Investigation the role of Plasma-Activated Water Washing and Storage on the Shelf Life of Cestrum nocturnum L. (Night-Blooming Jasmine) Flowers

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Research Article

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Title

Investigation the role of Plasma-Activated Water Washing and Storage on the Shelf Life of *Cestrum nocturnum* L. (Night-Blooming Jasmine) Flowers

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Abstract

This study investigates the impact of Plasma-Activated Water (PAW) washing and storage on the shelf life of Night-Blooming Jasmine (NBJ) flowers (*Cestrum nocturnum* L.). NBJ flowers, known for their fragrant properties and diverse applications, have a short natural shelf life. PAW is generated using a pencil plasma jet with varying plasma treatment times (10 to 30 minutes) and is utilized for washing and storing NBJ flowers. The flowers undergo microbial analysis, sensory evaluation, weight loss assessment, spoilage evaluation, color analysis, and antioxidant activity measurement after PAW washing and storage.

PAW exhibits an increasing acidity and oxidative potential, along with a higher concentration of reactive species (NO_3^- ions, NO_2^- ions, H_2O_2, dissolved O_3) with longer plasma treatment times. Washing NBJ flowers with PAW significantly reduces microbial growth, weight loss, and spoilage, maintaining better sensory properties compared to control-washed flowers. However, PAW, when used as a storage medium, negatively impacts the flowers, causing oxidative damage, compromised membrane integrity, and higher spoilage. PAW-washed NBJ flowers retain color intensity and delay browning while preserving antioxidant activity.

PAW, as a washing medium, proves effective in preserving the quality of flowers. Caution is advised when using PAW as a storage medium, as prolonged exposure leads to oxidative damage and compromises the overall quality of flowers. Further optimization of plasma treatment times is crucial for balancing the positive and negative effects of PAW on flower preservation.

Keywords: Plasma Activated Water, reactive oxygen-nitrogen species, night blooming jasmine flowers, shelf-life enhancement, washing medium

1. Introduction

Night-Blooming Jasmine (NBJ) (*Cestrum nocturnum* L.) flowers are renowned for their mesmerizing and heavy fragrance, which permeates the environment during the evening and night. Commonly known as the queen of the night, lady of the night, or night-scented jessamine, these flowers are native to West Indies, Central and South America, as well as found in regions like South Asia (India, Bangladesh, Southern China), Australia, and tropical/subtropical areas worldwide (1-6).

NBJ flowers are soft and fragrant (7), appearing greenish white or light greenish yellow. The calyx, the green part at the bottom with five teeth, is smaller (about one-third) compared to the main flower part (corolla) measuring 2.0–2.5 cm. The corolla lobes, or petal edges, are approximately 5–6 mm long, somewhat blunt, and can either stick up or spread out (4, 8). The flowers are valued for their uses in aromatherapy, ornamentation, decoration, antimicrobial properties against bacteria, fungi, viruses, food preservation, and traditional medicinal applications, especially in South Asian countries like China, India, Bangladesh, and Thailand, etc. (1-4, 6, 7). Medicinally, NBJ flowers have been used for various purposes such as treating abdominal pain, conjunctivitis, fever, diarrhea, uterine bleeding, toothache, asthma, abscess, arterial hypotension, acting as an antispasmodic, dyspeptic, and potentially against various cancers like breast cancer and malignancy tumors, etc. (3, 6, 7) These applications are attributed to the presence of bioactive compounds like essential oils, phenols (e.g., eugenol), alkaloids, fatty acids, alcohols (phenylethyl alcohol, benzyl alcohol), flavonol glycosides, benzaldehyde, cytotoxic steroids, etc. (1, 2, 4, 6)

Despite their significance, naturally falling NBJ flowers have a short shelf life, lasting only a few hours to a day. Preserving them requires maintaining suitable conditions such as temperature, moisture, dry storage, and protection from direct light exposure, among other

factors. The microbial load on the flowers also contributes to spoilage. However, past literature lacks information on the preservation of post-harvested NBJ flowers. Hence, it become the motivation of the present investigation.

Some of the general techniques/methods used for the preservation of post-harvest flowers including low-temperature storage, drying, controlled atmosphere storage, and edible coatings, are known. However, specific preservation methods depend on factors like flower firmness, intended use, membrane intensity, temperature sensitivity, and susceptibility to dry/humid conditions and microbial infection, etc. (9-14)

This study explores a novel technique for enhancing the shelf life of flowers, specifically Night-Blooming Jasmine, using plasma-activated water (PAW). The use of PAW for flowers preservation or enhancement of shelf-life reported for the first time in present investigation as per best of authors literature survey. The study aims to unravel the role of PAW in augmenting the shelf life of NBJ flowers. Two distinct PAW preservation modes are implemented to enhance the flowers' shelf life. In the first mode, PAW acts as a washing medium, exposing NBJ flowers to PAW for a brief period. In the second mode, PAW serves as a storage medium, where NBJ flowers are kept and their shelf life meticulously studied.

Plasma-activated water (PAW) technology has gained global attention in the past decade due to its versatile applications spanning healthcare, medicine, food preservation, and agriculture, etc.(15-36) Plasma, recognized as the fourth state of matter, interacts with water, leading to changes in its physicochemical properties through the formation and dissolution of various reactive species (37-42). The resulting water, modified by plasma on a physiochemical level, is termed plasma-activated water (PAW) (15, 41, 43-46). The technology encompassing the use of PAW, including pathogen inactivation (fungi, viruses, bacteria, pests, etc.), food preservation, medicinal applications (wound healing, selective cancer cell killing, etc.), and

agricultural benefits (enhanced germination, nutrient provision to plants, etc.), is collectively known as PAW technology (15-23, 26, 27, 30, 31, 33, 44, 46-48). The interaction between plasma and water generates stable reactive species such as nitrate (NO_3^-) ions, nitrite (NO_2^-) ions, hydrogen peroxide (H_2O_2), dissolved ozone (O_3), peroxynitrite ($ONOO^-$) ions, etc. This combination of reactive species renders PAW a valuable product for a wide array of applications (15-36, 49).

In this study, PAW is prepared by subjecting it to different plasma treatment times. It is then employed as both a washing and storage medium to enhance the shelf life of Night-Blooming Jasmine (NBJ) flowers. The physicochemical changes in PAW, along with the identification and measurement of reactive species with increasing plasma treatment time, are discussed in detail. The health assessment of NBJ flowers following PAW washing or storage is based on changes in microbial load, weight loss, spoilage, color, sensory characteristics, bioactive compounds, and other relevant factors.

2. Material and Methods

2.1 PAW as Washing and Storage Medium for NBJ Flowers

The schematic in Figure 1 illustrates the production of plasma-activated water (PAW) with varying plasma treatment times using ultrapure milli-Q water for 10 minutes (PAW-10), 20 minutes (PAW-20), and 30 minutes (PAW-30). Plasma was generated using a device called a dielectric barrier discharge pencil plasma jet (DBD-PPJ) (27, 50). A volume of 100 ml of water was used for PAW production, with air serving as the plasma-forming gas.

The prepared PAW, along with controls (ultrapure milli-Q water (DM water)), was exposed to Night-Blooming Jasmine (NBJ) flowers, as depicted in Figure 1. PAW exposure with NBJ flowers was conducted in two different modes: PAW as a washing medium and PAW as a storage medium.

In the PAW as a washing medium mode, a 2-minute (120s) exposure/washing of NBJ flowers was carried out using PAW and control. After the washing period concluded, the NBJ flowers were transferred to a dry petri dish.

In the PAW as a storage medium mode, NBJ flowers were kept in PAW and control, as shown in Figure 1. The PAW and control-washed or stored flowers were covered with food-grade polyethylene film and stored in a refrigerator at 4 °C.

2.2 Dielectric Barrier Discharge Pencil Plasma Jet (DBD-PPJ) Setup

The DBD-PPJ operates based on the principle of dielectric barrier discharge, utilizing quartz as the dielectric, copper as the power electrode, and tungsten attached to a brass bucket as the ground electrode. This setup features a co-axial cylindrical configuration, with a central tungsten rod as the electrode and a hollow cylindrical copper assembly as the outer electrode. A quartz tube is tightly fitted inside the copper assembly, maintaining a distance of 1.2 mm between the inner surface of the quartz tube and the central electrode.

Air is supplied from the top of the DBD-PPJ setup using an air pump, with the airflow rate controlled and set at 20 liters per minute using an air flow controller. The air discharge occurs in the gap between the dielectric and central electrode, and the resulting plasma radicals/species are pushed downstream by the air flow, directing them towards the water interface. Additional details about the DBD-PPJ setup and its geometry can be found in supplementary material Figure S1.

The DBD-PPJ is powered by a high voltage high-frequency (HVHF) power supply (power rating 10 kVA, frequency range 10-40 kHz (operating frequency of present work – 40 kHz)) operating in the current limiter mode to prevent the transition of DBD discharge to arc discharge at atmospheric pressure. The schematic and details of the electrical characterization of plasma and determination of plasma species/radicals using optical emission spectroscopy

are presented in supplementary material Figure S2 and Table S1. The voltage-current waveform and emission spectra of air plasma are illustrated in supplementary material Figures S3 and S4. The calculated plasma discharge power was approximately 5 W, which was used for PAW production.

2.3 Physicochemical Properties and Reactive Species of PAW

The interaction between plasma and water induces changes in the physicochemical properties of water, including pH, electrical conductivity (EC), total dissolved solids (TDS), and oxidation-reduction potential (ORP). These alterations result from the formation and dissolution of species such as NO_3^- ions, NO_2^- ions, H_2O_2, dissolved O_3, etc. Measurements of pH, EC, TDS, and ORP were conducted using a digital pH meter, digital EC meter, digital TDS meter, and digital ORP meter, respectively (detailed specifications of these meters are provided in supplementary material Table S2).

The dissolved reactive species in PAW were determined semi-quantitatively using strip/colorimetry test kits and quantitatively using UV-visible spectroscopy (refer to supplementary material Figure S5). The concentration of NO_3^- ions was determined using UV screening methods, NO_2^- ions concentration using the Griess reagent system, dissolved O_3 concentration measured using indigo colorimetry methods, and H_2O_2 concentration determined using the titanium oxysulfate method (27, 30, 40, 50). Detailed information about these methods is available in supplementary material Table S3.

2.4 Preparation of NBJ Flowers

Night-blooming jasmine (NBJ) flowers are sustainably harvested from NBJ plants in Gandhinagar, Gujarat, India (23°14’28.5’’N, 72°37’32.0’’E). The flowers are carefully plucked to ensure the preservation of the plant and its natural environment. A total of 1200

NBJ flowers (600 for washing experiments and 600 for storage experiments) undergo washing with ultrapure milli-Q water to remove any settled dirt.

The NBJ flowers are organized into sets as follows: 150 for the control group (50 (set) × 3 (replicate)), 150 for PAW-10 (50 (set) × 3 (replicate)), 150 for PAW-20 (50 (set) × 3 (replicate)), and 150 for PAW-30 (50 (set) × 3 (replicate)). Each set comprises 50 flowers, and the entire process is replicated three times to gauge the repeatability of the results.

2.5 Microbial Analysis

A 100 mg sample of NBJ flowers was mixed with 10 ml of sterile phosphate-buffered saline (PBS) solution. The mixture was vortexed to facilitate the transfer of microbes into the solution. This solution was then mixed with nutrient broth, and serial dilutions were performed in an incubator at 37 °C. Microbial colonies were counted using a colony counter (21, 27, 30). The normalized microbial growth is calculated using the following expression:

$$Normalized\ microbial\ growth = \frac{(CFU/g)}{(CFU/g)_{max}} \quad (1)$$

2.6 Sensory Analysis

Sensory evaluation of NBJ flowers after PAW washing and PAW storage was conducted at regular intervals. These assessments encompassed visual inspection, aroma analysis, texture analysis, and overall quality. A group of 20 individuals, ranging in age from 25 to 55, rated these sensory properties on a scale of 1 to 9, where 1 corresponds to "very bad," 3 to "bad," 5 to "fine," 7 to "good," and 9 to "very good," respectively.

2.7 Spoilage and Weight Loss

NBJ flowers that displayed a dull appearance and experienced a significant loss of aroma were deemed spoiled in the present investigation. The spoiled NBJ flowers are continually monitored at regular intervals, and the expression used to calculate the spoilage percentage is given by:

$$\%\, Spoilage = \left(\frac{N_i - N_t}{N_i}\right) \times 100 \quad (2)$$

Where N_i represents the initial number of unspoiled NBJ flowers,

N_t represents the unspoiled NBJ flowers at time 't' (days).

Expression used to calculate weight loss is given as:

$$Weight\ loss\ (\%) = \left(\frac{W_i - W_t}{W_i}\right) \times 100 \quad (3)$$

Where W_i represents the initial weight of NBJ flowers,

W_t represents the NBJ flowers at time 't' (days).

2.8 CIELAB Color Analysis

The color analysis of NBJ flowers was performed using a colorimeter (model number NR100). The measured color levels were represented by L* × a* × b*, where L* represents lightness and darkness, a* represents greenness and redness, and b* represents blueness and yellowness, respectively.

The expression used to calculate the color difference of NBJ flowers over time is given by:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

The expression used to calculate the chromatic aberration index (C*) is given by:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

The hue angle of color is calculated by the following expression:

$$h_{ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (6)$$

2.9 Phenol and Flavonoids Analysis

To analyze phenol and flavonoids, 100 mg of Night-blooming jasmine (NBJ) flowers were subjected to extraction using ethanol as the solvent. The NBJ flowers were combined with 2 ml of warm aqueous 80% ethanol (volume/volume). The resulting solution with NBJ flowers underwent heating in a boiling water bath for 10 minutes. Subsequently, the mixture was centrifuged at 12,000 rpm for 10 minutes, and the supernatant was collected and cooled in an ice bath for phenol and flavonoids analysis.

For total phenol analysis, 0.1 ml of the extracted supernatant was mixed with 0.5 ml of a folin-ciocalteu reagent (diluted 1:10 with water) and incubated for 1 minute. After the incubation period, 0.4 ml of 7.5% sodium carbonate was added and further incubated for 60 minutes. The absorbance of the solution was measured at 765 nm, and the phenol concentration was determined using a standard curve prepared with gallic acid (21, 51).

For flavonoids analysis, 0.1 ml of the extracted supernatant was mixed with 0.5 ml of water and 30 µl of 1:20 weight/volume sodium nitrate. After a 5-minute incubation period, 1:10 weight/volume aluminium chloride was added, and the solution was further incubated for 6 minutes at room temperature. Subsequently, 0.2 ml of 1 M sodium hydroxide was added, and the solution was vortexed. Finally, the absorbance was recorded at 510 nm, and the flavonoids concentration was determined using a standard curve prepared with quercetin (21, 52).

2.10 Data Analysis

All experiments were conducted at least three times, and the results were expressed as the mean ± standard deviation ($\mu \pm \sigma$). Statistical analysis of the results was performed using analysis of variance, followed by a post-hoc test named Fisher's Least Significant Difference (LSD).

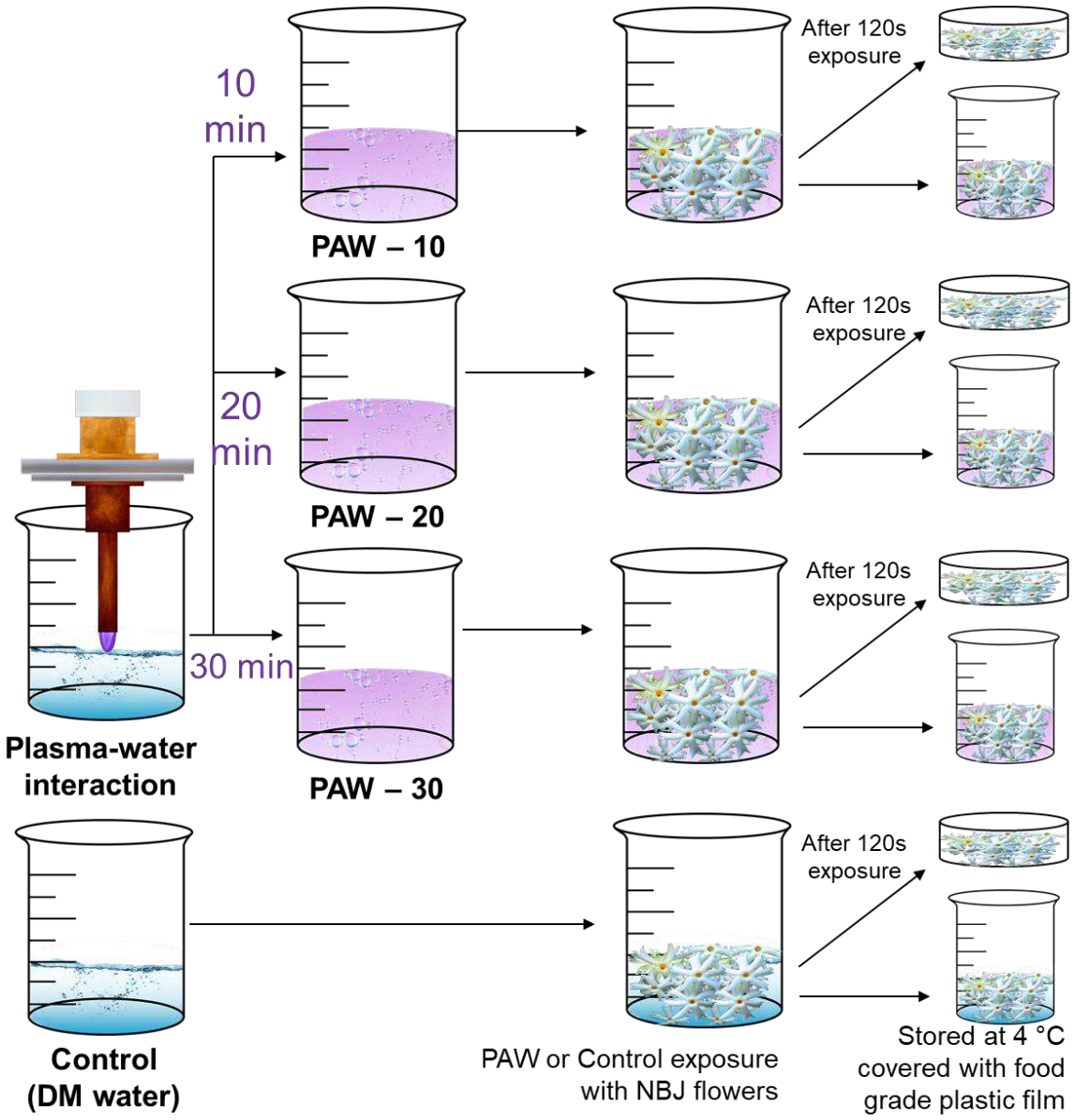

Figure 1. Schematic of the preparation of PAW with different plasma treatment. The exposure/treatment of NBJ flowers with prepared PAW and control. A portion of NBJ flowers removed after 120s, named as PAW/control-washed, and the remaining NBJ flowers in PAW/control are named as PAW/control-stored.

3. Results and Discussion

3.1 Physicochemical Properties and Reactive Species

Figure 2 shows the alterations in the physicochemical properties of plasma-activated water (PAW) and the concentration of reactive oxygen-nitrogen species (RONS) with varying plasma

treatment times. As the plasma treatment time increases, the water becomes progressively more acidic, coupled with a significant elevation in the oxidizing nature (oxidation-reduction potential (ORP)) of the water (Figure 2 (a)). The decreasing pH and heightened oxidizing potential of PAW contribute to its antimicrobial activity by oxidizing the cell walls of pathogenic microbes, leading to microbial inactivation post-PAW treatment. Following 30 minutes of plasma treatment, the pH decreased from 6.9 to 4.9, and the oxidizing potential of water increased from 250 mV to 385 mV. This pH reduction and ORP increase after plasma-water interaction align with previous studies by Thirumdas et al. (53), ten Bosch et al. (48), Laurita et al. (54), Shen et al. (55), Lukes et al. (56), Xiang et al. (57), Hongzhuan et al. (46), and Tian et al. (58), respectively.

As the pH of water decreases after plasma-water interaction, this is attributed to the dissolution of various reactive oxygen-nitrogen species (RONS). These dissolved ions also contribute to the increase in electrical conductivity (EC) and dissolved solids (TDS) of PAW, as shown in Figure 2 (b). Ultrapure milli-Q water exhibits non-conductivity with an EC of 1 $\mu S\ cm^{-1}$ and is free from dissolved impurities, as indicated by a TDS value of 0 ppm. Following 30 minutes of plasma treatment, the EC and TDS of PAW rose to 170 $\mu S\ cm^{-1}$ and 95 ppm, respectively. These increases in EC and TDS signify the formation and dissolution of ionic compounds in PAW due to plasma-water interaction (25, 53, 55, 57, 58). Dominating dissolved ionic species include H^+, NO_3^- ions, and NO_2^- ions, occurring in the form of nitric and nitrous acid, contributing to the observed decrease in pH after plasma interaction (25, 53, 55, 57, 58) as observed in Figure 2 (a). Previous reports by Thirumdas et al. (53), Tian et al. (58), and Xiang et al. (57), etc. have also documented an increase in water EC after plasma treatment.

The measured concentration of dissolved NO_3^- and NO_2^- ions in PAW is presented in Figure 2 (c). Both NO_3^- and NO_2^- ions exhibited a consistent increase in concentration with plasma treatment time. However, the concentration of dissolved NO_3^- ions (13.8 mg L^{-1} at 30

minutes) was substantially higher compared to NO_2^- ions (1.4 mg L^{-1} at 30 minutes). This discrepancy is attributed to the higher stability of NO_3^- in PAW compared to other dissolved species like NO_2^-, H_2O_2, and dissolved O_3, as indicated in equations (7-11) (27, 37, 43, 49, 59-61).

$$NO_2^- + H_2O_2 \rightarrow NO_3^- + H_2O \quad (7)$$

$$NO_2^- + O_3(aq.) \rightarrow NO_3^- + O_2 \quad (8)$$

$$H_2O_2 + O_3(aq.) \rightarrow HO\cdot + HO_2\cdot + O_2 \quad (9)$$

$$2HO\cdot \rightleftharpoons H_2O_2 \quad (10)$$

$$2HO_2\cdot \rightleftharpoons H_2O_2 + O_2 \quad (11)$$

As H_2O_2 and dissolved O_3 react with each other and NO_2^- ions, their concentrations decrease, as observed in Figure 2 (d). These reactions are more pronounced in a highly reactive environment, characterized by low pH and high oxidizing potential, which occurs at longer plasma treatment times (27, 50, 54, 56, 61). This phenomenon explains the observed rise and fall behavior in H_2O_2 and dissolved O_3 concentrations. Initially, their concentrations increase with time, and later, they decrease due to the reactions outlined in equations (7-11).

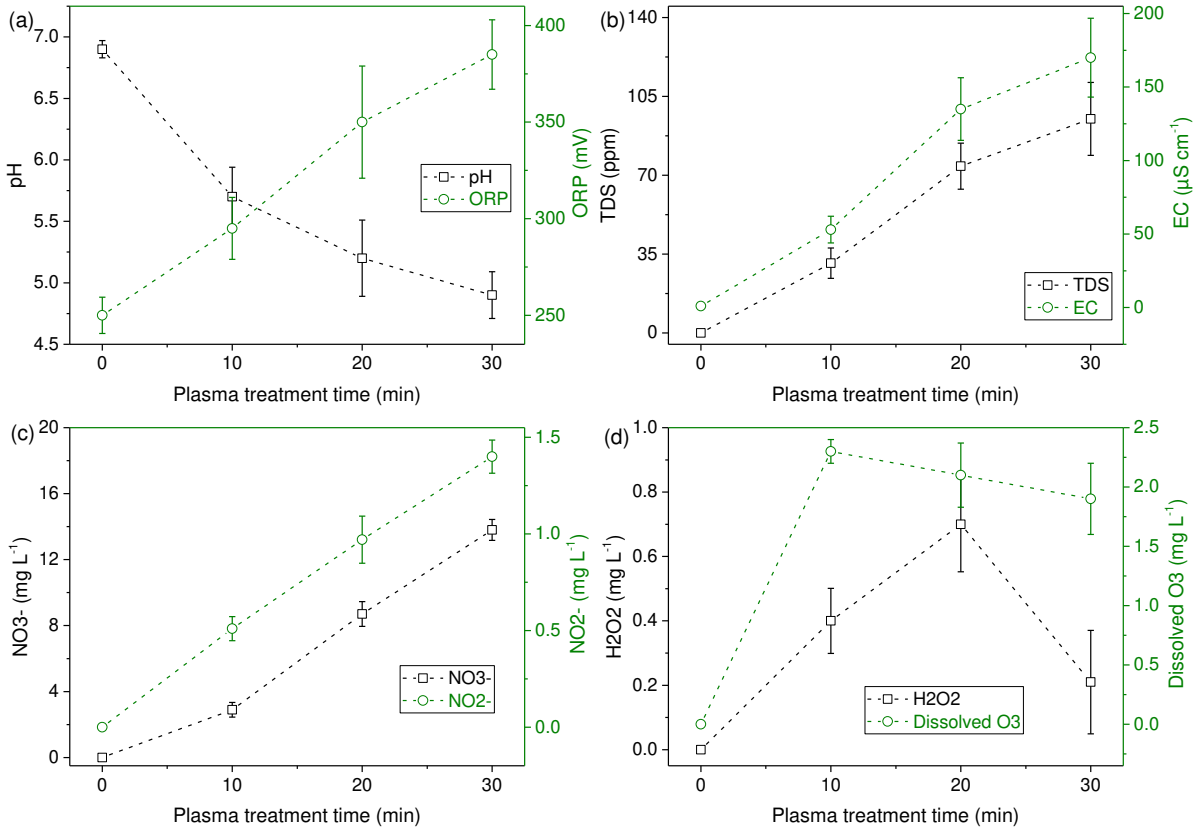

Figure 2. (a, b) Physicochemical properties (pH, oxidation-reduction potential (ORP), total dissolved solids (TDS), electrical conductivity (EC)) and (c, d) concentrations of reactive species (NO_3^- ions, NO_2^- ions, H_2O_2, Dissolved O_3) in PAW with increasing plasma treatment time.

3.2 NBJ Flowers Over Storage Duration

Figures 3 and 4 depict images of NBJ flowers when washed with PAW/control and stored in PAW/control over the storage duration. The PAW-washed flowers appear relatively fresh compared to control-washed flowers over the storage period, as illustrated in Figure 3. Furthermore, at the end of 15 days, the control-washed NBJ flowers are completely spoiled and covered with microbes. In contrast, the PAW-washed flowers remain in much better condition even after 15 days, showing no signs of complete spoilage or microbial coverage.

However, the use of PAW as a storage medium had a negative impact on NBJ flowers preservation, as indicated in Figure 4. PAW oxidized the NBJ flowers during storage, causing

ruptures that released various bioactive or organic compounds from the NBJ flower membranes. Consequently, the color of PAW-stored NBJ flowers significantly differed from control-stored NBJ flowers. PAW-stored NBJ flowers appeared brownish, while control-stored NBJ flowers maintained a greenish-yellow color closer to the original NBJ flower color. Previous studies by Sajib et al. (62) and Sivachandiran et al. (63) reported the negative effects of PAW on seeds and plants. Sajib et al. (62) found that higher plasma treatment times for PAW production negatively affected black gram seed germination and plant growth. Sivachandiran et al. (63) emphasized the importance of optimizing plasma treatment time for seeds since higher treatment damaged the seeds, leading to reduced germination and plant growth.

After 20 days of storage, PAW-stored NBJ flowers showed a substantially higher microbial load compared to control-stored NBJ flowers, as shown in Figure 4. Despite PAW's known antimicrobial properties, the observed results did not support PAW's antimicrobial behavior. The possible reason for this outcome could be the various nutrient compounds released by the damaged NBJ flowers, which supported microbial growth and neutralized the antimicrobial activity of PAW. Additionally, PAW is a rich source of nitrogen, which also promotes microbial growth, as observed in Figure 4.

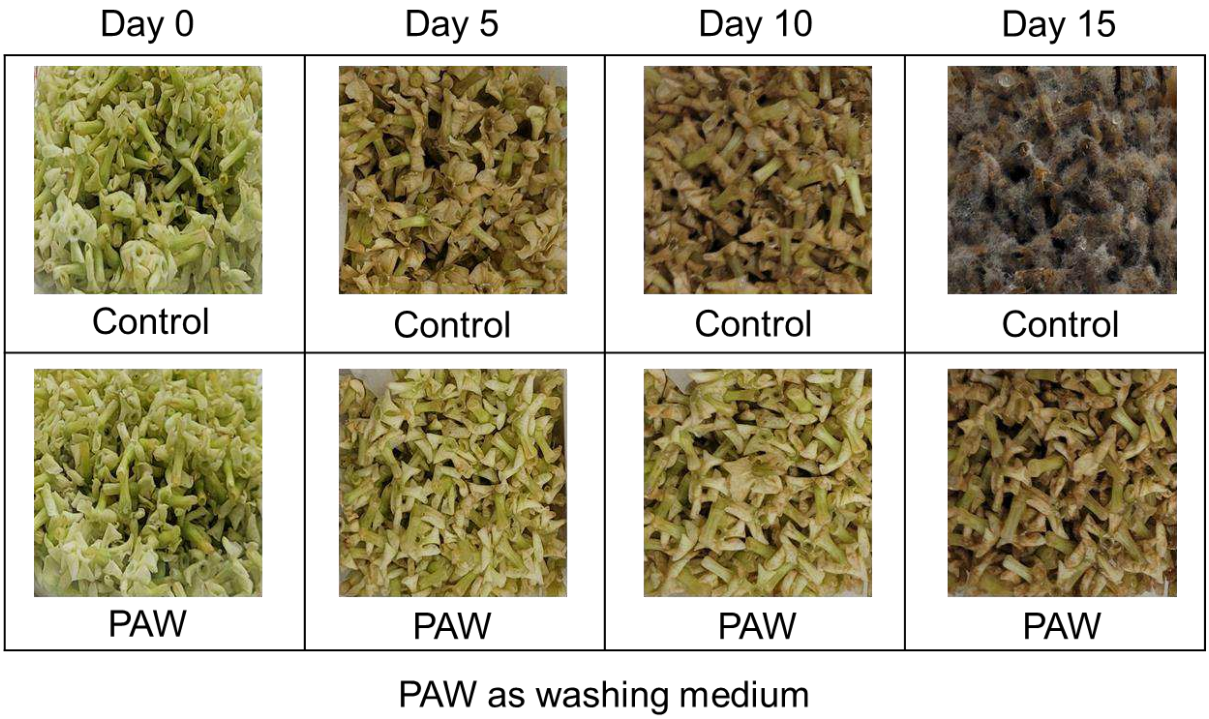

Figure 3. The images of PAW- and control-washed NBJ flowers with increasing storage time.

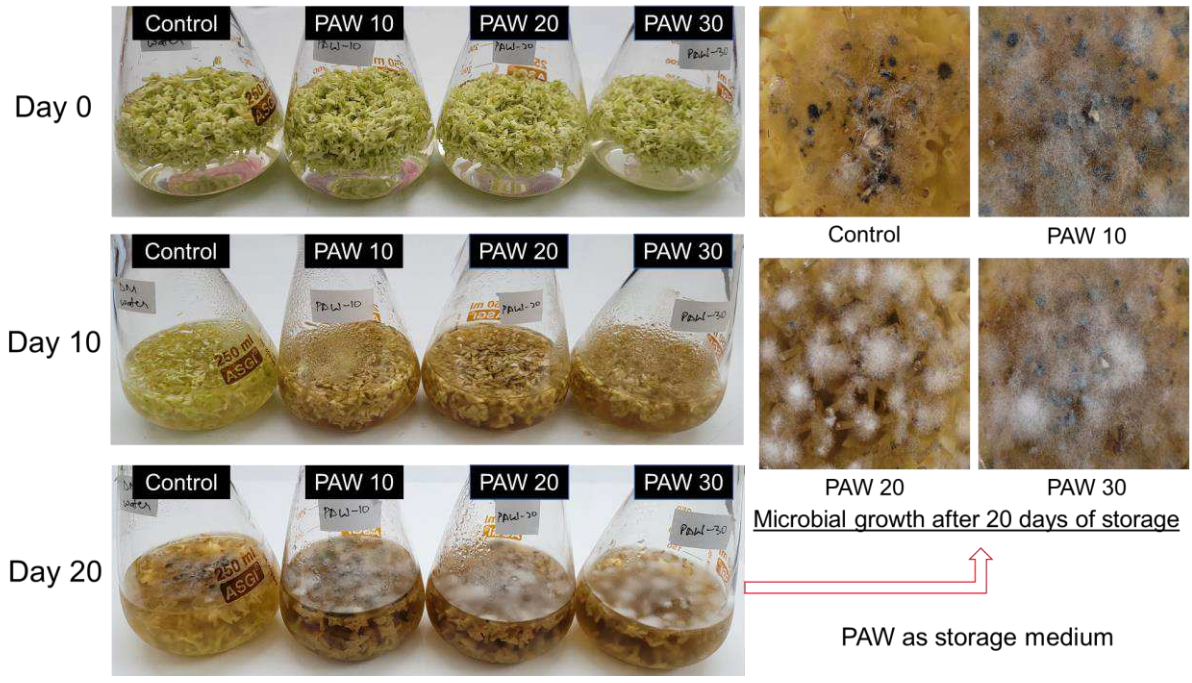

Figure 4. The images of PAW- and control-stored NBJ flowers with increasing storage time.

3.3 Microbial Growth on NBJ Flowers

Figure 5 illustrates the microbial growth on NBJ flowers after PAW washing and PAW as a storage medium. PAW washing of NBJ flowers significantly reduces microbial growth compared to the control, as depicted in Figure 5 (a). At the end of 15 days of storage, the normalized microbial growth after PAW-10, PAW-20 and PAW-30 washing of NBJ flowers were 0.71, 0.61 and 0.66 in comparison to control. However, using PAW as a storage medium for NBJ flowers shows high microbial growth compared to the control, as observed in Figure 5 (b). The quantitative estimation of microbial growth (Figure 5) aligns with the observed images in Figures 3 and 4.

The various reactive oxygen and nitrogen species (RONS) present in PAW, such as H_2O_2, dissolved O_3, etc., and the acidic environment of PAW act as antimicrobial agents during PAW washing, leading to a substantially lower observed microbial count compared to the control (Figure 5 (a)) (24-26, 33, 35, 55). However, these RONS, acidic and oxidizing environment when exposed to longer duration with NBJ flowers had a negative impact on health of NBJ flowers as observed when PAW was used as storage medium (Figure 5 (b)) (62, 63).

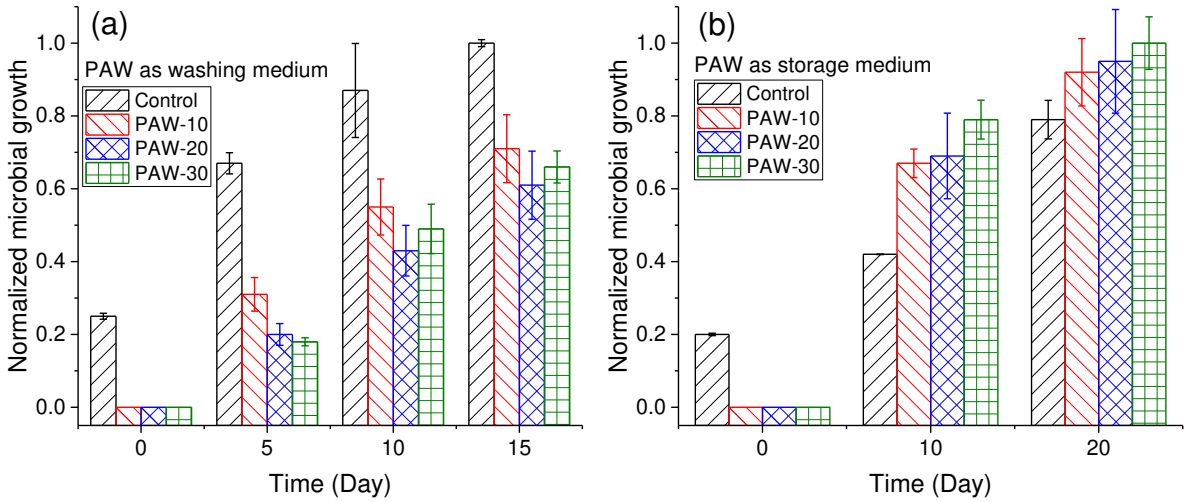

Figure 5. Normalized microbial growth on NBJ flowers with increasing storage time when (a) PAW is used as a washing medium and (b) PAW is used as a storage medium.

3.4 Sensory Evolution of NBJ Flowers

The sensory properties chart, including visual inspection, aroma analysis, texture analysis, and overall quality of NBJ flowers when washed with PAW, is shown in Figure 6. Figure 6 (a) demonstrates that PAW washing significantly enhances the appearance of NBJ flowers compared to control washing. Even after 5 days of storage, visual inspection showed a no significant ($p > 0.05$) reduction in the appearance of NBJ flowers.

The aroma analysis aligns with the results of visual inspection, indicating that PAW-washed NBJ flowers have a better aroma compared to control-washed, as shown in Figure 6 (b). Additionally, PAW washing helps in retaining the firmness of NBJ flowers compared to control, as shown in Figure 6 (c) (texture analysis). Hence, overall, PAW washing enhances the shelf-life of NBJ flowers, as depicted by the overall quality analysis (Figure 6 (d)).

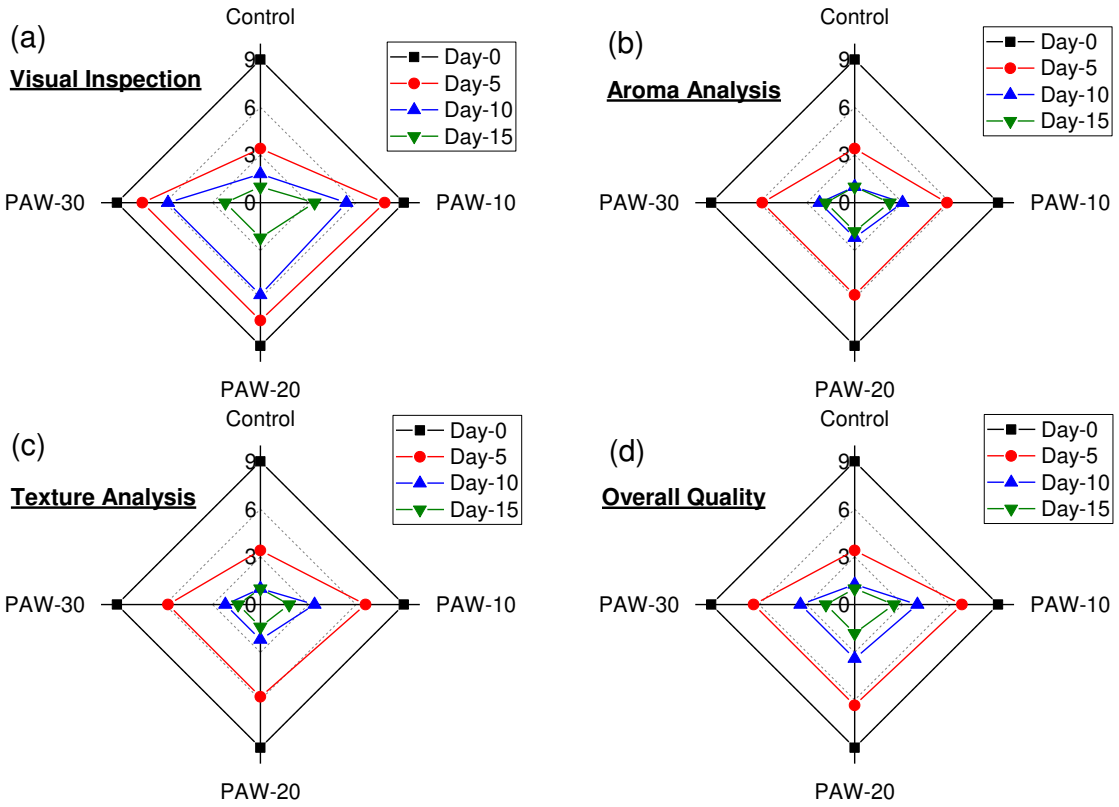

Figure 6. Sensory properties ((a) visual inspection, (b) aroma analysis, (c) texture analysis, and (d) overall quality) of NBJ flowers over time after PAW washing.

3.5 Weight Loss and Spoilage of NBJ Flowers

Figure 7 (a, b) shows the weight loss and spoilage of NBJ flowers over the storage duration when PAW was used as a washing medium and as a storage medium. The weight loss of NBJ flowers over storage, when washed with PAW, was lower than control (25.3% (PAW-10), 20.9% (PAW-20) and 23.9% (PAW-30) lower compare to control at the end storage duration), signifying less compromise in the membrane integrity of PAW-washed flowers compared to control. Hence, washing NBJ flowers with PAW significantly reduces moisture loss during storage and maintains the freshness of the flowers (Figure 7 (a)). Moreover, longer exposure of PAW to NBJ flowers (PAW as a storage medium) significantly compromises the membrane integrity, resulting in higher weight loss compared to control as a storage medium (Figure 7 (b)).

Similar results were observed in NBJ flowers spoilage analysis (Figure 7 (c, d)), where higher spoilage was observed in control compared to PAW washing (100% (control), 73% (PAW-10), 85% (PAW-20), 92% (PAW-30) spoilage at the end of storage duration). PAW washing retains the membrane integrity and reduces the microbial load, helping in lower NBJ flowers spoilage (Figure 7 (c)). However, the use of control and PAW as a storage medium is not suitable for NBJ flowers preservation due to a high percentage of spoilage during storage (Figure 7 (d)).

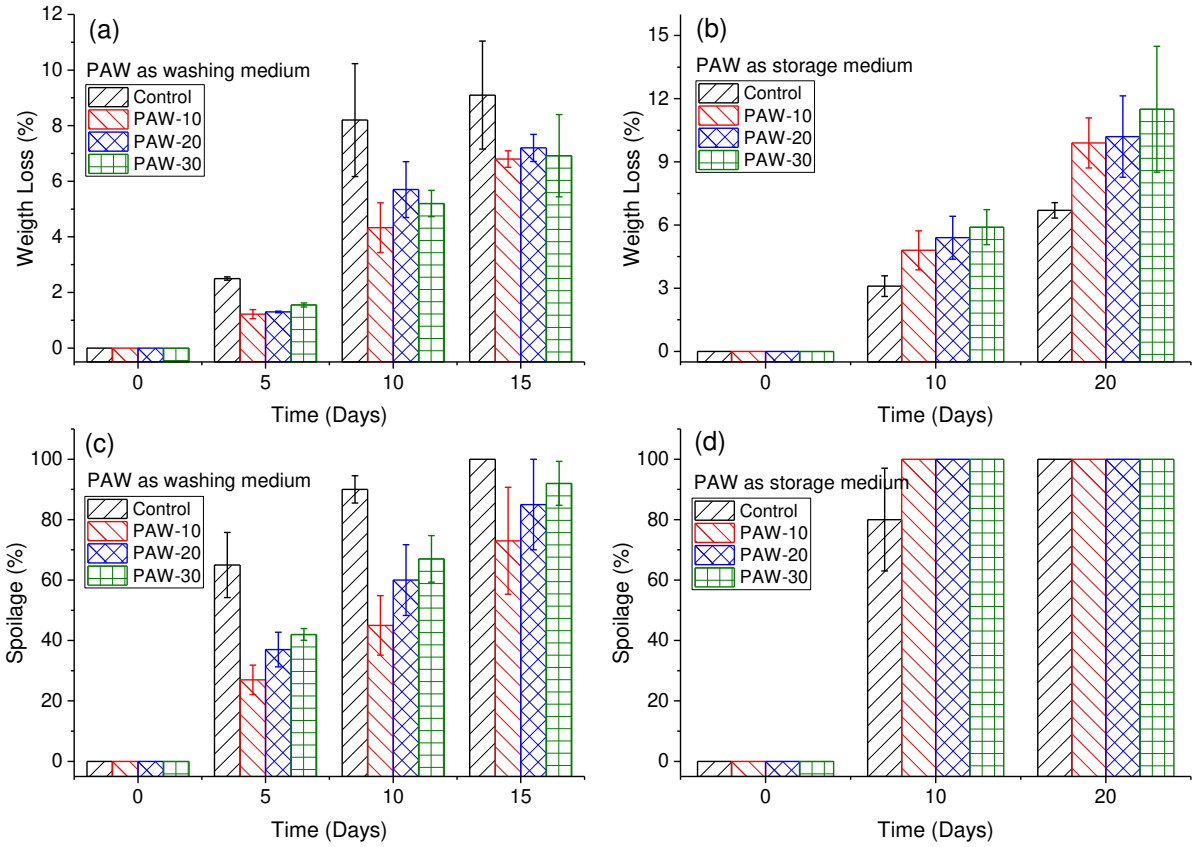

Figure 7. (a, b) Weight loss and (c, d) spoilage of NBJ flowers over time when washed with PAW and stored in PAW.

3.6 Color Characteristics of NBJ Flowers

Color is a key attribute for analyzing the freshness of flowers. The change in color parameters, such as L* (lightness: 100 to darkness: 0), a* (greenness: (-) to redness: (+)), b* (blueness: (-) to yellowness: (+)), ΔE* (color difference), C_{ab}* (chroma, relative saturation), and h_{ab} (hue angle) when PAW was used as a washing medium, is shown in Figures 8. Color analysis of NBJ flowers when PAW was used as a storage medium is shown in supplementary material Figure S6.

PAW washing delays the toning down of NBJ flowers compared to control, as shown in Figure 8 (a). Additionally, PAW-washed NBJ flowers showed lesser redness (Figure 8 (b)) and higher yellowness (Figure 8 (c)) compared to control, signifying a delay in browning or

decolorization of flowers compared to control washing. Moreover, the observed color difference in PAW-washed NBJ flowers is relatively low compared to control-washed flowers (Figure 8 (d)). The PAW-washed NBJ flowers showed better color intensity compared to control based on chroma and hue angle analysis (Figure 8 (e, f)).

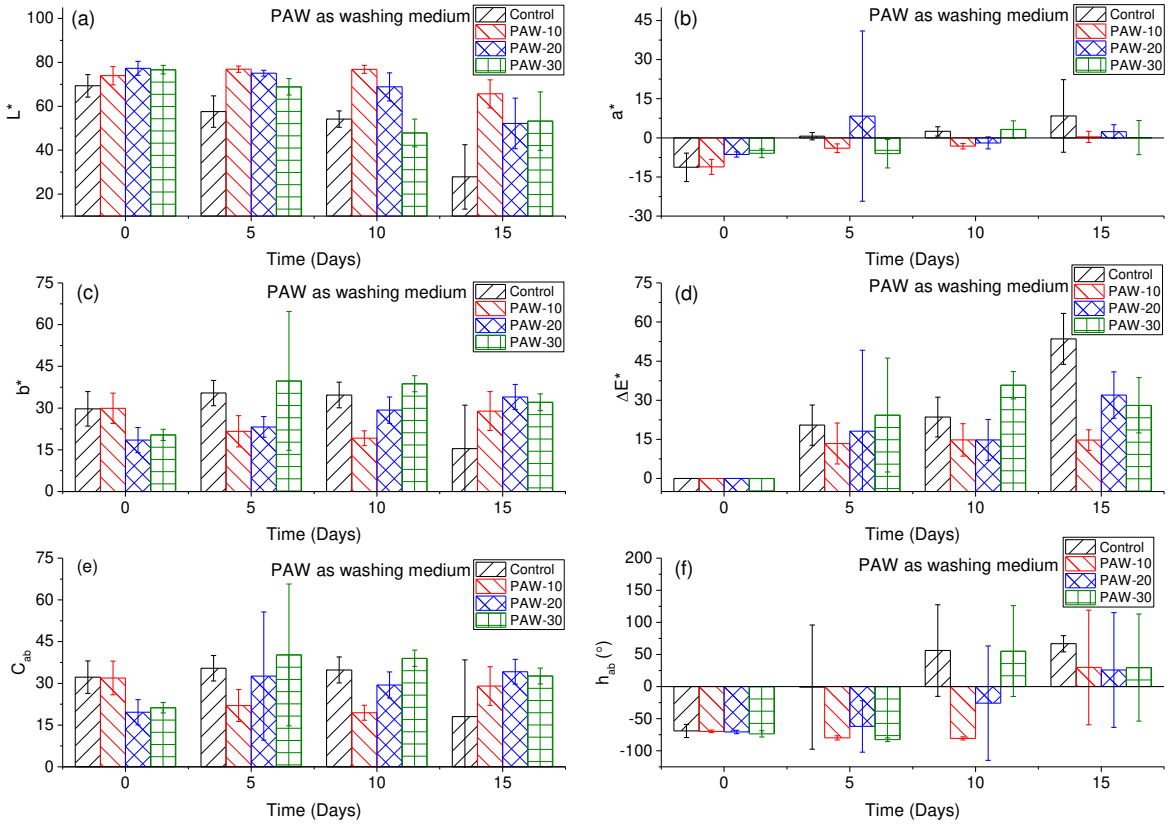

Figure 8. CIELAB color measurements ((a) L*, (b) a*, (c) b*, (d) ΔE*, (e) C_{ab}, (f) h_{ab}) of NBJ flowers over time when washed with PAW.

3.7 Antioxidant activity analysis of NBJ flowers

Figure 9 shows the variation in total phenol and flavonoids of NBJ flowers over the storage duration. PAW washing did not show a significant ($p > 0.05$) change in the phenol and flavonoids content of NBJ flowers compared to control, as observed in Figure 9 (a, c). However, when PAW and control were used as a storage medium, the phenol and flavonoids concentration of NBJ flowers decreased significantly over time (Figure 9 (b, d)). Moreover,

this decrease in phenol and flavonoids concentration was higher when PAW was used as a storage medium compared to control. This signifies the negative impact of PAW as a storage medium; the longer exposure of PAW reactive species significantly damages the NBJ flowers, resulting in antioxidant compounds leaking, and lower antioxidant activity observed in PAW as a storage medium compared to control (62, 63).

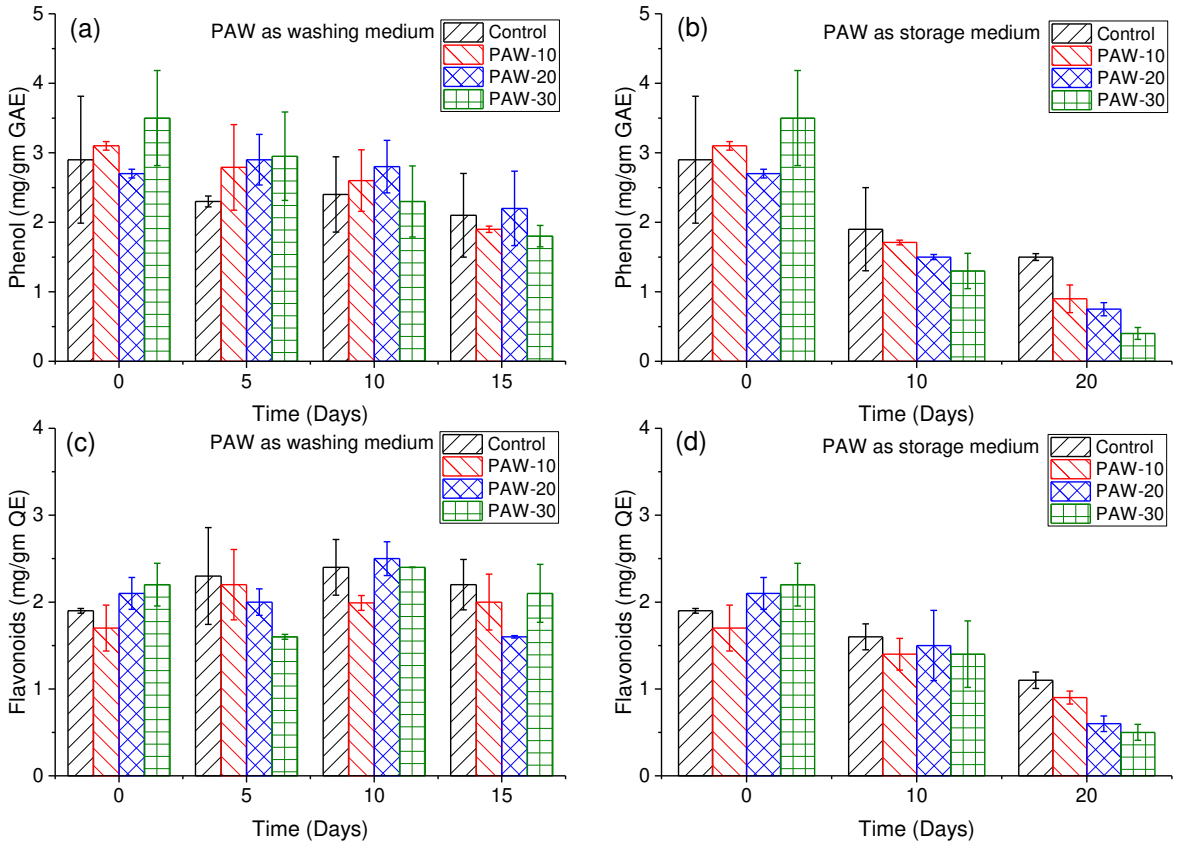

Figure 9. Variation in (a, b) total phenol and (c, d) flavonoids concentration of NBJ flowers over time when washed with PAW and stored in PAW.

The results indicate that PAW washing enhances the appearance, aroma, texture, and overall quality of NBJ flowers compared to control washing. PAW washing significantly reduces microbial growth, weight loss, and spoilage, indicating its potential as a preservative for night blooming jasmine flowers. However, the use of PAW as a storage medium has adverse effects, causing oxidative damage, color changes, higher microbial growth, weight loss, and spoilage

compared to control storage. The sensory evaluation supports the positive impact of PAW washing on NBJ flowers' shelf life. Color analysis reveals that PAW washing delays the toning down and browning of NBJ flowers compared to control washing. Antioxidant activity analysis shows a decline in phenol and flavonoids concentration over time, with a more significant decrease in PAW-stored flowers compared to control.

4. Conclusion

In conclusion, Plasma-Activated Water (PAW) washing proves beneficial for enhancing the shelf life of NBJ flowers by preserving their appearance, aroma, and texture. PAW washing also effectively reduces microbial growth, weight loss, and spoilage. However, caution is advised in using PAW as a storage medium for NBJ flowers, as it leads to oxidative damage and a decline in antioxidant compounds. Further optimization of PAW treatment times and conditions is necessary to maximize its benefits in flower preservation. This study contributes valuable insights into the application of PAW technology in the post-harvest preservation of delicate flowers like Night-Blooming Jasmine, offering potential benefits for the floral industry and related sectors.

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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