 in PAW is highly time-dependent, with significant degradation occurring after 5 days of storage even at 4 °C, indicating that low temperatures only slow but do not prevent decomposition (Zhang et al., 2024). According to Sun et al. (2012), H_2O_2 concentration is halved in 8-20 hours.

Nitrite concentration in PAW samples were decreased during storage while nitrate concentration slightly increased. This observation can be explained by the conversion of nitrite to nitrate during storage (Lukes et al., 2014). Conversely, nitrate concentrations increased throughout the storage period in all samples. For example, in the pH 2.5 sample, nitrate levels rose from 417 to 463 mg/L. This observation mirrors the findings of Zhang et al. (2024), who also reported increasing nitrate levels during prolonged storage. The accumulation of nitrate likely results from the progressive conversion of nitrogen-based intermediates such as NO and NO_2^-, contributing to the long-term stability of PAW. The stability of key parameters such as ORP and nitrate reinforces its potential as a shelf-stable, non-thermal antimicrobial solution

Antimicrobial Effects of PAW

Effect of PAW on Planktonic Cells

The antimicrobial efficacy of PAW samples with different pH values (2.5, 2.75, and 3.0) against *E. coli* and *B. cinerea* were shown in Figure 1 and 2.

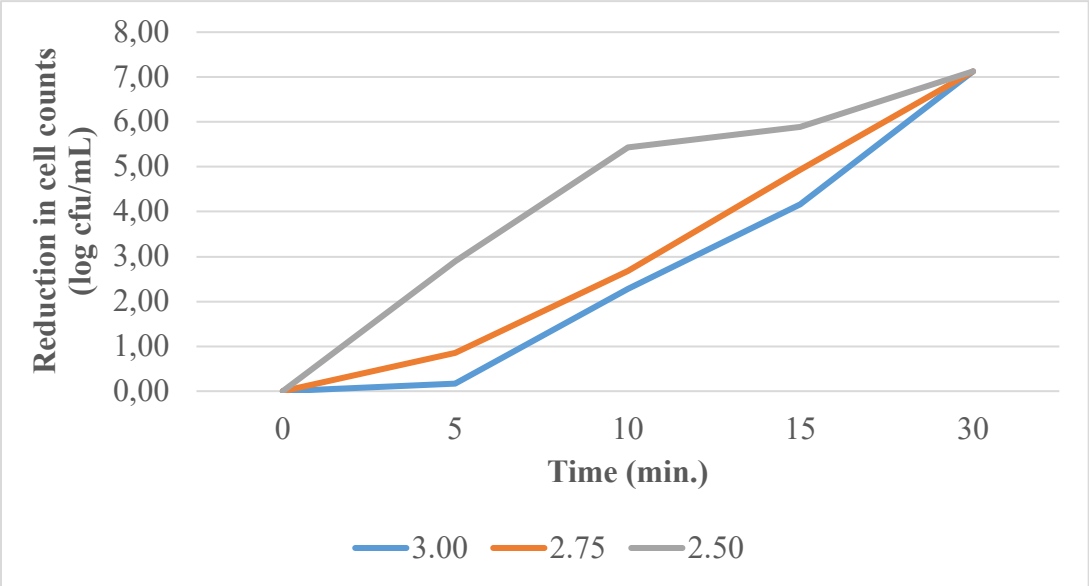

Figure 1. Effect of PAW's at different pH's on *E. coli* cells

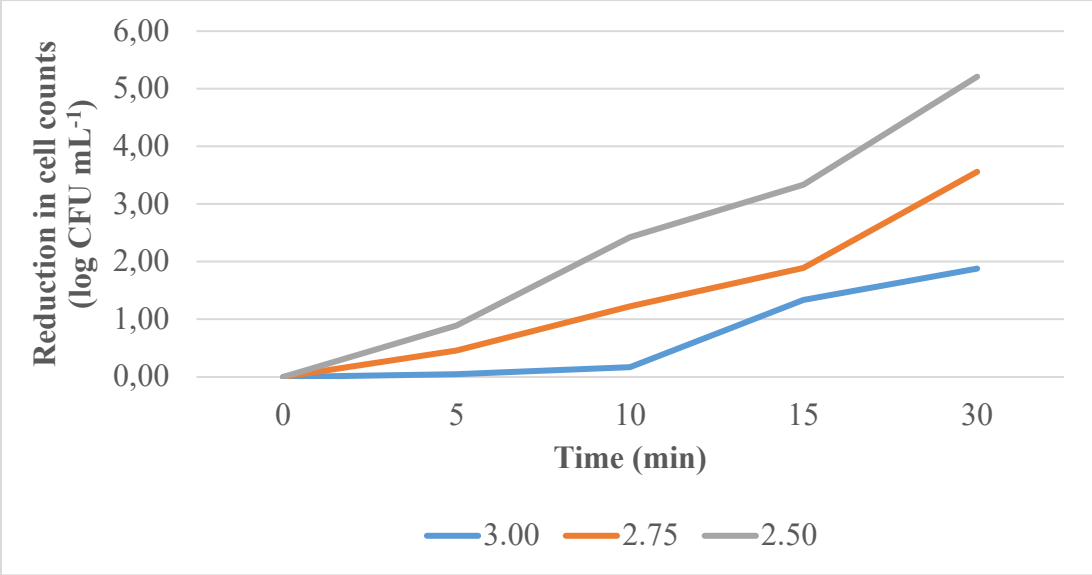

Figure 2. Effect of PAW's at different pH's on *B. cinerae* spores

The results showed that the antimicrobial efficacy of PAW increased with longer treatment times for *E. coli* and *B. cinerea*. pH 2.5 PAW sample exhibited stronger antimicrobial activity compared to pH 2.75 and pH 3.00 PAW samples. The resuts also showed that *E. coli* was more sensitive to PAW treatment than *B. cinerea*. Based on these results, pH 2.5 PAW was selected for further experiments due to its higher antimicrobial efficacy.

The higher antimicrobial effect at lower pH values can be attributed to the higher concentration of reactive species in more acidic PAW samples. The acidic environment could facilitates the penetration of reactive species into microbial cells by disrupting their membrane integrity (Shen et al., 2016). The difference in susceptibility between *E. coli* and *B. cinerea* is consistent with previous studies reporting that PAW is generally more effective against bacteria than fungi (Guo et al., 2022; Zhang et al., 2020). This difference can be explained by the complex composition of of fungal spore cell wall structure, which provides better protection antimicrobial agents.

Antimicrobial Effects of PAW on Inoculated Strawberries

The efficacy of pH 2.5 PAW in reducing the microbial load on strawberry surfaces was evaluated using strawberries artificially inoculated with *E. coli* and *B. cinerea* (approximately 6-7 log CFU/g). The inoculated strawberries were treated with pH 2.5 PAW for different contact times (5, 15, and 30 minutes), and the reduction in microbial load was determined. For *E. coli*-inoculated strawberries, the microbial load reductions after 5, 15, and 30 minutes of PAW treatment were 0.73, 3.13, and 3.60 log CFU/g, respectively (Figure 3). There was no statistically significant difference ($p > 0.05$) between the

reductions achieved after 15 and 30 minutes of treatment. Therefore, 15 minutes was selected as the optimal treatment time.

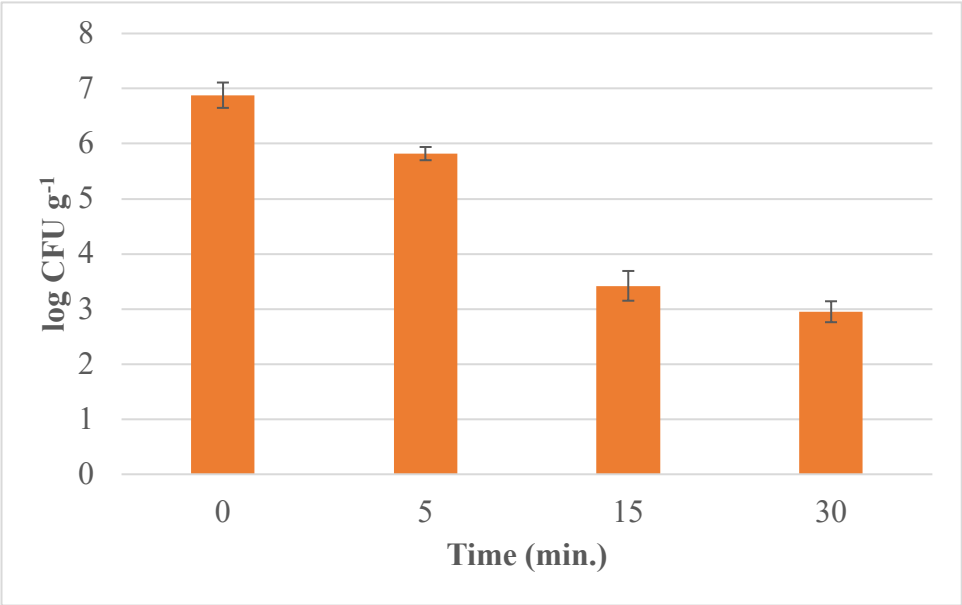

Figure 3. Antibacterial effect of PAW on strawberry samples inoculated with *E.coli*

For *B. cinerea*-inoculated strawberries, the microbial load reductions after 5, 15, and 30 minutes of PAW treatment were 0.49, 1.99, and 2.56 log CFU/g, respectively (Figure 4). Unlike *E. coli*, the differences in reduction between all treatment times were statistically significant ($p < 0.05$).

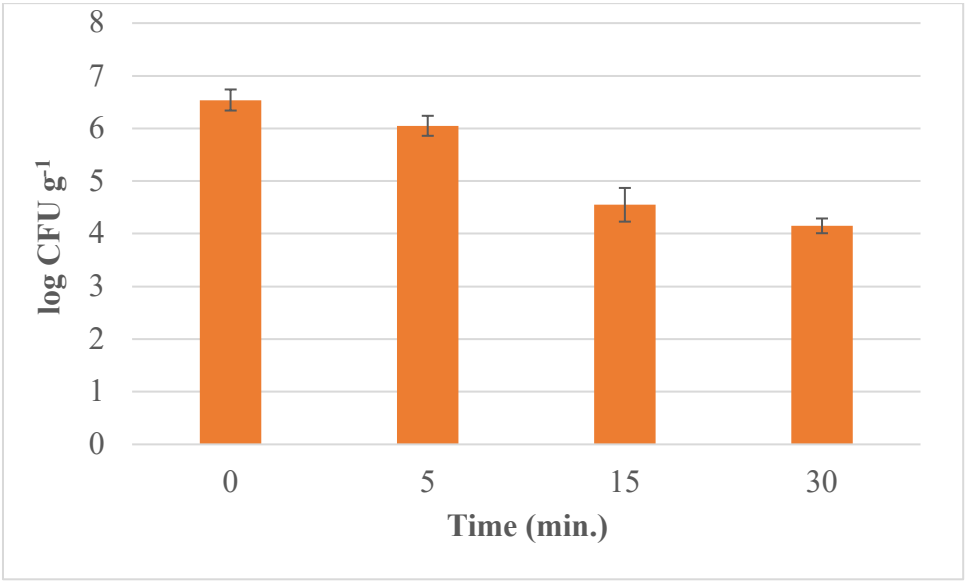

Figure 4. Antifungal effect of PAW on strawberry samples inoculated with *B. cinerea*

The effectiveness of PAW against *E. coli* on strawberry surfaces can be attributed to the reactive species present in PAW, particularly hydrogen peroxide, nitrate, and nitrite, which exert oxidative stress

on bacterial cells. The acidic nature of PAW (pH 2.5) likely enhances its antimicrobial activity by disrupting bacterial cell membranes and increasing their permeability to reactive species (Ghimire et al., 2024; Wang et al., 2021). The reduction levels observed in our study are comparable to those reported by Ortiz-Solà et al. (2022), who found that water-assisted UV-C treatment reduced Salmonella enterica by 2.2 log CFU/g on strawberries. Our results demonstrated that PAW treatment was more effective than traditional chlorine-based sanitizers, which typically achieve less than 2 log reductions on fruit surfaces (Lafarga et al., 2019; Wei et al., 2017).

In our study, *B. cinerea* was found to more susceptible to PAW treatment compared to *E. coli.* In general, spores are more resistant to antimicrobial treatments than vegetative cells due to differences in the structure of the cell wall. Fungi generally have more complex cell walls comprising chitin, glucans, and mannoproteins, which provide better protection against oxidative stress (Zhang et al., 2020). Our results align with previous studies reporting that PAW is generally more effective against bacteria comparing to fungi (Guo et al., 2022; Zhang et al., 2020). Although the antimicrobial efficacy against *B. cinerea* was limited, it is of great importance in extending the shelf life of strawberries. *B. cinerea* is a major cause of postharvest decay in strawberries, and reductions in its population can significantly extend shelf life by delaying the onset of visible mold growth (Petrasch et al., 2019).

Compared to planktonic cells, the antimicrobial effect of PAW was found to be lower on inoculated strawberries. The efficacy of antimicrobial agents or treatment is known to be higher in model systems than on food (Ortiz-Solà et al., 2022; Wei et al., 2017).

The lower microbial reduction observed on strawberry surfaces compared to planktonic cells can be attributed to several factors. The complex structure of the fruit surface can reduce the effect of PAW treatment on microorganisms on the surface. Additionally, organic substances in foods can react with antimicrobial agents and reduce the PAW's antimicrobial efficacy (Han et al., 2020).

Based on the results, PAW1 (pH 2.5) with a 15-minute contact time was selected as the optimal treatment condition for the shelf-life study. This treatment time provided significant reductions in both bacterial and fungal populations without being excessively long from a processing perspective.

Effect of PAW Treatment on Microbial Quality of Strawberries During Storage

The effect of the optimal PAW treatment (pH 2.5, 15 minutes contact time) on the microbial quality of strawberries during 1 week of storage at 4°C was evaluated by monitoring the total aerobic psychrophilic bacteria count and total mold and yeast count (Table 3).

Table 3. Changes in total aerobic psychrophilic bacteria count and total mold and yeast counts (log CFU g^{-1}) in PAW-treated and untreated strawberries during storage.

Sample	Days			
	0	**2**	**4**	**7**
Total aerobic psychrophilic bacteria (log CFU g^{-1})				
Untreated	4,61±0,19 ^{Aa*}	4,83±0,21 ^{Aa}	5,21±0,24 ^{Ba}	6,03±0,34 ^{Ca}
PAW treated	2,43 ±0,35 ^{Ab}	2,54±0,24 ^{Ab}	2,81±0,31 ^{Ab}	3,22±0,28 ^{Bb}
Total mold and yeast (log CFU g^{-1})				
Untreated	4.40±0.29 ^{Aa}	4.51±0.14^{Aa}	5.29±0.27 ^{Ba}	6.43±0.23 ^{Ca}
PAW treated	2.15±0.21^{Ab}	2.34±0.14 ^{Ab}	3.43±0.11 ^{Bb}	5.16±0.35 ^{Cb}

*Different uppercase letters in the same column indicate significant differences ($p < 0.05$) between treatments. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) between treatments.

The initial total aerobic psychrophilic bacteria count in control strawberries was 4.61 log CFU g^{-1}, which increased to 6.03 log CFU g^{-1} after 7 days of storage, representing an increase of approximately 1.4 log CFU g^{-1}. In contrast, PAW-treated strawberries had an initial count of 2.43 log CFU g^{-1}, which increased to 3.22 log CFU g^{-1}after 7 days, an increase of only about 0.8 log CFU/g. Throughout the storage period, the bacterial counts in PAW-treated strawberries were significantly lower ($p < 0.05$) than those in control samples.

Similar trends were observed for the total mold and yeast counts. The initial count in control strawberries was 4.40 log CFU g^{-1}, which increased to 6.43 log CFU g^{-1}after 7 days of storage, representing an increase of approximately 2 log CFU/g. In PAW-treated strawberries, the initial count was 2.15 log CFU g^{-1}, which increased to 5.16 log CFU g^{-1}after 7 days, an increase of about 3 log CFU g^{-1}. Despite the larger increase in mold and yeast counts in PAW-treated samples during storage, their counts remained significantly lower ($p < 0.05$) than those in control samples throughout the storage period.

The lower initial microbial counts in PAW-treated strawberries compared to controls demonstrate the immediate antimicrobial effect of PAW treatment. The slower microbial growth rate in PAW-treated samples during storage suggests that PAW treatment not only reduces the initial microbial load but also has a residual antimicrobial effect that persists during storage. This residual effect may be attributed to the continued action of reactive species that remain on the fruit surface after treatment. PAW has been shown to effectively inhibit the growth of various microorganisms, including bacteria, molds, and yeasts. For example, PAW treatment reduced the microbial load on fresh walnuts by 1.15 log CFU g^{-1} immediately after treatment and prevented microbial growth during storage (Xiao et al., 2023). Dallagi

et al. (2023) demonstrated that PAW treatment effectively delayed microbial growth in fresh-cut apple samples during refrigerated storage, with significantly lower total aerobic and yeast/mold counts observed in optimized activation conditions (e.g., PAW30) compared to control samples. These findings confirm that the antimicrobial activity of PAW can be maintained throughout storage, depending on the activation time and reactive species concentration.

The more pronounced increase in mold and yeast counts compared to bacterial counts during storage, particularly in PAW-treated samples, confirms that fungi are generally more resistant to PAW treatment than bacteria, as observed in our earlier experiments with inoculated strawberries.

Effect of PAW Treatment on Physical and Chemical Quality of Strawberries

Ascorbic Acid Content

The ascorbic acid (vitamin C) content of PAW-treated and control strawberries was measured during 7 day storage at 4 °C (Table 4).

Table 4. Changes in ascorbic acid, total antioxidant activity and total anthocyanin content of PAW-treated and control strawberries during storage.

Sample	Days			
	0	**2**	**4**	**7**
Ascorbic acid (mg kg^{-1})				
Untreated	518±3.48^{Aa*}	508±2.11^{Aa}	511±1.99 ^{Aa}	514±3.24 ^{Aa}
PAW treated	521±1.96^{Aa}	520±3.74^{Ab}	517±3.12 ^{Aa}	521±2.35 ^{Ab}
Total antioxidant activity (mmol kg^{-1})				
Untreated	1.25±1.02 ^{Aa}	1.39±0.56 ^{Aa}	1.35±0.59 ^{Aa}	1.03±0.85 ^{Aa}
PAW treated	1.27±0.87 ^{Aa}	1.41±0.72 ^{Aa}	1.33±1.04 ^{Aa}	1.06±1.12 ^{Aa}
Total anthocyanin content (mg kg^{-1})				
Untreated	324±5.24 ^{Aa}	318±2.12 ^{Aa}	331±2.62 ^{Aa}	312±4.11^{Aa}
PAW treated	321±3.06 ^{Aa}	323±1.85 ^{Aa}	327±3.21 ^{Aa}	317±2.52 ^{Aa}

*Different uppercase letters in the same column indicate significant differences ($p < 0.05$) between treatments. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) between treatments

No significant differences in ascorbic acid content were observed between PAW-treated and control strawberries throughout the storage period. The initial ascorbic acid content was approximately 520 mg/kg fresh weight, which remained relatively stable during storage in both groups.

Bozkurt (2014) reported that direct atmospheric plasma application in a model system resulted in a significant reduction in ascorbic acid content due to interactions with reactive species and UV radiation. However, in our study, PAW treatment did not significantly affect the ascorbic acid content of strawberries. This difference can be attributed to the indirect nature of PAW application, where the plasma is not directly applied to the fruit but is used to activate water that subsequently contacts the fruit.

Yang et al. (2023) also found that PAW treatment did not significantly affect the ascorbic acid content of strawberries during storage, which is consistent with our findings. Similarly, in fresh-cut pears, PAW treatment did not significantly alter the soluble solid content or titratable acidity, which includes vitamin C levels, but it did help in maintaining quality and reducing microbial growth (Chen et al., 2019). The stability of ascorbic acid content during storage can be attributed to the low storage temperature (4°C), which helps minimize oxidative degradation of vitamin C (Lee & Kader, 2000).

Total Antioxidant Activity

The total antioxidant activity of PAW-treated and control strawberries was measured during storage (Table 4).

No significant differences in total antioxidant activity were observed between PAW-treated and control strawberries throughout the storage period. Both groups showed a slight decrease in antioxidant activity by day 7, which may be related to the natural degradation of antioxidant compounds during storage. Natural degradation of antioxidant compounds during storage is a complex process influenced by various factors such as temperature, light, humidity, and the presence of oxygen (Ali et al., 2018).

Ayala-Zavala et al. (2004) reported that storage temperature influences the changes in antioxidant activity in strawberries, with higher temperatures leading to more pronounced changes. In our study, the relatively stable antioxidant activity can be attributed to the low storage temperature (4 °C), which helps preserve antioxidant compounds. Similar to our findings, Yang et al. (2023) found that PAW treatment helped maintain antioxidant activity in strawberries during storage, suggesting that PAW may have a protective effect on antioxidant compounds.

Total Anthocyanin Content

The total anthocyanin content of PAW-treated and control strawberries was measured during storage (Table 4). No significant differences in total anthocyanin content were observed between PAW-treated and control strawberries throughout the storage period. The anthocyanin content remained relatively stable in both groups, with only minor fluctuations.

Anthocyanins are important pigments responsible for the red color of strawberries and contribute to their antioxidant capacity (Timberlake & Bridle, 1982; Wang et al., 1996). Ayala-Zavala et al. (2004)

reported that anthocyanin content in strawberries can be influenced by storage temperature and duration. In their study, anthocyanin content decreased during the first 5 days of storage at 0 °C and 5 °C but increased in strawberries stored at 10 °C.

In our study, the stable anthocyanin content observed in both PAW-treated and control strawberries may be attributed to the vacuum packaging used during storage, which prevented moisture loss and minimized oxidative degradation of anthocyanins. This finding suggests that PAW treatment does not negatively impact the anthocyanin content of strawberries, which is important for maintaining their visual appeal and nutritional value.

Color Changes

The color parameters (CIE L*a*b*) of PAW-treated and untreated strawberries were measured during storage (Table 5). PAW treatment did not affect the color parameters of strawberries compared to control samples. PAW treated and untreated samples showed similar changes during storage, including a decrease in L* values (indicating darkening) and increases in a* values (indicating increased redness) and b* values (indicating increased yellowness). These changes are consistent with the normal ripening process of strawberries.

Table 5. Changes in color parameters of PAW treated and untreated strawberries during storage.

Sample	Color Values	Days			
		0	2	4	7
Untreated	L*	60.20±0.34	58.43±0.33	56.92±0.65	56.12±0.48
PAW treated		60.41±0.42	57.98±0.25	56.83±0.18	56.31±0.39
	ΔL	0.34	-0.77	-0.16	0.34
Untreated	a*	24.35±0.08	26.38±0.33	28.11±0.21	29.25±0.28
PAW treated		24.47±0.15	26.42±0.07	28.07±0.06	29.29±0.31
	Δa	0.49	0.15	-0.14	0.13
Untreated	b*	25.65±0.09	24.98±0.31	26.44±0.21	27.21±0.34
PAW treated		25.59±0.23	24.95±0.17	26.35±0.14	27.26±0.16
	Δb	-0.23	-0.12	-0.34	0.18
	ΔE	0.64	0.79	0.40	0.40

ΔE values less than 1 indicate that there is no distinguishable difference between PAW treated and untreated samples with human eye. Similar to our findings, Yang et al. (2023) reported that PAW treatment did not cause significant changes in the color of strawberries during storage. As an important quality criteria, the preservation of color in PAW-treated strawberries is important for consumer acceptance. Similar to our results, the ΔE of Broccoli, Alfalfa and Clover sprouts washed by PAW was higher than or equal to 2, hence the human eye would be capable of noticing the color difference between the washed sprouts and unwashed sprouts (Rivero et al., 2022).

CONCLUSION

The results of this study indicate that plasma-activated water (PAW) is highly effective for microbial decontamination on strawberries while preserving their physical and chemical properties. Using the pH 2.5 PAW for decontamination led to significant reductions in both *E. coli* and *B. cinerea* counts on the surface of strawberries. Notably, *B. cinerea* was found to be more resistant to PAW treatment than *E. coli*.

Except from reducing the microbial population, PAW treatment also slowed down the microbial growth during refrigerated storage. Comparing with the untreated samples, microbial counts on PAW treated samples were significantly lower after 7 days of storage. This prolonged antimicrobial effect indicated that PAW showed antimicrobial acitivity during storage. More importantly, it showed this antimicrobial effect without affecting physical or chemical quality of the strawberries. Ascorbic acid content, total antioxidant activity, anthocyanin levels, and color remained stable throughout storage. Maintaining these quality criteria is of great importance in terms of customer preferences and preserving nutritional quality of the strawberries.

Comparing with traditional chemical decontamination methods PAW offers several advantages. PAW is accepted as environmtally friendly, as it breaks down into water without leaving harmful residues. Despite its advantages, there is a need for further studies for the adoption of PAW technology. The efficiency of PAW should be tested in various products and standardized production and treatment parameters should be optimized for commercial use.. Also, the potential of combining PAW with other preservation techniques should be investigated to improve its effectiveness and extend the shelf life of fresh produce.

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