

Enhancement of shelf life of Citrus Limon L. (Lemon) using plasma activated water

Vikas Rathore (✉ rohangavasker007@gmail.com)

Institute for Plasma Research

Sudhir Kumar Nema

Research Article

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Authors

Vikas Rathore^{1,2*}, Sudhir Kumar Nema^{1,2}

1. Institute for Plasma Research (IPR), Gandhinagar, Gujarat, 382428, India
2. Homi Bhabha National Institute, Mumbai, Maharashtra, 400094, India

*Email: vikas.rathore@ipr.res.in

Abstract

The present study investigates the impact of plasma-activated water (PAW) on the shelf life of Lemon (*Citrus Limon* L.). PAW is generated through atmospheric pressure air dielectric barrier discharge using a pencil plasma jet. The plasma interacted with water for different time intervals to produce PAW of varying reactivity. The voltage-current waveform of the plasma is characterized, and the species/radicals formed in the plasma are detected using optical emission spectroscopy.

The results of the study indicate that a 30-minute exposure of lemons to PAW results in a significantly higher reduction of microbial load on the lemon surface compared to the control (ultrapure milli-Q water). In addition, the PAW exposure of lemons resulted in lower weight loss and spoilage compared to the control over a long duration. The sensory evaluation (smell, appearance, taste) and color evaluation (L^* , a^* , b^*) showed that PAW exposed lemons have shown improved acceptability compared to lemons exposed to the control. The surface firmness test also revealed that PAW exposure did not damage the lemon surface. The analysis of lemon juice nutrients (titratable acidity, sugar, carbohydrate, protein, and vitamin C) and antioxidant activity (total phenols, flavonoids, DPPH and ABTS assay) indicated that the interaction of PAW with lemons did not result in significant changes in these aspects compared

to the control. Lastly, the 2-month long-term storage of lemons after PAW and control exposure showed that lemons exposed to PAW had acceptable quality, wherein lemons exposed to control were completely spoiled and covered with mold. The study reveals that PAW has the potential to be used as an environmentally friendly alternative to chemical food preservatives.

Keywords: plasma activated water, plasma characterization, reactive oxygen-nitrogen species, *Citrus limon* (lemon), food preservation

1. Introduction

Microbial contamination and infection are the leading cause of food spoilage worldwide. To prevent this, various preservation methods are employed, such as low temperature preservation (refrigeration and freezing), thermal processing (sterilization and pasteurization), chemical preservation (using alcohol, acids, and benzoates), and irradiation (using X-rays and gamma rays). However, these methods have drawbacks such as decreased food quality, dehydration, loss of nutrients, reduced sensory quality, lipid oxidation, chemical and radiation poisoning, and failure to eliminate pathogens, etc (1-8). One emerging technology in food preservation is cold plasma, which avoids issues like toxicity from chemicals and radiation, dehydration, and loss of quality and nutrients. However, cold plasma systems have their own limitations, such as the requirement for high voltage, high frequency power supplies, so proper precautions must be taken while operating (1, 9-13). Therefore, it is limited to industrial use only. The solution to this problem is provided by plasma activated water (PAW) technology, which can be generated at a central location and transported to various locations, including households. (3, 14-29)

The food preservation efficacy of PAW previously reported by Berardinelli et al. (9), Frías et al. (29), Guo et al. (21), Han et al. (27), Inguglia et al. (17), Jung et al. (18), Kang et al. (30), Liao et al. (24), Liu et al. (25), Ma et al. (22), Zhai et al. (26), Yong et al. (20), Xu et

al. (14), Xiang et al. (3), Schnabel et al. (4), Royintarat et al. (28). These reports highlight the role of PAW in extending the shelf life of various fruits, vegetables, dairy, and meat products, including tofu, chicken meat, Korean rice cake, fresh-cut apples, shrimp, strawberries, grapes, ham, beef, Chinese bayberries, and button mushrooms. The presence of reactive species such as hydroxyl radicals ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), dissolved ozone (O_3), peroxyxynitrite (ONOO^-), and an acidic environment (including nitric and nitrous acids) gives PAW its antimicrobial efficacy (14, 17, 20, 21, 24, 26, 29, 31, 32). The antimicrobial action of PAW on food reduces spoilage and makes it a promising alternative to the previously discussed preservation techniques. This work focuses on the efficacy of PAW in preserving *Citrus limon* (*C. limon*).

India is one of the largest producers and consumers of *C. limon*. It is widely consumed in India as well as in other parts of the world, as a major source of vitamins (ascorbic acid), folic acid, potassium, and pectin. Some of the major regions in India where *C. limon* is grown are Gujarat, Bihar, Delhi, Assam, Arunachal Pradesh, Chhattisgarh, and Punjab (33, 34). According to the Food and Agriculture Organization (FAO) of the United States, India is the largest producer of *C. limon* with 3.7 million tons in 2020, followed by Mexico, China, Argentina, Brazil, and Turkey (35). However, more than 40% of the *C. limon* post-harvest is spoiled during storage, mainly due to naturally occurring fungal flora (31). Our previous study extracted the fungi causing *C. limon* spoilage and evaluated the fungicidal efficacy of PAW. The results showed that a 10-minute PAW treatment resulted in a $6+ \log_{10}$ CFU/ml reduction and maintained this fungicidal efficacy over a long duration (31). Furthermore, no research has been reported on using PAW to enhance the shelf-life of *C. limon* and retain its quality attributes, nutritional value, and antioxidant activity, based on a literature review by the authors. Therefore, this present work addresses this gap by focusing on enhancing the shelf-life of *C. limon* using PAW as a treatment medium.

The present study utilizes dielectric barrier discharge air plasma for PAW production. The impact of plasma-water treatment time on PAW production and the effect of PAW treatment time on the shelf-life of *C. limon* are explored. The health of *C. limon* after PAW treatment is evaluated based on the following parameters: sensory evolution, microbial analysis, physical attributes, nutritional analysis, and antioxidant activity.

2. Materials and Methods

2.1 Preparation and Characterization of PAW

The PAW was prepared using air plasma, with a pencil plasma jet. The electrical and optical emission characterization of the pencil plasma jet can be found in our previous work (36, 37). A 250 ml of water was added to a 500 ml glass beaker for PAW production (as seen in supplementary figure S1). The plasma-water treatment time was varied from 1 to 4 hours (1 hour (PAW-I), 2 hours (PAW-II), 4 hours (PAW-III)) for water activation (Figure 1). The physical and chemical properties (pH, oxidation-reduction potential (ORP), total dissolved solid (TDS), electrical conductivity (EC)) and reactive species concentrations (NO_3^- , NO_2^- , H_2O_2 , dissolved O_3) of PAW were measured using methods described in previous reports (23, 36, 38, 39).

2.2 PAW Treatment with *C. limon*

The *C. limon* used in this study was obtained from a local vegetable and fruit market in sector 21, Gandhinagar, Gujarat, India. For comparison, *C. limon* of similar shape, size, and weight (horizontal diameter: 3.55 ± 0.13 cm, vertical diameter: 3.23 ± 0.19 cm, weight: 22.43 ± 1.06 g) was chosen. A total of 192 *C. limon* were divided into 4 groups (control, PAW-I, PAW-II, PAW-III, $192 = 48 \times 4$) and each group was further divided into 3 subgroups ($48 = 16 \times 3$) for repetition.

The treatment procedure is shown in Figure 1. The PAW treatment time was varied from 10 to 30 minutes. The excess water was removed using a UV-sterilized paper towel. The treated *C. limon* were stored in a sterile plastic container (Low-density polyethylene) at 4°C in the refrigerator and covered with food-grade cast polypropylene film. The analysis of the *C. limon* and its juice was performed for 15 days.

2.3 Sensory Evaluation

The sensory evaluation of the *C. limon* and its juice after control and PAW treatment was performed over the storage duration, including smell, taste, appearance, and overall acceptance. A group of 15 people (male and female) aged 25 to 55 rated the *C. limon* health and juice quality on a scale of 1 to 9, where 1 represented very bad, 3 bad, 5 fine, 7 good, and 9 very good.

2.4 Microbial Analysis

For microbial analysis, 100 mg of the *C. limon* peel was removed using a sterile knife and mixed with 10 ml of sterile phosphate buffer saline (PBS) solution (procedure described in previous publication) (31)). A 0.1 ml of the solution (after serial dilution) was transferred to nutrient broth, and colonies were counted using a colony counter. The expression to calculate the percentage reduction compared to the control is given as:

$$\% \text{ microbial reduction} = \left| \frac{(CFU/g)_{PAW} - (CFU/g)_{control}}{(CFU/g)_{control}} \right| \times 100$$

2.5 Color Characterization

The color of *C. limon* was measured during storage after PAW and control treatments using a colorimeter (model number NR100, 3nh, CIE LAB color space, observed angle 10°, D65 light source, ϕ8 double aperture). The color levels were recorded as CIE L*×a*×b*, where 'L*' represents lightness (0 represents black and 100 represents white), 'a*' represents the color

between red and green (-a* represents greenness, +a* represents redness), and 'b*' represents the color between blue and yellow (-b* represents blueness, +b* represents yellowness). The chromatic aberration index (C*) was calculated as $(a^{*2} + b^{*2})^{1/2}$ to provide visual observation of color purity. The degreening of *C. limon* was calculated using the citrus color index (CCI = $(1000 \times a^*) / (L^* \times b^*)$). The perceived color was calculated using the hue angle ($h^\circ = \tan^{-1}(b^*/a^*)$).

2.6 *C. limon* Physical Analysis

The physical characteristics of *C. limon*, such as weight loss, firmness/hardness, and spoilage, were analyzed during storage after control and PAW treatments. Weight loss was calculated using the following expression:

$$\text{Weight Loss (\%)} = \left(\frac{W_t - W_{t_0}}{W_{t_0}} \right) \times 100$$

Where, W_t is weight of *C. limon* at time 't (day)', W_{t_0} is weight of *C. limon* at time t = 0 day.

The firmness/hardness of *C. limon* during storage was monitored regularly using Digital Fruit Hardness Tester (LUTRON ELECTRONIC, Model Name/Number: FR5120). The spoilage of *C. limon* were analysed on the base of occurrence of brown color on its *C. limon* surface, smell, firmness and juice taste.

2.7 *C. limon* juice nutritional analysis

The juice volume of the *C. limon* was initially measured during the storage period. The total dissolved solids were measured using a Smart Digital Refractometer (SCM-1000, HM Digital India Pvt. Ltd.). 0.1 ml of juice was diluted with 0.9 ml of ultrapure milli-Q water to measure titrable acidity, sugar, protein, and ascorbic acid (vitamin C) if needed. The titrable acidity of the juice was determined using volumetric titration with 0.1 N or 0.01 N NaOH solution and phenolphthalein as an indicator (25).

To analyze sugar, 2 ml of warm ethyl alcohol (80% v/v) was added in 1 ml of juice. The solution was heated in a boiling water bath for 10 minutes, then cooled. A freshly prepared 0.2% ice-cold anthrone reagent was then added. The solution was incubated in a boiling water bath and placed on ice after the incubation period. The color of the solution was measured at 590 nm, with appropriate dilution performed for dense color solutions (40). The standard curve of a sugar solution was prepared using dextrose with a molar extinction coefficient $0.0064 \text{ (mg l}^{-1}\text{)}^{-1} \text{ cm}^{-1}$.

The protein concentration in *C. limon* juice was determined using Lowry et al. (41). The protein was extracted from 1 ml of juice using 2 ml of extraction buffer consisting of 10% trichloroacetic acid and 0.07% β -mercaptoethanol in acetone. 0.1 ml of the protein extraction solution was mixed with 0.1 ml of 2N sodium hydroxide, then hydrolyzed in boiling water for 10 minutes and cooled to 25°C. 1 ml of complex reagent (2% Na_2CO_3 , 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2% $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ in a 100:1:1 ratio) was added and the solution was incubated at room temperature for 10 minutes. 0.1 ml of freshly prepared folin reagent was added, and the absorbance was measured at 750 nm after a 30 to 60 minutes wait period. A standard curve for protein was prepared using bovine serum albumin solution with a molar extinction coefficient of $0.7 \text{ (}\mu\text{g l}^{-1}\text{)}^{-1} \text{ cm}^{-1}$.

For the determination of ascorbic acid (vitamin C) in juice, 0.1 ml of juice was mixed with 0.4 ml of 10% TCA and shaken at 3000 rpm for 5 minutes. The solution was diluted with 1 ml of ultrapure milli-Q water, and 0.1 ml of freshly prepared folin reagent was added. The solution was shaken for 10 minutes and incubated for an additional 10 minutes before measuring absorbance at 760 nm (42). The standard curve was determined using various concentrations of ascorbic acid and the resulting molar extinction coefficient was found to be $0.0008 \text{ (mg L}^{-1}\text{)}^{-1} \text{ cm}^{-1}$.

2.8 Antioxidant Activity in *C. limon* Juice

For the estimation of total phenols in juice, 0.1 ml of juice was mixed with 0.5 ml of a folic-ciocalteu reagent (diluted 1:10 with water) and incubated for 60 seconds. After incubation, 0.4 ml of 7.5% sodium carbonate (Na_2CO_3) was added and incubated for 60 minutes before measuring absorbance at 765 nm. A standard curve was prepared using various concentrations of gallic acid, with a molar extinction coefficient of $0.0062 \text{ (mg L}^{-1}\text{)}^{-1} \text{ cm}^{-1}$, to quantify total phenols in juice.

For the determination of total flavonoids, 0.1 ml of diluted juice was mixed with 0.5 ml water and 30 μl of 1:20 w/v NaNO_2 . After a 5 minute incubation, 0.3 ml of 1:10 w/v AlCl_3 was added, and the solution was incubated for 6 minutes at room temperature. 0.2 ml of 1M NaOH was then added, and the solution was mixed by shaking or vortexing. The absorbance was measured at 510 nm (43). The standard curve was prepared using quercetin (molar extinction coefficient was $0.0007 \text{ (mg L}^{-1}\text{)}^{-1} \text{ cm}^{-1}$) to determine unknown concentration of flavonoids.

For the DPPH assay, 0.1 ml of juice was mixed with 0.3 ml of DPPH $\cdot$ solution (3.2 μl of standard DPPH, consisting of 250 mg DPPH in 2 ml CH_3OH , diluted in 10 ml of CH_3OH), and 0.6 ml of CH_3OH . After an incubation period of 30 minutes, the absorbance was recorded at 517 nm. For the ABTS assay, the ABTS^+ solution was prepared by mixing equal volumes (10 ml each) of ABTS (7 mM) and $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM). This mixture was incubated in the dark for 12 to 16 hours. Then, 400 μl of the solution was added to 20 μl of diluted juice, and the absorbance was recorded at 732 nm after an incubation period of 8 minutes. (44, 45). To quantify the activity of DPPH and ABTS, a standard curve was prepared using different concentrations of Trolox solution ($\% \text{ inhibition} = (\text{A}_{\text{DPPH or ABTS}} - \text{A}_{\text{sample}}) / \text{A}_{\text{DPPH or ABTS}} \times 100$).

All the chemicals used in this investigation were of analytical grade and sourced from various suppliers including High Purity Laboratory Chemicals Pvt. Ltd., Sigma Aldrich, and TCI Chemicals (India) Pvt. Ltd.

2.9 Data Analysis

The collected data from the experiment was presented as mean $\pm$ standard deviation in graphs and tables. The experiments were conducted a minimum of three times ($n \geq 3$). The statistical significance ($p < 0.05$) of the results was analyzed using an analysis of variance followed by a post-hoc test (Fisher's Least Significant Difference). Significant differences in the tables and figures were indicated by different lowercase letters.

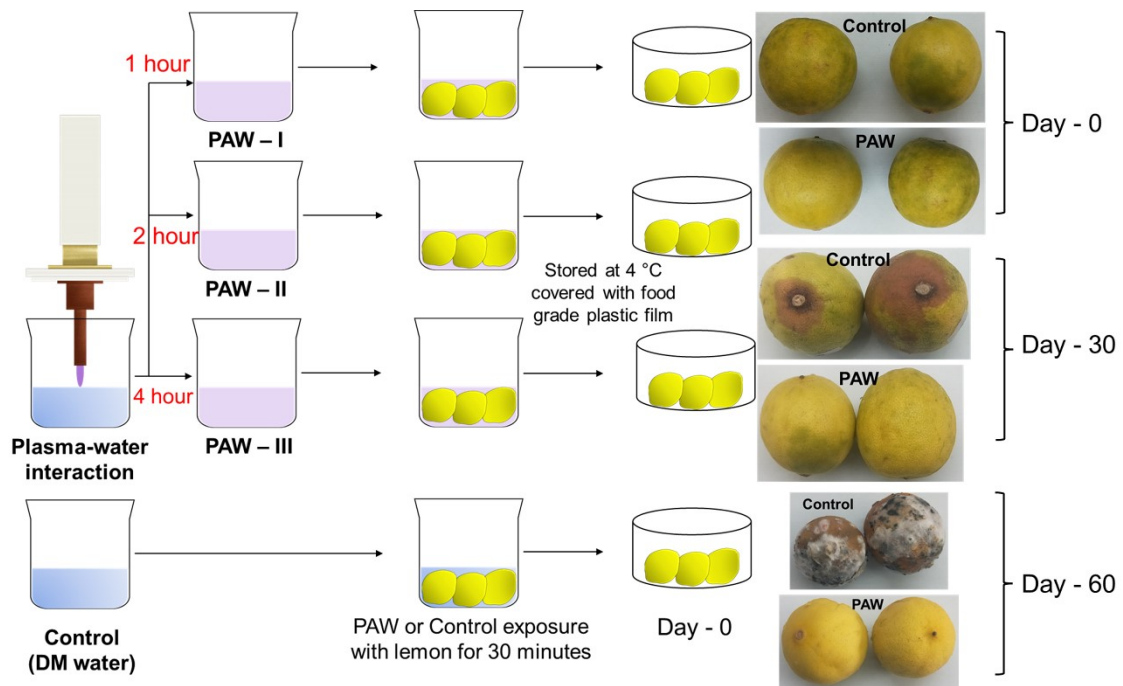


Figure 1. Schematic of treatment of *Citrus limon* using control and PAW

3. Results

3.1 Plasma Characterization and Species Identification

The voltage-current waveform of PPJ air plasma is shown in Figure 2(a). The observed peak-to-peak voltage was 4.2 kV and the peak-to-peak current was 54.67 mA. The current profile, as shown in Figure 2(a), is a combination of conduction current and discharge current. The conduction current is depicted as a continuous sine wave, and the discharge current is shown as a collection of spikes over the sine wave. These spikes, which occur during the peak of the

rising and falling half-cycles of the voltage profile, represent the generation of reactive species/radicals during air discharge (Figure 2(a)). The spikes have a duration in the nanosecond range, and the collection of nanosecond pulses has a duration in the microsecond range, indicating a filamentary micro-discharge. Hence, the observed air plasma discharge was a DBD filamentary micro-discharge (46). The results of the plasma discharge showed a power consumption of 7.60 W during air discharge, calculated using the transported charge-voltage Lissajous figure (not shown in present manuscript).

The spikes observed in the current waveform indicated the mobility of charged species and generated electrons due to air discharge. The emission spectra of the air plasma showed strong emission band peaks of N_2 systems and weak N_2^+ systems, which were identified in Figure 2 (b). The emission band peaks of N_2 ($C^3\Pi_u \rightarrow B^3\Pi_g$) systems were 296.1 nm ($3 \rightarrow 1$), 313.0 nm ($2 \rightarrow 1$), 315.4 nm ($1 \rightarrow 0$), 337.0 nm ($0 \rightarrow 0$), 353.7 nm ($1 \rightarrow 2$), 357.7 nm ($0 \rightarrow 1$), 371.3 nm ($2 \rightarrow 4$), 375.5 nm ($1 \rightarrow 3$), 380.5 nm ($0 \rightarrow 2$), 394.1 nm ($2 \rightarrow 5$), 399.8 nm ($1 \rightarrow 4$), 405.9 nm ($0 \rightarrow 3$), 426.8 nm ($1 \rightarrow 5$), 434.1 nm ($0 \rightarrow 4$) and N_2^+ ($B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$) system were 391.1 nm ($0 \rightarrow 0$) and 419.8 nm ($2 \rightarrow 3$), respectively (47). The high-energy reactive species and radicals produced during the air discharge contributed to the alteration of the chemistry of water during plasma-water interaction.

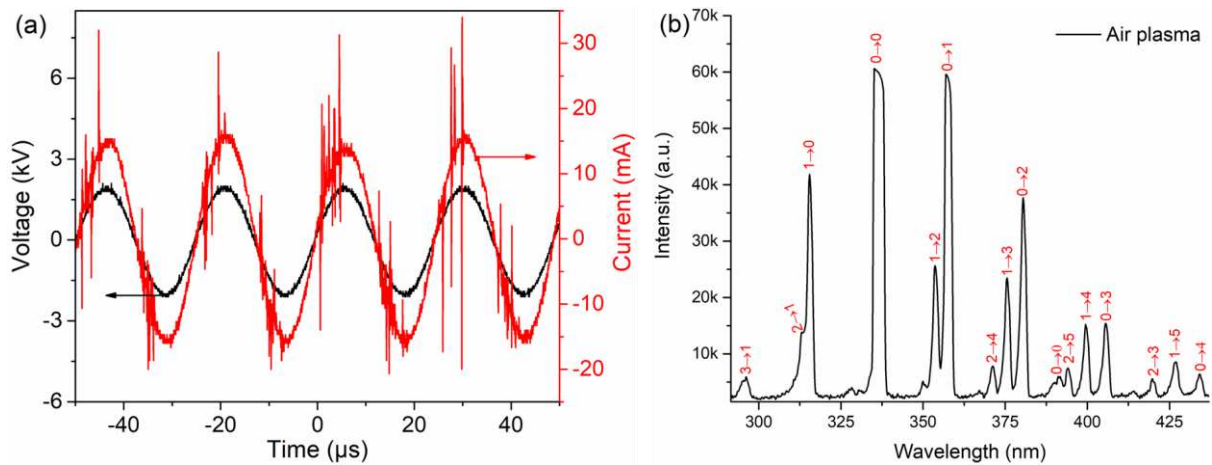


Figure 2. (a) Electrical characterization and (b) identification of reactive species/radicals in air plasma produced in pencil plasma jet

3.2 Properties of PAW used for *C. limon* treatment

The plasma-water interaction results in the generation of various reactive species. Figure 2 (b) shows the emission spectra of the reactive species formed in the plasma. When these reactive species interact with water, they lead to the formation of various stable reactive species, as reported in earlier studies (23, 32, 36, 38). This interaction leads to a decrease in pH and an increase in oxidizing potential, inorganic solids, and electrical conductivity, as shown in Figure 3 (a, b). These changes are due to the formation of species such as dissolved ozone (O_3), hydrogen peroxide (H_2O_2), and nitrogen-containing acids like nitrous and nitric acids (HNO_2 and HNO_3), which are identified as nitrite and nitrate ions in plasma-activated water (PAW), as shown in Figure 3 (c, d).

Figure 3 shows that as plasma-water treatment time increases, there is substantial enhancement in the properties of PAW. For example, a 4-hour plasma treatment of water (ultrapure milli-Q water, demineralized water) decreased the pH of water from 6.8 to 2.8, increased the ORP, TDS, and EC from 240 mV to 602 mV, 0 ppm to 900 ppm, and $1 \mu S cm^{-1}$ to $2430 \mu S cm^{-1}$, respectively. Additionally, the concentration of NO_2^- , NO_3^- , H_2O_2 , and dissolved O_3 after 4 hours of water activation was $2.64 mg l^{-1}$, $11.92 mg l^{-1}$, $5.3 mg l^{-1}$, and $2.47 mg l^{-1}$, respectively. This trend of enhancement in the physicochemical properties and reactive species in PAW with treatment time has also been shown in previous studies on food preservation (4, 9, 14, 20, 22, 25, 26, 30).

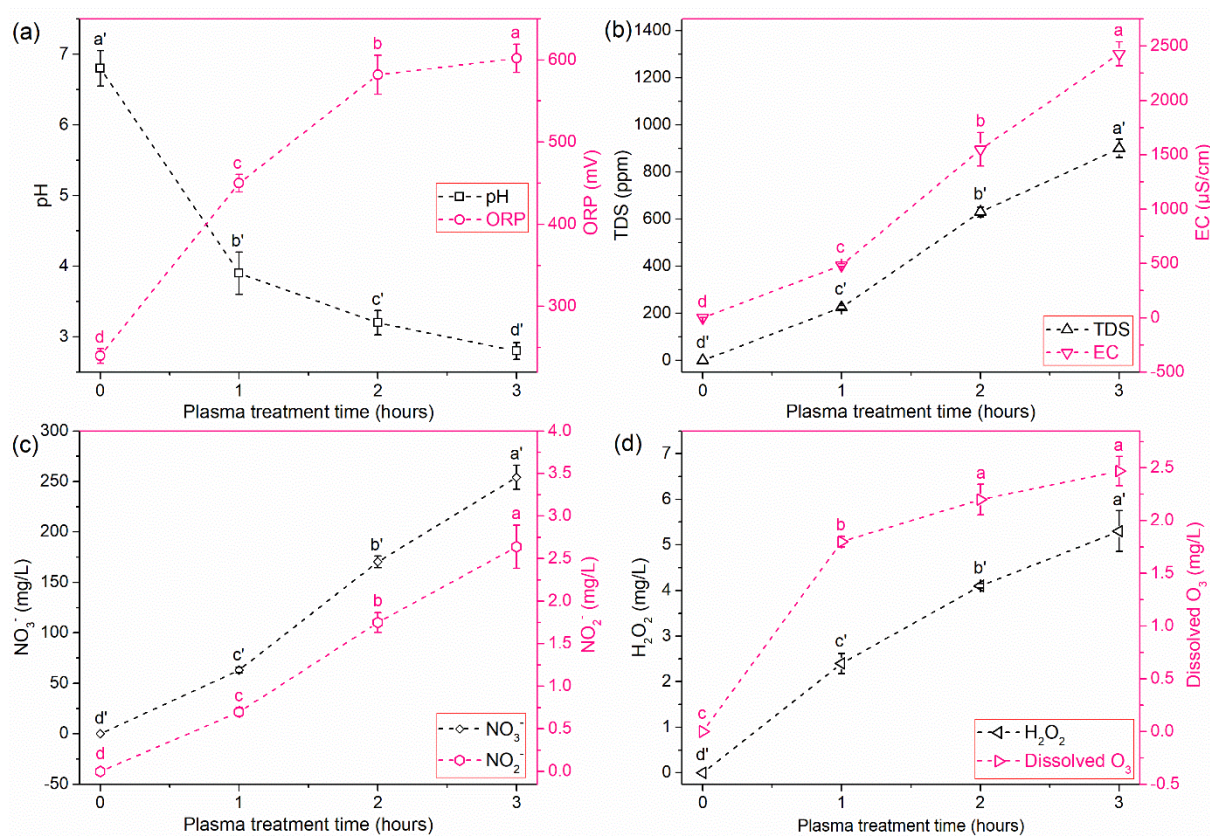


Figure 3. Variation in properties of PAW with varying plasma treatment time using water

3.3 Effect of PAW treatment on the properties of *C. limon*

The present work focuses on the effect of PAW treatment on the properties of *C. limon* (lemon) with the aim of enhancing its shelf-life. The study includes evaluating the change in microbial load, weight loss, surface hardness, surface color parameters, spoilage, nutritional analysis, and antioxidant activity of the lemon over time. The effect of PAW treatment on lemon was explored by considering two important parameters: plasma-water treatment time and duration of exposure of PAW with lemon. The first step was to investigate the effect of plasma-water treatment time on the health of the lemon, and then the impact of the duration of exposure of PAW with lemon was explored after obtaining the optimum plasma-water treatment time.

3.3.1 Role of plasma-water treatment time on *C. limon* shelf life

Sensory Properties

The change in sensory properties of *C. limon* when treated with control (ultrapure milli-Q water) and PAW prepared using different plasma-water exposure times from 1 to 4 hours (PAW-I to PAW-III) is shown in Table 1. At the end of 15 days, the control-treated *C. limon* showed degradation in its sensory properties (smell, taste, appearance, and overall acceptability) compared to the PAW-treated *C. limon*. The smell, taste, and appearance of the control-treated *C. limon* changed from very good (9) to fair (around 6) over the 15-day storage period, leading to a drop in overall acceptability below the recommended level. On the other hand, the PAW-treated *C. limon* retained its sensory properties over the 15-day storage period without significant change. This result is supported by previous reports by Liu et al. (25), Zhai et al. (26), etc. who also reported the retention of sensory attributes of food during storage after PAW treatment. The present study did not investigate plasma-water treatment times above 4 hours, as higher treatment times may damage the food due to the higher reactivity in the form of hydroxyl ($\cdot\text{OH}$) radicals, dissolved ozone (O_3), and high acidity in the form of nitrous and nitric acids (NO_2^- ions and NO_3^- ions), etc. Kang et al. (19) reported a slight reduction in sensory attributes of food due to these reactive species compared to the control.

Table 1: Study of sensory evolution (smell, appearance, taste, overall acceptability) of *Citrus limon* after control and PAW treatment.

Day	Smell						
		30 min treatment time			PAW – III treatment time		
	Control	PAW - I	PAW - II	PAW - III	10 min	20 min	30 min
0	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
5	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
10	8.25 ^a ±1.04	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
15	6.11 ^b ±1.05	8.5 ^a ±0.92	8.71 ^a ±0.76	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
	Appearance						
		30 min treatment time			PAW – III treatment time		

	Control	PAW - I	PAW - II	PAW - III	10 min	20 min	30 min
0	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
5	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
10	7.50 ^b ±0.92	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
15	5.67 ^c ±1.00	8.00 ^a ±1.07	8.43 ^a ±0.98	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
	Taste						
		30 min treatment time			PAW – III treatment time		
	Control	PAW - I	PAW - II	PAW - III	10 min	20 min	30 min
0	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
5	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
10	8.25 ^a ±1.04	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
15	6.55 ^b ±0.88	8.5 ^a ±0.92	8.43 ^a ±0.98	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
	Overall acceptability						
		30 min treatment time			PAW – III treatment time		
	Control	PAW - I	PAW - II	PAW - III	10 min	20 min	30 min
0	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
5	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
10	8.00 ^a ±1.07	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00
15	6.11 ^b ±1.05	8.25 ^a ±1.04	8.43 ^a ±0.98	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00	9.00 ^a ±0.00

Microbial analysis

The growth of microbes (bacteria, fungi, etc.) on the surface of food is a major cause of spoilage. Figure 4 (a) shows the microbial analysis of the surface of *C. limon* after PAW treatment compared to the control. The *C. limon* treated with PAW showed significantly lower microbial growth compared to the control. Furthermore, PAW prepared using a plasma treatment time of 4 hours (PAW-III) showed a higher reduction in microbes compared to PAW prepared using 1 hour (PAW-I) and 2 hour (PAW-II) treatment, as shown in Figure 4 (a). At the start of the study (t = 0 day), PAW-III showed a 17.5% and 6.2% lower microbial growth

compared to PAW-I and PAW-II, respectively. By the end of 15 days, PAW-III showed a 45.8% and 8.9% lower microbial growth compared to PAW-I and PAW-II, respectively. Previous studies by Liu et al. (25), Liao et al. (24), Kang et al. (19) and Han et al. (27), etc. have also reported a reduction in microbial growth on fresh-cut apples, shrimps (*Metapenaeus ensis*), chicken breast, and Korean rice cake, respectively, supporting the findings of the present investigation.

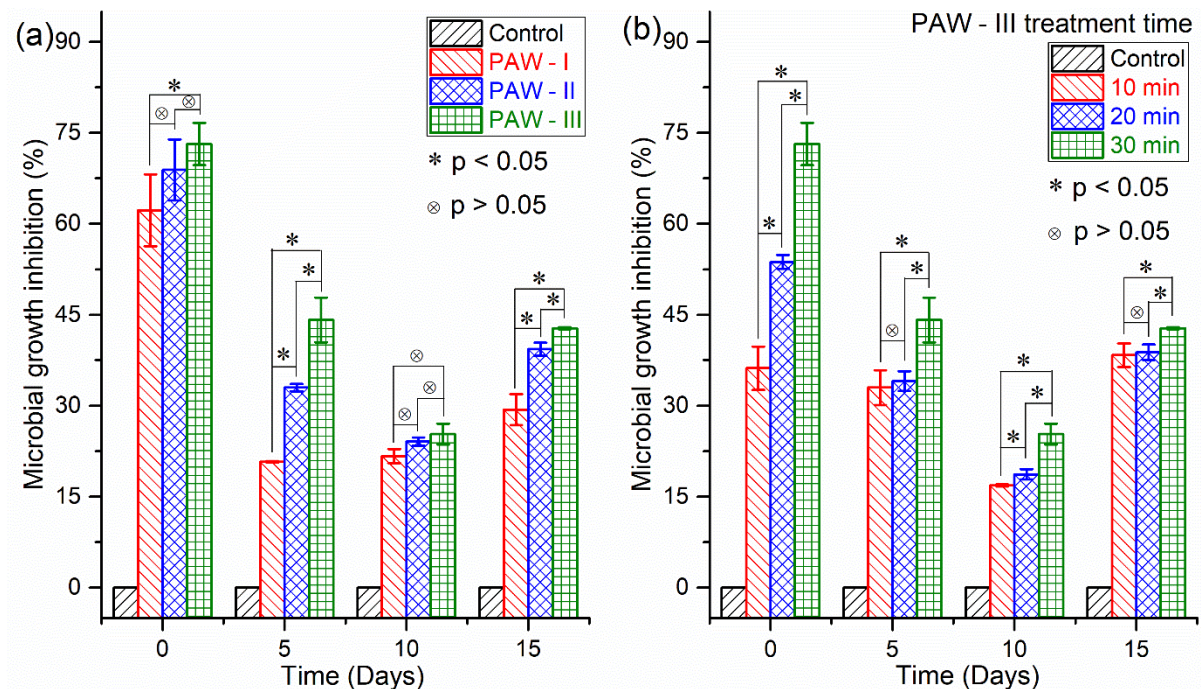


Figure 4. Variation in microbial load inhibition on *Citrus limon* when treated with PAW compared to the control.

Physical Characteristics

Color is a key physical attribute to determine the freshness of food (fruits, vegetables, meats, etc.). Results from analysis of the color of *C. limon* after control and PAW treatment are shown in Figure 5. The lightness level (L^*) of *C. limon* treated with PAW (prepared with different plasma treatment times PAW-I to PAW-III) was slightly higher (Days 5 & 10: L^* - 82.4 & 83.97 (control), 84.33 & 85.31 (PAW-I), 84.36 & 85.66 (PAW-II), 84.73 & 87.53 (PAW-III))

and the chromatic aberration index was lower (Days 5 & 10: C^* - 61.59 & 62.65 (control), 53.82 & 55.61 (PAW-I), 59.45 & 59.78 (PAW-II), 59.94 & 58.82 (PAW-III)) compared to control (Figure 5 (a)). Figure 5 (c) shows that degreening occurs during storage and PAW-III treated lemon showed substantially less degreening (a^* & CCI: -3.34 & -0.64) compared to control and other PAW treatments (a^* & CCI: -0.55 & -0.10 (control), -0.67 & -0.14 (PAW-I), -1.04 & -0.20 (PAW-II)) after 15 days of storage. PAW treated *C. limon* also showed lower yellowness (b^* & h° : 55.15 & -89.30 (PAW-I), 59.41 & -89.00 (PAW-II), 61.65 & -86.90 (PAW-III)) compared to control (b^* & h° : 62.28 & -89.49 (control)) over the 15 day experiment duration (Figure 5 (e)). In conclusion, PAW treated *C. limon* showed less ripening compared to control.

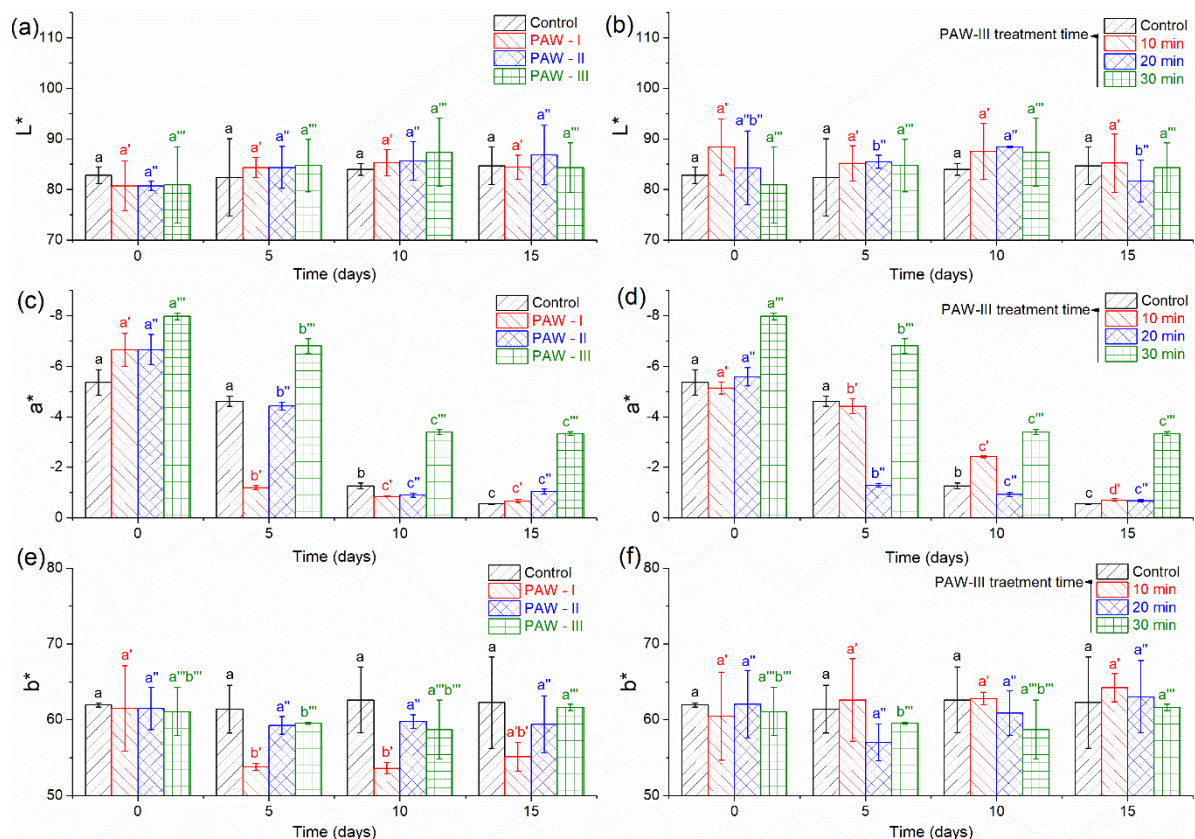


Figure 5. Changes in the surface color (L^* , a^* , b^*) of *Citrus limon* during storage after PAW and control treatments.

The variation in firmness (hardness), weight loss, and spoilage of *C. limon* over time after control and PAW treatment is shown in Figure 6. The PAW-treated *C. limon* showed significantly lower weight loss compared to the control, indicating less compromise in the epidermis, mesocarp, and exocarp's leakage permeability (Figure 6 (a)). As a result, the moisture loss from the PAW-treated lemon was lower than the control. This was further supported by the surface hardness results (Figure 6 (c)), where the surface hardness of PAW-treated *C. limon* remained intact. However, at the end of 15 days, the firmness of control-treated *C. limon* had reduced significantly compared to PAW. The PAW treatment did not affect the firmness, as shown in previous studies of Xiang et al. (3), Ma et al. (22), Ma et al. (15), Zheng et al. (48).

The spoilage of *C. limon* during storage after control and PAW treatment is shown in Figure 6 (e). After 15 days, approximately 45% of the *C. limon* had spoiled. The duration of plasma treatment with water during PAW preparation was found to play a significant role in *C. limon* spoilage. As the plasma-water treatment time increased, a reduction in *C. limon* spoilage was observed, as shown in Figure 6 (e). The spoilage of *C. limon* after PAW-I, PAW-II, and PAW-III treatments was 17.78%, 13.33%, and 0.00%, respectively. Hence, a 4-hour plasma interaction with water (PAW-III) for PAW preparation is recommended to enhance the shelf life of *C. limon* based on its physical attributes. Ma et al. (22) also reported a substantial reduction in spoilage of strawberries after PAW treatment, which is in line with the current results.

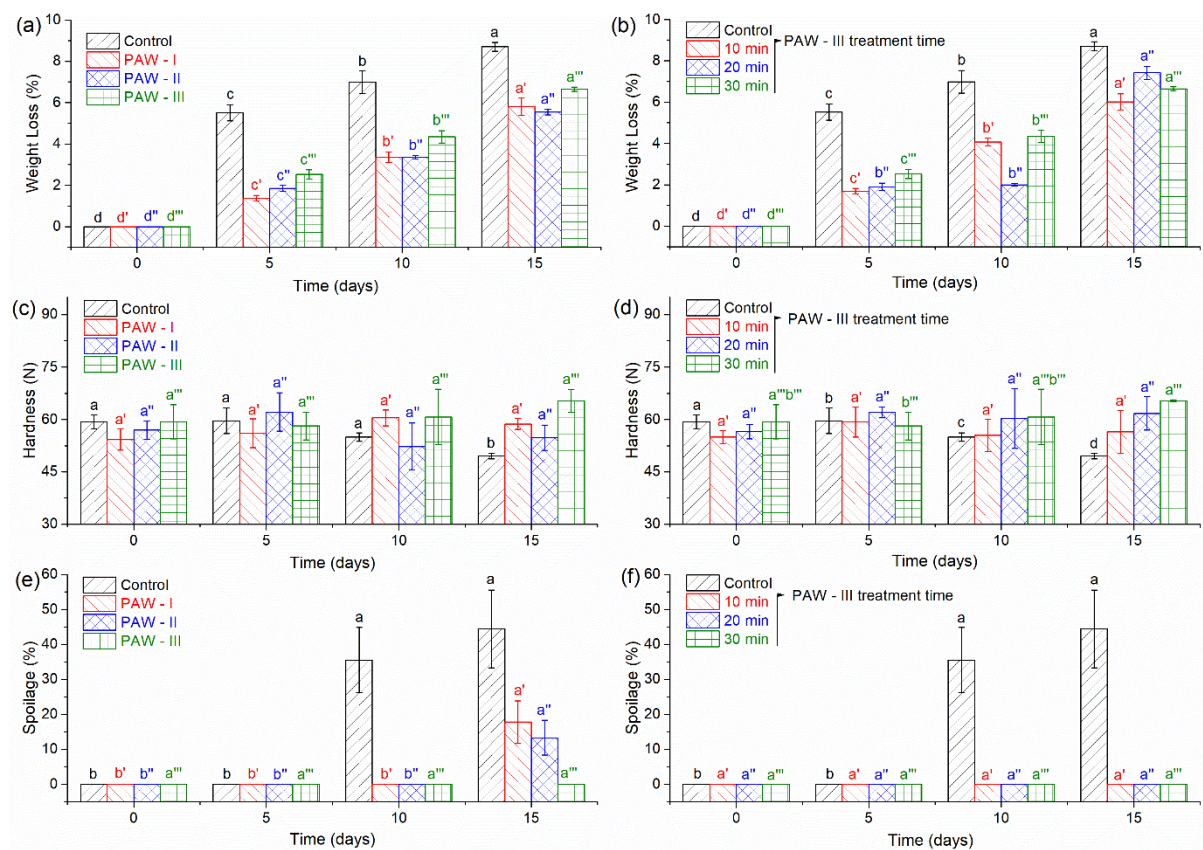


Figure 6. Changes in weight loss, hardness, and spoilage of *Citrus limon* during storage after PAW and control treatments.

C. limon Juice Analysis

The variation in the volume of *C. limon* juice during storage after treatment with control and PAW (PAW-I to PAW-III) is shown in Figure 7 (a). The volume of *C. limon* juice after control and PAW treatment varied between 7.5 ml and 14 ml. No clear trend was observed in the juice volume after control and PAW treatment during the experimental duration due to differences in juice content among the *C. limon*.

The variation in the titrable acidity of *C. limon* juice during storage is shown in Figure 7 (c). It showed a rise and fall in its percentage over the storage duration for both the control and PAW-treated *C. limon* (ranging from 5% to 7% citric). However, there was no statistically significant difference between the control and PAW-treated titrable acidity of *C. limon* juice

over time, as shown in Figure 7 (c). Hence, the PAW treatment did not affect the titrable acidity of the *C. limon*. Xiang et al. (3) also reported the effect of PAW on the titrable acidity and showed that the PAW treatment did not change the titrable acidity of grapes, supporting the results of the present investigation.

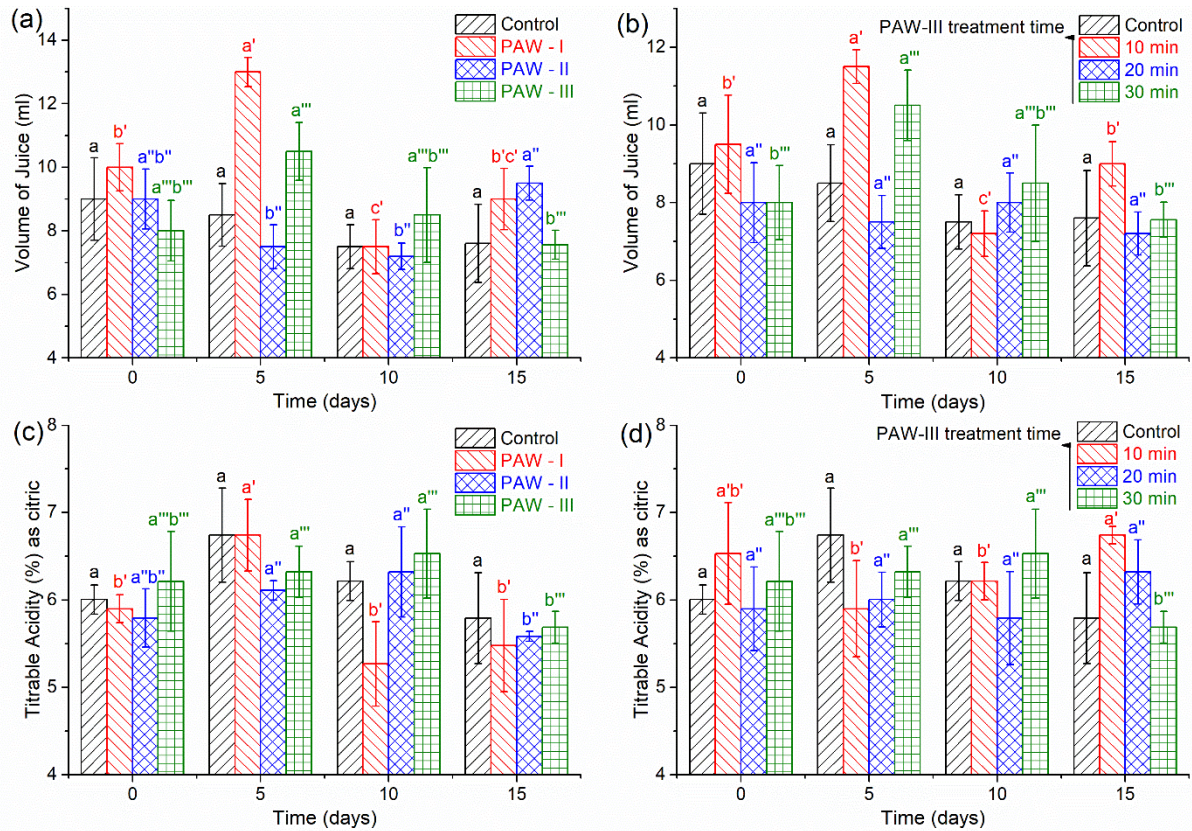


Figure 7. Changes in volume and titrable acidity of *Citrus limon* juice during storage after control and PAW treatment.

The biochemical analysis of *C. limon* juice after treatment with control and PAW is shown in Figure 8. The variation in soluble solids, sugar, protein, and vitamin C is shown. The PAW-II and PAW-III treatments showed slightly higher soluble solids (maximum value during storage: 8.2 °Brix) compared to the control (maximum: 7.9 °Brix), but this difference was not substantial. Therefore, PAW treatment did not change the soluble solids content of the *C. limon* juice (Figure 8 (a)). However, at the end of 15 days, the total sugar concentration in the juice

of PAW-II and PAW-III treated *C. limon* was higher (maximum sugar concentration: 72.31 mg L⁻¹ and 72.18 mg L⁻¹ during storage) compared to the control and PAW-I (maximum sugar concentration: 68.87 mg L⁻¹ and 66.1 mg L⁻¹ during storage), indicating an elevated nutritional level of PAW treated *C. limon* juice compared to the control (Figure 8 (c)). Previously, Xiang et al. (3) showed that PAW treatment did not significantly affect the concentration of dissolved solids and sugar in grapes, which is in line with the results of the present study.

The protein concentration of PAW treated *C. limon* juice was either higher or similar (ranging from 1.52 to 1.87 mg L⁻¹) to the control (1.47 mg L⁻¹ to 1.56 mg L⁻¹) up to day 10 of *C. limon* storage. However, at the end of 15 days, a substantial decrease in the soluble protein concentration was observed in the *C. limon* juice treated with PAW-III (1.41 mg L⁻¹). Meanwhile, a considerable increase in soluble protein concentration was observed in the *C. limon* juice treated with PAW-II (2.33 mg L⁻¹) (Figure 8 (e)). Earlier research by Royintarat et al. (28) reported no change in chicken meat protein after PAW treatment.

The control treated *C. limon* juice showed a higher ascorbic acid (vitamin C) concentration (288.75 mg L⁻¹) compared to PAW (170 mg L⁻¹ for PAW-III) with increasing storage time, as shown in Figure 8 (g). This elevated concentration of ascorbic acid in the control treated *C. limon* may cause health problems like nausea, diarrhea, headache, fatigue, and heartburn upon consumption. Hence, the elevated concentration of ascorbic acid in the control treated *C. limon* is not fit for consumption. The PAW treatment with grapes did not change the ascorbic acid concentration, as previously demonstrated in the work of Xiang et al. (3). This is in line with the present investigation.

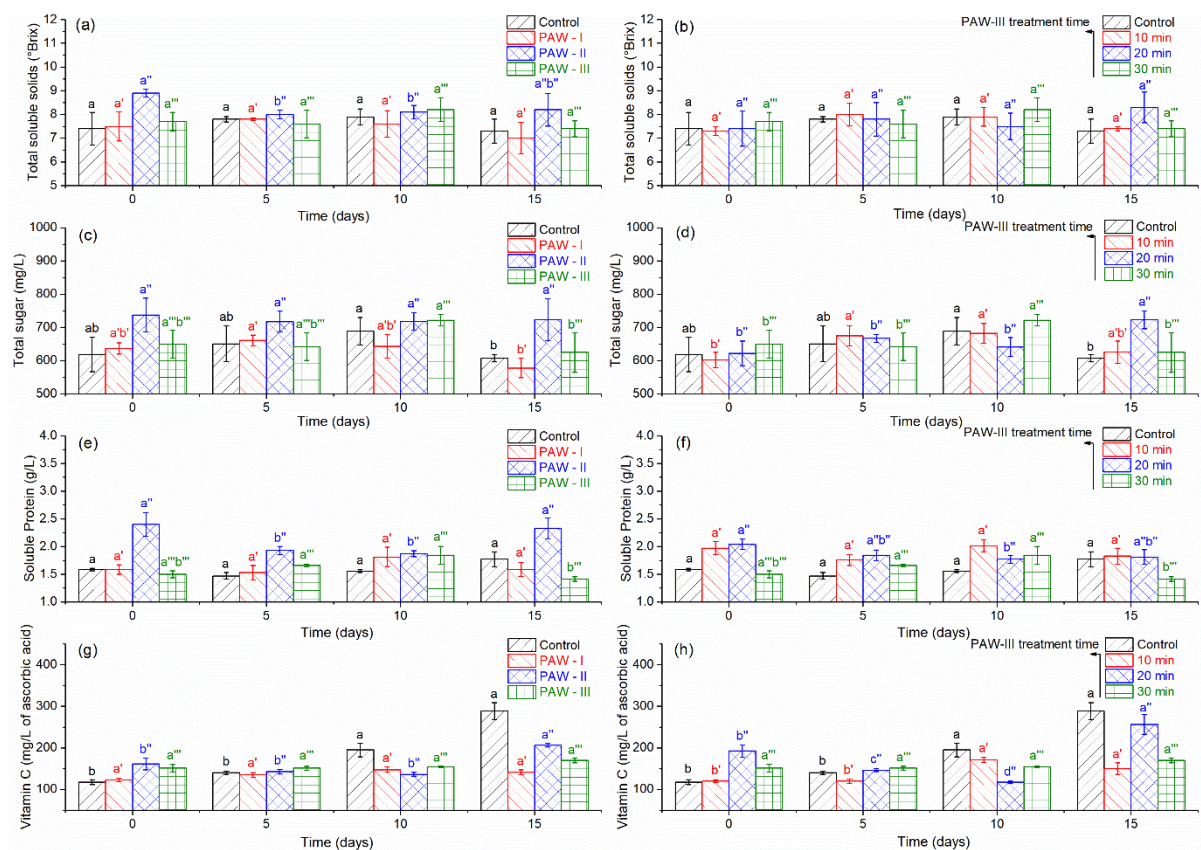


Figure 8. Variation in total soluble solids, total sugar, soluble protein, and vitamin C of *Citrus limon* Juice during storage after control and PAW treatment.

Antioxidant activity

The antioxidant activity of *C. limon* juice was analyzed based on phenols, flavonoids, ABTS⁺, and DPPH[•]. The variation in the concentration of phenols and flavonoids during storage is shown in Figure 9 (a-d). Both the control and PAW-treated *C. limon* juice showed an increase, decrease, or remained unchanged in phenol and flavonoid concentration during storage (Figure 9 (a, c)).

The ABTS⁺ and DPPH[•] antioxidant activity of the control and PAW-treated *C. limon* juice either decreased over time or showed a fluctuation, as shown in Figure 9 (e, g). Previous research did not find significant changes in these ABTS⁺ and DPPH antioxidant activities in

food after PAW treatment (3, 25). Additionally, the ABTS⁺ and DPPH[•] activities decreased over storage, which is consistent with the findings of this investigation (25).

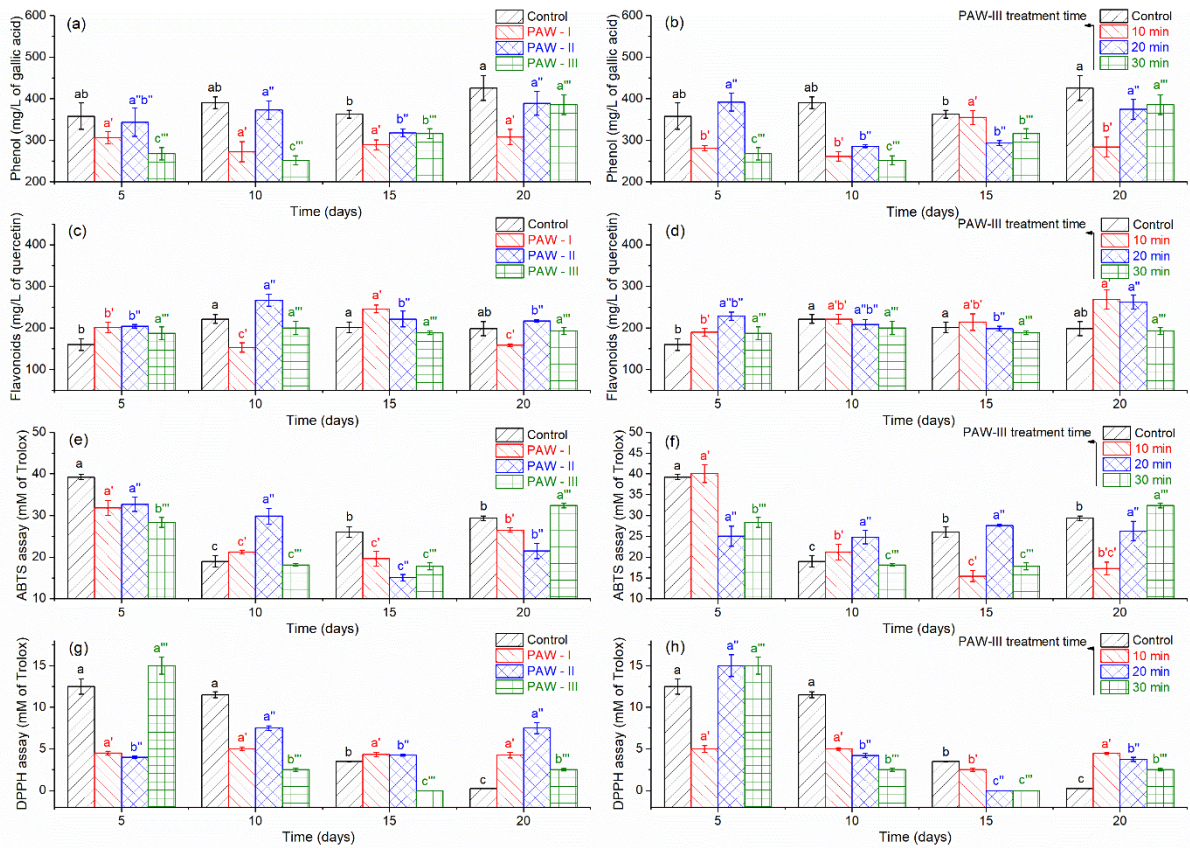


Figure 9. The change in phenols, flavonoids, ABTS assay, and DPPH assay of *Citrus limon* juice during storage after control and PAW treatment.

3.3.2 Role of PAW treatment time on *C. limon* shelf life

The results in the previous section showed that PAW-III is superior to PAW-I, PAW-II, and the control in enhancing the shelf-life of *C. limon*. PAW-III treatment resulted in higher microbial reduction, reduced spoilage, and better physical attributes. The previous section also discussed the effect of increasing plasma-water treatment time (1 hour for PAW-I, 2 hours for PAW-II, and 4 hours for PAW-III) on the shelf life of *C. limon*. This section further investigates the significance of PAW treatment time on *C. limon* using PAW-III. The results of the 30-

minute PAW-III treatment with *C. limon* were promising, so this section looks at the role of lower treatment times, such as 20 minutes and 10 minutes, respectively.

A 10-minute PAW-III treatment on *C. limon* showed similar sensory attributes (smell, appearance, taste, and overall acceptability) as the 30-minute treatment, as shown in Table 1. The microbial load reduction on the *C. limon* surface after a 10-minute PAW-III treatment was lower than the 30-minute treatment but was similar to PAW-II and higher than PAW-I, as shown in Figure 4. Additionally, the weight loss and surface firmness of *C. limon* after 10 minutes of PAW-III treatment was similar to the 30-minute treatment, as shown in Figure 5 (b, d). Furthermore, there was no spoilage of *C. limon* during the storage period after a 10-minute PAW-III treatment, as shown in Figure 5 (f).

However, the degreening of *C. limon* over storage after a 10-minute treatment was substantially higher compared to the 30-minute treatment, as shown in Figure 6 (d). In addition, a higher titrable acidity was found in the *C. limon* after 10 minutes of treatment compared to the 30-minute treatment, as shown in Figure 7 (d).

The concentration of soluble solid, sugar, and ascorbic acid showed similar concentrations in both the 10-minute and 30-minute PAW-III-treated *C. limon*. However, a 20-minute PAW-III treatment significantly increased these solids, sugar, and vitamin C concentrations in *C. limon*, as shown in Figure 8 (b, d, f). Furthermore, the concentration of soluble protein in the 30-minute PAW-III treatment was significantly lower than that in the 10-minute and 20-minute treatments, as shown in Figure 8 (h).

The phenol and ABTS antioxidant activity increased with increasing PAW-III treatment time on *C. limon*, while the flavonoid and DPPH antioxidant activity decreased with PAW-III treatment time, as shown in Figure 9 (b, d, f, h).

4. Discussion

The current study investigates the effectiveness of plasma activated water (PAW) in extending the shelf life of *C. limon*. The quality of the treated *C. limon* was evaluated based on factors such as color, sensory changes, nutritional analysis, and antioxidant activity. PAW is a widely recognized antimicrobial solution with the ability to inactivate bacteria, fungi, viruses, and pests, as reported in previous literature (31, 32, 49, 50). Microbial infection is a major factor in the spoilage of *C. limon*, and PAW has been used to improve its shelf life by reducing the microbial load. Previous research has shown that PAW treatment can effectively reduce microbial growth. For example, Liu et al. (25) found that PAW reduced microbial growth to less than 1 log₁₀ CFU g⁻¹ from 2.3 log₁₀ CFU g⁻¹ on fresh-cut apples. Ma et al. (15) demonstrated a maximum microbial reduction of 1.1 log CFU g⁻¹ in Chinese bayberries during storage using PAW. Xu et al. (14) showed that PAW produced using a 10-minute plasma treatment time resulted in a 1.5 log reduction in button mushroom microbial growth. The current study also showed a substantial reduction in the microbial load on *C. limon* after PAW treatment during storage, in agreement with previous research findings (Figure 4). This resulted in a significant improvement in *C. limon* spoilage during storage, with a storage duration of up to 60 days (Figure 1). The study showed similar results to the work of Ma et al. (15), which found that PAW-treated Chinese bayberries had less spoilage (21% to 25%) compared to the control (~46%).

The antimicrobial efficacy of PAW is due to the formation of various reactive species as a result of plasma-water interaction. The plasma generates high energy species and radicals, as demonstrated by the emission spectra of air plasma (species such as N₂, N₂⁺, etc. (Figure 2 (b)). These reactive species interact with water to form stable reactive oxygen-nitrogen species in the water. The formation of these species occurs through three major steps (14, 21, 23, 24, 29, 32, 36, 38, 39).

- Dissociation of O₂, N₂, and H₂O (surrounding moisture) in air plasma leads to the formation of radicals such as O, N, ·H, ·OH, etc.
- Recombination occurs at the plasma-liquid interface to form O₃, N_xO_y, H₂O₂, etc.
- In the aqueous phase, NO₃⁻, NO₂⁻, H₂O₂, and dissolved O₃ are formed through the dissolution of gases such as O₃, N_xO_y, H₂O₂, etc. in water and subsequent reactions among them (Figure 3 (c, d)).

The above text discusses the antimicrobial efficacy of PAW, which is attributed to the reactive species formed during the plasma-water interaction. With increasing plasma-water treatment time, the concentration of these reactive species increases and the resulting PAW shows improved physical and chemical properties. The PAW-III treatment (4 hours) was found to have higher microbial reduction compared to other treatments and the control. The PAW treatment also delays the degreening of the *C. limon*, enhances its shelf life, and does not significantly impact its nutritional values and antioxidant activity.

Based on the results, it can be concluded that PAW treatment is more effective for food preservation than direct plasma treatment or chemical treatment. This is because the latter methods can lead to etching, degradation of the food surface, dehydration, and a decrease in quality. Additionally, PAW is a more environmentally friendly and economically viable alternative to traditional food preservation techniques, as it doesn't leave any toxic residue on the food or enter the wastewater during food processing (15, 27). The only cost associated with producing PAW is the electrical discharge power, which ranges from a few watts to less than 100 W (23, 32, 37).

5. Conclusion

The study has demonstrated the effectiveness of plasma activated water (PAW) in improving the shelf life of *C. limon*. The PAW treatment was found to reduce the microbial load on the

C. limon surface, leading to less spoilage and improved sensory properties in comparison to the control. It is observed that 10-minute PAW treatment was sufficient to enhance the shelf life of *C. limon*. The important finding was the PAW treatment did not have any negatively impact on the nutritional value or antioxidant activity of the *C. limon* juice. This can makes plasma activated water a promising alternative to conventional food preservatives and a potential solution to reducing food spoilage during storage to meet future global food demand.

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Conflict of interests

The authors declare that there are no conflicts of interests

Author Contributions

V.R. and S.K.N. were involved in the conceptualization and design of the study. V.R. handled material preparation, data collection, and analysis. V.R. wrote the initial draft of the manuscript and both authors provided feedback on subsequent versions. The final manuscript was reviewed and approved by both authors

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