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Research article

Effect of plasma-activated water on chlorophyll retention in detached Tejpat (*Cinnamomum tamala*) leaves

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ABSTRACT

In this work, the atmospheric pressure air gliding arc discharge has been produced for the generation of plasma-activated water (PAW) and studying its effect on the chlorophyll retention and greenness of Tejpat (*Cinnamomum tamala*) leaves. The discharge is characterized via electrical and optical methods to calculate the electron excitation temperature (1.38 eV) and density (3.46×10^{17} cm^{-3}) of the plasma. The influence of plasma exposure time on the physico-chemical properties of PAW and the aging effect of electrical conductivity, pH, total dissolved solids, oxidation-reduction potential, and concentration of nitrite and nitrate are presented. It is found that the modification in physico-chemical properties of water has a considerable effect on chlorophyll retention and the greenness of detached leaves. The chlorophyll retention is highest for the leaves that are treated with 12 minutes of PAW as compared to the other plasma exposure time as well as untreated water. Moreover, the greenness of leaves for 12 minutes of plasma treatment remains for a longer duration (576 ± 34 hrs) as compared to treatment times of 0, 4, 8, and 16 minutes due to prolonged activeness of the physical parameters. This work is significant in that PAW plays an important role in maintaining greenness in detached green leaves.

1. Introduction

Non-thermal plasma technology is expected to provide maximum efficiency at the lowest practicable environmental cost, hence promoting sustainable societal and economic development. It can be produced under atmospheric conditions via different configurations of plasma reactor sources: gliding arc discharge, glow discharges, plasma jet, and dielectric barrier discharge [1–3]. The electron temperature of atmospheric pressure plasma (non-thermal and cold plasma) is obtained in the order of 1 to 10 eV making their wide ranges of growing applications in diverse fields such as agriculture, food industry, material processing, and bio-medicine [4–7]. In recent years, non-thermal plasma technologies could be an alternative approach instead of using different chemicals, for food preservation and agricultural products without affecting the nutritional value [8,9]. The use of many different chemicals as preserving agents on nutritional and medicinal leaves causes chemical traces that can be harmful to the food and the consumer. Thus, the production of PAW using a non-thermal plasma reactor can be considered as an alternative to different harmful chemicals

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[8,9].

There are several ways to produce PAW such as: (a) directly into the water; (b) directly into the water using multiple electrodes; (c) gaseous phase discharge onto the water's surface; (d) gaseous phase discharge into the water; (e) gas phase discharge onto the water's surface using plasma produced by a forward vortex flow reactor; and (f) gas phase discharge into the water in the form of bubbles [10]. In all cases, when gas plasma comes in contact with water, it changes water's chemical and physical properties. During the gas-liquid interface interaction, both short-lived (for example, hydroxyl radicals, nitric oxide, superoxide, etc.) and long-lived reactive species (NO_2^-, H_2 O_2, NO_3^-, etc.) are accumulated in bulk [4]. The resulting blend of plasma species and water is referred to as plasma-activated water (PAW). It is reported that PAW is useful in the treatment of contaminated or polluted water [11], the antimicrobial effect [12], applicability in the cancer treatment [13], wound healing [14], dentistry fields [15], seeds germination and seedling growth [16], food decay, post-harvest of food and vegetables [17,18] and many more. However, the true significance of the reactions relies on several factors, including the non-thermal plasma system being utilized, the environment, and the continuous or pulsed excitation regime [19]. The electrical conductivity (EC), potential of hydrogen (pH), oxidation-reduction potential (ORP), and concentrations of nitrate and nitrite in the water are influenced by reactive species formation in PAW by plasma discharge [6].

To generate PAW, even though we have various types of laboratory discharges, each of these discharges has a similar basic production principle and can be used for different purposes [20]. The gliding arc, generated at ambient temperature and air pressure, serves as an intermediary system between thermal and non-thermal discharges. It can deliver high plasma density, power, and operational pressure concurrently with a high degree of non-equilibrium [21]. The gliding arc discharge can be produced by using either a DC or AC power supply and the system can power up to an industrial size. The remarkable change in the chemical composition and increased reactivity of the heavy activated species generated in the plasma are the reasons for the growing interest in gliding arc discharges. Because it requires less energy and equipment, and is more efficient, it is seen as a more appealing and efficient oxidation method [22]. Therefore, we have produced atmospheric pressure air gliding arc discharges for making the PAW.

The *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm, locally known as Tejpat, is a medicinal plant with significant distribution in the Himalayas. The leaves of Tejpat have been widely utilized as a spice around the world. It is employed in various Ayurvedic formulations, conventional medicinal products, and culinary, Rheumatism, colic, diarrhea, nausea, and vomiting are treated with leaves because of their fragrant, astringent, stimulant, and carminative properties. The demand for *C. tamala* is growing daily due to its great therapeutic value and role as an essential spice ingredient [23]. Also, it is an evergreen plant in all seasons. Chlorophyll pigment is responsible for the conversion of light energy to biochemical energy in plants and is also responsible for providing greenness to the leaves. Therefore, retention of chlorophyll pigments is the key to maintaining greenness in leaves [24].

Since, the leaf chlorophyll content is affected by various factors like nutrient availability, and biotic and abiotic stresses [25], it is an indicator of a plant's photosynthetic capacity and overall physiological status of the plant [26]. Upon detachment from the plants, the leaves get wounded at the base of the petiole. Gene expression leading to the production of phytohormone ethylene is one of the responses of plants to wounding [27]. Since ethylene is one of the phytohormones that accelerate senescence in mature leaves [28], it leads to gradual and rapid degradation of chlorophyll thereby causing decreased leaf chlorophyll content in detached leaves. Mainly invasive [29] and noninvasive [30] techniques have been proposed earlier to measure the chlorophyll of leaves. The chlorophyll content meter (CCM-300; Opti-Sciences Inc., USA) is a device for non-invasive measurement of chlorophyll content in leaves. It gives an accurate measurement of chlorophyll in mg/m^2 of leaf surface by measuring the ratio of chlorophyll fluorescence at 700 and 735 nm (F735/F700) [31]. In several studies involving various agronomic and horticultural crops, the CCM-300 readings have been reported to correlate significantly with the total chlorophyll content of leaves measured by invasive extraction methods [32–34]. Various studies have been done covering the effects on chlorophyll retention in leaves by using treatments like heating [35] and kinetic model [36–38]. However, there have been no studies on chlorophyll retention in leaves by using PAW. In the present work, we have studied the effect of PAW in maintaining the greenness in detached *Cinnamomum tamala* leaves. Furthermore, the effect of aging on the physical properties of PAW is also systemically illustrated.

2. Material and methods

The block diagram for the production of gliding arc discharge at the atmospheric pressure and using it to produce the PAW is shown in Fig. 1. The rate of airflow is controlled by a flow meter [Fig. 1(a)] and 15.0 liters per minute (LPM) of air is allowed to flow between two diverging electrodes. In the experiment, we have used copper electrodes having a length of 132.0 mm, and a diameter of 5.8 mm with electrode separation of 2.8 mm and 13.9 mm at the air injection and exit positions respectively. The locally assembled pulsating DC power supply has been used for the discharge production, whose voltage can be varied from 0 to 15.00 kV [16]. The voltage probe and oscilloscope, namely PINTEK HVP-40 and Tektronix TBS 1052B have been used to measure the discharge voltage. On the other hand, we connect a 100 Ω resistor in series with a ground electrode for the measurement of current, which is shown in Fig. 1(a). To identify the reactive species produced in the discharge, we have employed the spectroscopic technique using optical emission spectroscopy (device name: HR1-high-resolution spectrometer, ASEQ Instrument) by placing the tip of the optical fiber cable at a distance 10.0 mm below the exit position of the electrodes. The average of spectra lines of discharge has been obtained for 30 observations with an integration time of 300 ms.

The Boltzmann plot method has been employed to calculate the electron excitation temperature (T_{ex}) in which the produced discharge is considered in the local thermal equilibrium defined by the equation (1) [39]:

$$\ln\left(\frac{\lambda_{ki} I_{ki}}{A_{ki} g_k}\right) = -\frac{E_k}{k_B T_{ex}} + C \tag{1}$$

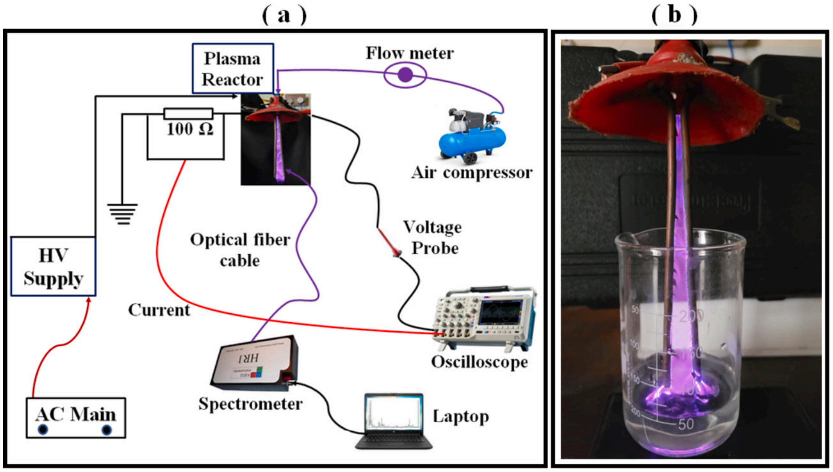

Fig. 1. (a) Block diagram of experimental arrangement along with discharge characterization and (b) production of PAW from gliding arc in a glass beaker.

where I_{ki} represents the relative line intensity of upper and lower energy levels k to i state, λ_{ki} is the respective wavelength for the transition, A_{ki} is the transition probability, g_k is the statistical weight factor, E_k is the excited energy level, and C is the constant for adopted spectral lines. As we plot the graph of $\ln\left(\frac{\lambda_{ki} I_{ki}}{A_{ki} g_k}\right)$ as the function energy E_k, the slope of the Boltzmann fitted line plot gives the electron excitation temperature. Once we obtain the electron temperature, the density of arc discharge is determined by using the Saha-Boltzmann equation [40].

We have taken 50.0 mL of de-ionized water on the beaker and discharge is produced on the surface of the water with different plasma exposure times 4, 8, 12, and 16 minutes, and the corresponding PAW is represented by 4 PAW, 8 PAW, 12 PAW, and 16 PAW, whereas 0 PAW represents the untreated de-ionized water so-called controlled case. The image of PAW production by using gliding arc discharge is shown in Fig. 1(b). The formation of nitrate and nitrite concentrations in PAW is measured via a qualitative approach in which a color chart test strip has been used having a strip efficiency of 99.6%. The color code values (in mgL^{-1}) are 0, 1, 5, 10, 20, 40, and 80 for nitrite and 0, 10, 25, 50, 100, 250, and 500 for nitrate. The values were noted corresponding to the color change observed in the test kit. Thus, we obtained a single-color code value during the experiment, and the procedure aligns with earlier reported research [3,16,41]. The various physical properties of PAW such as the concentration of hydrogen ions (pH), and the presence of organic and inorganic materials measured in terms of TDS, EC, and ORP are measured by the water quality tester (7-in-1 RCYAGO). The buffer solution having pH 9.18 has been used to calibrate the water quality tester. The physical parameters of PAW are measured every 24 hrs continuously until 5 days, where PAW is put in an ambient environment of plastic glass without covering it. Tejpat leaves are plucked from the Tejpat tree from the Central Department of Botany, Kirtipur, Kathmandu, Nepal, and selected similar types of 25 leaves having petioles for each experiment divided into 5 leaves for each set of the experiment: control or 0 min, 4 min, 8 min, 12 min, and 16 min PAW, and repeated 3 times in same conditions. Each leaf is kept inside a plastic zip bag and wrapped in its petiole by tissue paper soaked in 10.0 ml of 0, 4, 8, 12, and 16 min PAW as shown in Fig. 2. The leaves are kept in a dark room with a day/night variation in temperature of 12 to 25 °C and 55 to 81% relative humidity range.

Leaf chlorophyll content of the leaves was measured before and after dipping them in plasma-treated water by using a chlorophyll content meter (CCM-300, Opti-Sciences, USA). This device measures chlorophyll content in leaves up to 675 mgm^{-2} and the measurement diameter of the device is 3.0 mm with its 4.0 mm outer diameter. However, the device works very accurately for the smaller plants as well. Unlike the absorption technique that requires full coverage of the aperture and a flat surface, this method does not need that [42]. We have performed the five different independent measurements for each parameter and the obtained results are presented as mean value ± error. The chlorophyll of each leaf was measured five times and averaged for a more accurate and precise result of the chlorophyll measurement. The chlorophyll content in these leaves is assessed every three days until it completely disappears, and the duration it takes for the chlorophyll to vanish is known as the greenness period. For chlorophyll retention, the measured chlorophyll is converted into a ratio with its initial value.

3. Results and discussion

The electrical and optical methods have been used to characterize the produced gliding arc discharge and the current-voltage characteristics and optical emission spectra (OES) are shown in Fig. 3. During the discharge production, the peak-peak discharge voltage is measured to be 1.52 kV DC, and the discharge current of 154.00 mA which is depicted in Fig. 3(a) and the blow-up of the discharge voltage-current waveform of a single peak is shown in Fig. 3(b). OES is an important technique in the characterization of plasma that measures the emission spectra by the excited molecular species present in the discharge. In order to identify the presence of various species on the discharge, the obtained optical emission spectrum is shown in Fig. 3(c). The second positive system of nitrogen species is observed in the wave band 310-380 nm, whereas the nitrogen species of first negative system are found in the 390-440 nm wave band. Moreover, in the spectrum of gliding arc discharge, the hydroxide radical OH is found at 309 nm and this

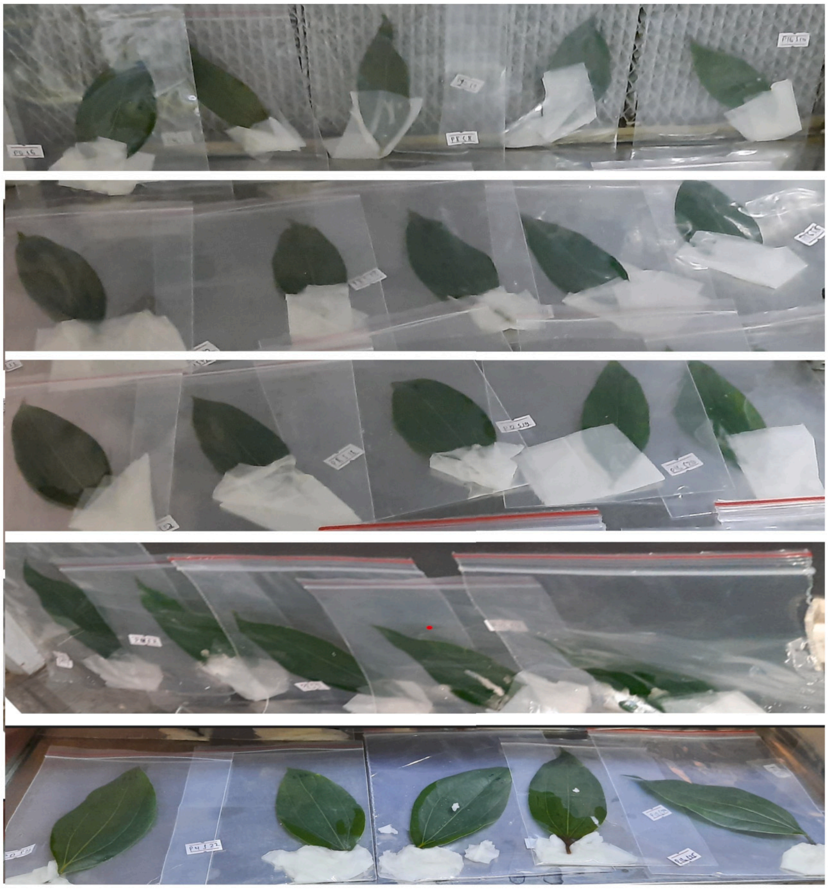

Fig. 2. Experimental setup for 25 Tejpat leaves in a plastic zip bag with soaked tissue paper.

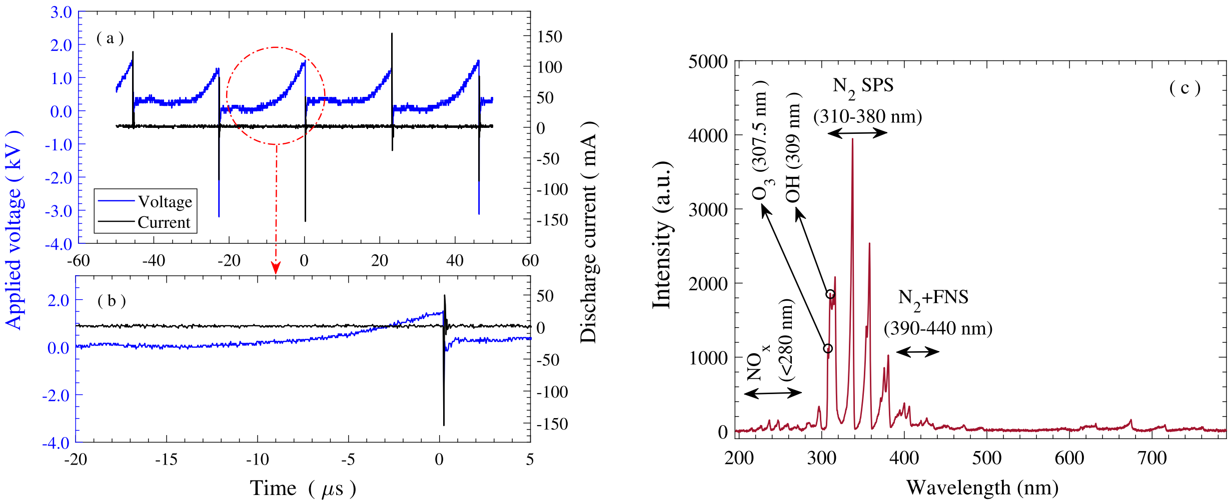

Fig. 3. (a) Real-time voltage–current waveform diagram, (b) blow up of voltage-current waveform for single peak, and (c) optical spectra of gliding arc discharge at atmospheric pressure with an airflow rate of 15.0 liters/minute.

radical is important for different plasma chemical processes, including the oxidation of gas and liquid contaminants. The reactive oxygen (O) radical is found at wavelength 715.9 nm. The nitric oxide gamma band NO_γ is also observed in the spectrum of discharge in the wave band 200 to 280 nm [43,44].

The Boltzmann fitted line using equation (1) and obtained data points are depicted in figure (4). Electron excitation temperature of

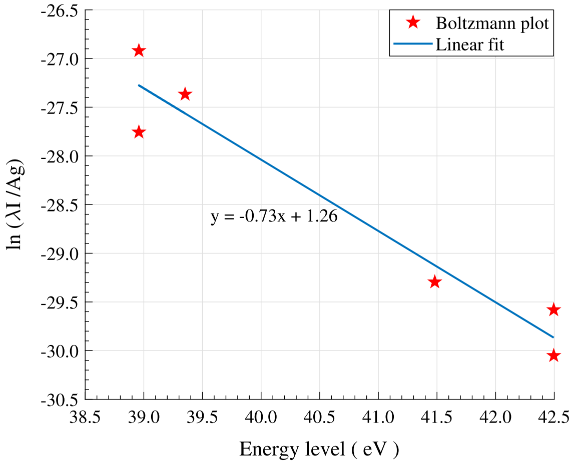

Fig. 4. Boltzmann plot of gliding arc discharge at atmospheric pressure and airflow rate 15.0 LPM.

the produced discharge is found to be 1.38 eV and density of electrons is calculated from the Saha-Boltzmann equation [40] and is found to be 3.46 $\times 10^{17}$ cm^{-3}.

3.1. Physico-chemical properties of PAW

When the atmospheric pressure plasma is exposed to the water, the formation of various primary and secondary reactive nitrogen and oxygen species changes the physico-chemical properties of PAW. The understanding of these parameters is crucial for various purposes of plasma applications such as bacterial inactivation, food preservation, seed germination, freshness of leaves, etc. In this work, we have measured the physico-chemical parameters such as pH, electrical conductivity, total dissolved solids, oxidation-reduction potential, temperature, and nitrite and nitrate concentration of PAW for different plasma exposure times which are shown in Fig. 5. When the discharge's highly active electrons interact with the gas molecules, dissociation takes place, primarily producing oxygen and nitrogen species [45]. As water treated with gliding arc discharge, the presence of additional ions like nitrates (NO^{-2}), nitrites (NO^{-3}), etc. affects the physical properties such as pH, EC, TDS, ORP, and temperature as well. This is because additional nitrate and nitrite ions have been added, increasing the water's acidity. The addition of discharge causes the pH value to decrease over the increase in exposure time. The variation of pH value against plasma exposure time is shown in Fig. 5 (a). The variation of electrical conductivity of PAW versus treatment time is shown in Fig. 5 (b). Application of discharge on the water surface increases the electrical conductivity to a certain extent and further application decreases it. The value of EC is found to be maximum for 12 PAW and its numerical value is found to be 51.60 ± 0.66 μ S/cm. The variation of total dissolved solid of PAW versus treatment time is presented in Fig. 5(c). It is observed that the addition of discharge on water causes to increase in total dissolved solids in water as well. The value of TDS is maximum for 12 PAW and its numerical value is found to be 26.0 ± 0.2 ppm. The variation of oxidation-reduction potential of PAW versus treatment time is shown in Fig. 5(d). It is found that the oxidation-reduction potential of treated water increases with the increase in treatment time. The variation of temperature of PAW versus treatment time is shown in Fig. 5(e). It is found that the treatment of the gliding arc causes to slowly increases the temperature of the water. The temperature is found to be to a maximum $28.0 \pm 0.6\,°C$ for the 16 PAW as compared to the control with a temperature of $24.3 \pm 0.2\,°C$. Since the plasma is produced from air atmospheric pressure, the concentration of nitrate and nitrite significantly changes with exposure to a gliding arc in the water. The variation of nitrate and nitrite concentration of PAW versus treatment time is shown in Fig. 5(f). It is found that the nitrate and nitrite concentration of treated water increase with the increase in plasma treatment time. The concentrations are found to be maximum for the 16 PAW with numerical values of nitrite and nitrate concentrations are 150 and 60 ppm respectively.

The primary method of absorbing nitrogen is nitrate [46], which has a major function in the synthesis of proteins, enzymes, nucleotide, and chlorophyll in plants, making it a crucial macro-nutrient for plant growth and development [47]. Nitrate is the primary form of nitrogen that plants receive, and it is also the nutrient element that they absorb the fastest. As a result, most plants rely on nitrate as their primary nitrogen source for morphological development, growth, and maintenance. The ability of plants to respond to changing environmental conditions is enhanced by nitrate, which can quickly boost the transport and digestion of nutrients [48]. The process of nitrate absorption and transformation is the foundation of plant growth and development. A shortage of nitrate quickly results in reduced photosynthetic efficiency, slowed root growth, and organ senescence, all of which hurt the quality and yield of fruit [49]. Nitrate decreased reactive oxygen species generation by increasing photosynthetic pigments [50]. Antioxidative genes may be upregulated by nitrate, which would then lead to an increase in the manufacture of antioxidative enzymes. Nitrate also boosts the metabolism of nitrogen overall and provides nitrogen continually for the synthesis of other photosynthetic enzymes, including chlorophyll [51].

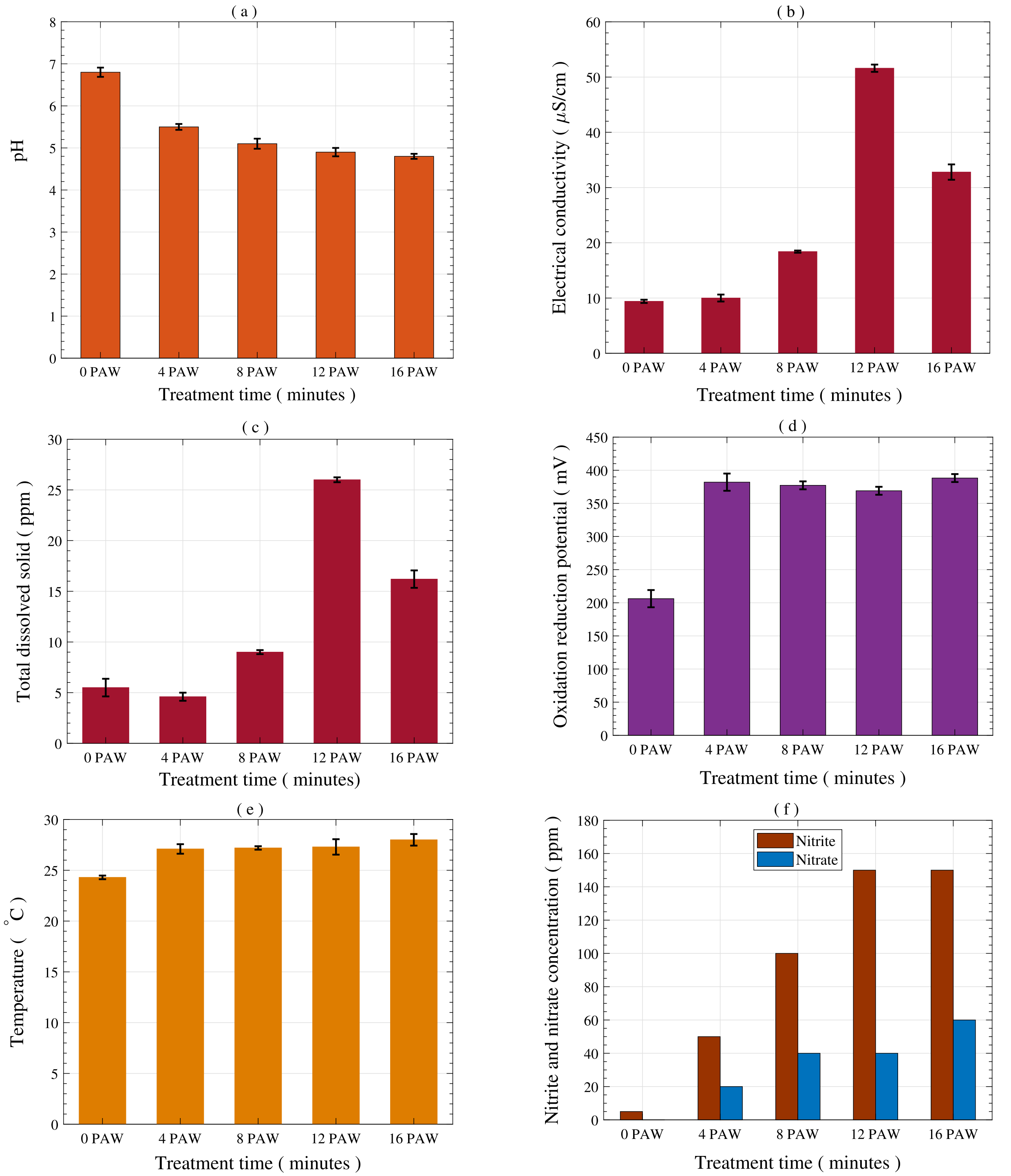

Fig. 5. The physical and chemical parameters of PAW by using gliding arc discharge for different plasma exposure time (a) pH, (b) electrical conductivity, (c) total dissolved solid, (d) oxidation-reduction potential, (e) temperature, and (f) nitrite and nitrate concentrations.

3.2. Aging effect

To study the aging effect of PAW, the change in physical parameters is measured for up to 5 days. The variation of pH, EC, TDS, and ORP as the function of the storage days is shown in Fig. 6. It is found that the pH and ORP have slowly regained their original value, whereas the EC and TDS go on increasing for the increase in storage days from their initial value. The regaining rate of pH

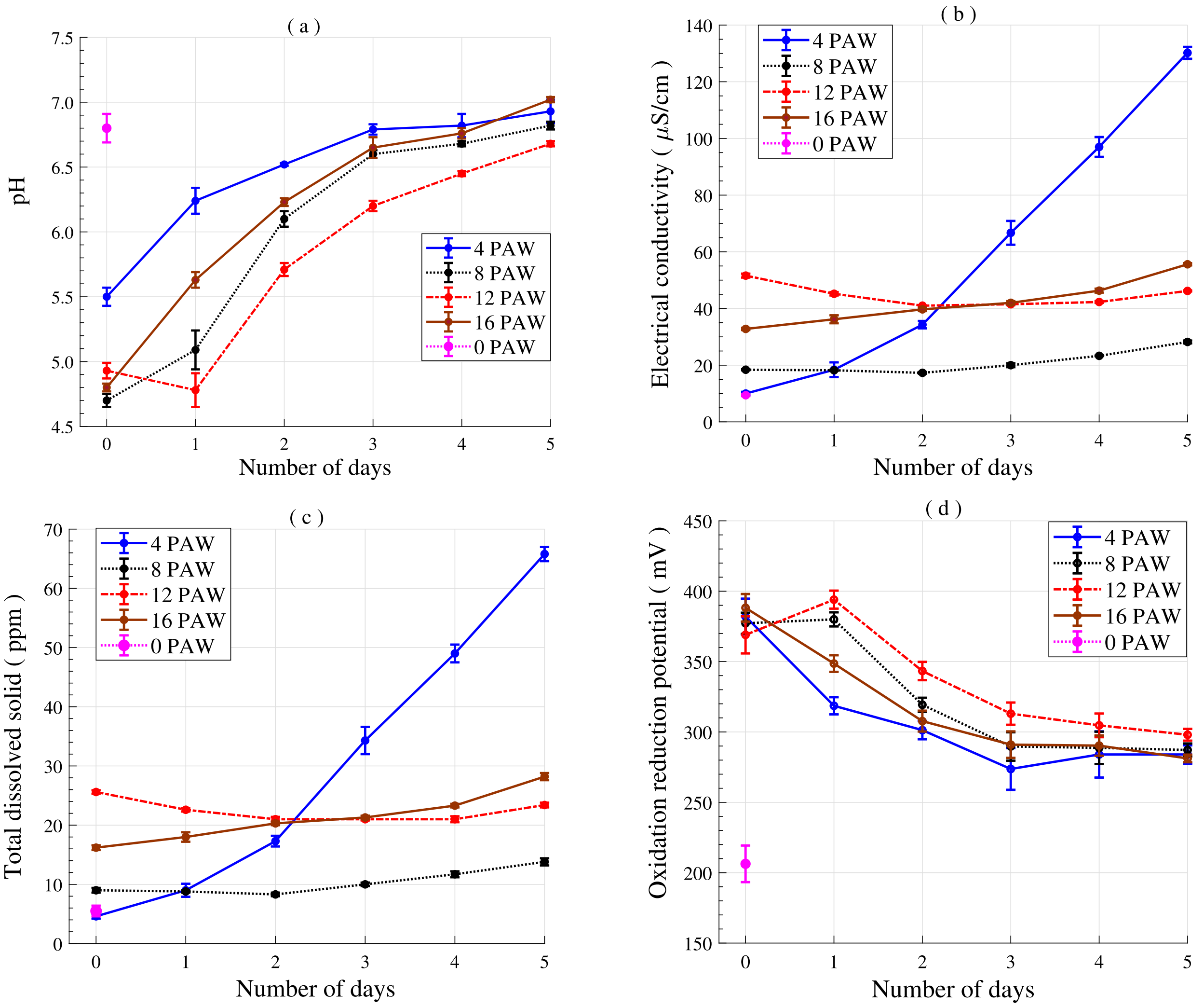

Fig. 6. Variation of (a) pH, (b) electrical conductivity, (c) total dissolved solids, and (d) oxidation-reduction potential during 5 days.

and ORP is found to be slower for the 12 PAW than that of other cases. For the same plasma exposure time (12 PAW), the increasing rate of EC and TDS is also lower. The changes in the physical properties of PAW explicitly indicate that there should be a significant effect on leaves as they absorb treated water for nutrients.

3.3. *Chlorophyll retention*

The chlorophyll retention of Tejpat leaves for various plasma exposure duration's is shown in Fig. 7. As the number of storage days increase, the chlorophyll retention (%) decreases; the lowest value was found after 21 days. The chlorophyll retention (%) is higher for the leaves that have been used with PAW for 12 min compared to the other various plasma-activated as well as untreated water. This indicates that the PAW can effectively preserve the chlorophyll content in the leaves, especially the PAW of 12 min, which is the best candidate for this purpose in our case.

3.4. *Greenness of leaves*

The average number of days for the leaves to reach zero chlorophyll content when treated with PAW is shown in Fig. 8. It is found that the leaves kept in 12 min PAW stayed green for 576 ± 34 hrs (24 days), whereas the leaves that we used untreated water is 456 ± 31 hrs (19 days). Similarly, the leaves kept in 4 min PAW stayed green for 504 ± 17 hrs (21 days), 8 min PAW stayed green for 552 ± 23 hrs (23 days), and 16 min PAW-treated leaves stayed green for 408 ± 19 (17 days). The obtained results show that 12 min PAW is more effective in preserving the chlorophyll content of Tejpat leaves.

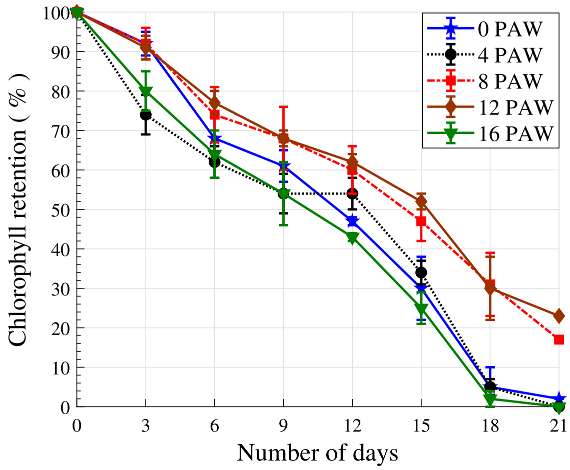

Fig. 7. The variation of chlorophyll retention versus the number of storage days.

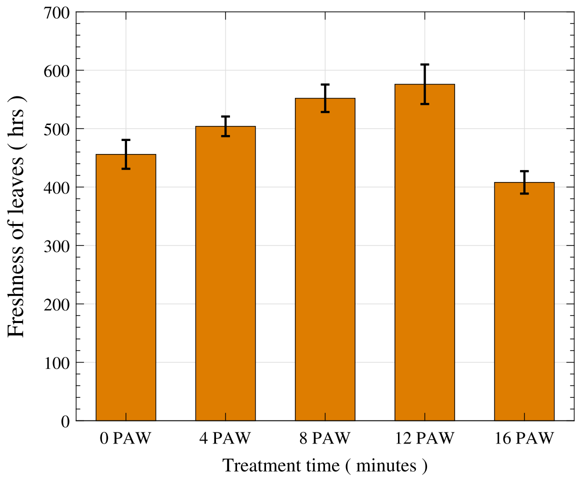

Fig. 8. Greenness of leaves in hours for different plasma treatment times of water.

3.5. Plasma-water interaction and freshness of agricultural products

The most popular and extensively used technique for creating PAW involves producing a discharge that comes into contact with the water's surface and is capable of delivering highly reactive species, primarily reactive oxygen and nitrogen species (RONS) [6,52]. The formation of these reactive species depends on the plasma exposure time and applied voltage. The formation of reactive nitrogen species (nitrite and nitrate ions) plasma-based technology is taken as an anti-bacterial agent due to an acidic environment and alternate fertilizer to enhance plant growth (instead of using other chemical fertilizers) [53–55]. The reactive nitrogen species play a significant role in the agricultural applications of PAW as they are absorbed by plants as a nutrient and control the plant metabolism and overall development of plants, resulting in keeping the freshness of leaves as well [55,56]. However, to effectively use the PAW as a long-term product, we have to investigate the post-discharge storage properties of PAW. The shelf life of reactive oxygen and nitrogen species presence in PAW has a crucial role in antibacterial activities, killing cancer cells, sterilization of fruits and green vegetables [57–59]. The reduction of chlorophyll content due to implications of PAW is attributed to the degradation of type II chlorophyll caused by the induction of reactive species. The prolongation of the freshness of green plant leaves and foods is probably caused by degradative enzyme inactivation. The pigment degradation and change in tissue microstructure can determine the color of fresh agricultural products [60] and our present work demonstrates the usefulness of PAW on Tejpat leaves; however, further detailed investigation into other parameters is to be considered as well.

4. Conclusion

In this study, a high voltage (1.52 kV) was applied between two electrodes to generate an atmospheric pressure gliding arc discharge and produce plasma-activated water (PAW). Electrical and optical techniques were employed to characterize the discharge, allowing us to determine the plasma temperature (1.38 eV) and electron density (3.46×10^{17} cm^{-3}) at a constant airflow rate of

15.0 liters/min. The physical and chemical parameters of PAW such as pH, EC, TDS, ORP, temperature, and nitrates and nitrites concentration have been studied. The effect of PAW on the chlorophyll of Tejpat (*Cinnamomum tamala*) is studied by presenting the chlorophyll retention and greenness of leaves. Before investigating the impact of PAW on Tejpat leaves, we examined the aging effect on the physical parameters of PAW. The pH value of PAW is reduced to 4.80 ± 0.06 for 16 minutes of plasma treatment time, whereas the electrical conductivity and total dissolved solid increased and reached the maximum for 12 minutes of plasma treatment time. Their maximum values are found to be 51.60 ± 0.66 μ S/cm and 26.0 ± 0.2 ppm respectively. The oxidation-reduction potential temperature of PAW increases and their maximum numerical value is found to be 388.2 ± 6.0 mV and $28.0 \pm 0.5\,^{\circ}$C respectively for 16 minutes of plasma treatment time. However, these changes in the physical properties did not last long for a long period. The pH value retained its original value in 5 days, whereas the total dissolved solids and electrical conductivity were found to be increasing. On the other hand, the oxidation-reduction potential gets reduced and becomes shifted to a stable domain after 5 days. The change in physical parameters during the storage time is slower for 12 minutes of PAW than in other cases which indicates the activeness of these parameters for a longer period. Furthermore, as the exposure time increases, the concentrations of nitrate and nitrite ions also rise. Specifically, their numerical values increase from 0 to 60 ppm and 0 to 150 ppm, respectively, when the treatment time extends from 0 to 16 minutes. When the (0, 4, 8, 12, and 16 minutes) PAW is used in detached Tejpat leaves, it is found that the chlorophyll retention % of the leaves is higher for 12 minutes of PAW. The 12-minute PAW used for leaves remains green for 576 ± 34 hrs, whereas the leaves exposed to untreated water remain green for 456 ± 31 hrs. This shows that 12 minutes of activated water are crucial to preserve the leaves for 120 more hours than control water.
Our findings demonstrate that changes in the physico-chemical properties of PAW have a crucial role in preserving the greenness of detached leaves, with practical implications for improving the shelf life of leafy vegetables.

CRediT authorship contribution statement

Roshan Chalise: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ashish Dahal:** Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Suresh Basnet:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Formal analysis, Data curation. **Sangat Sharma:** Writing – original draft, Software, Methodology. **Deepak Raj Pant:** Validation, Conceptualization. **Raju Khanal:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Raju Khanal reports financial support was provided by Research Coordination and Development Council, Tribhuvan University, Kirtipur, Nepal. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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