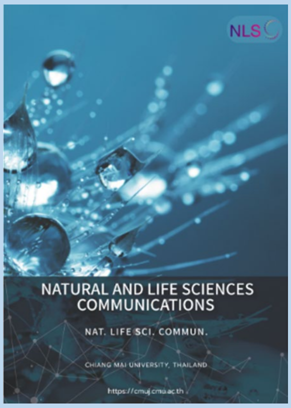
Research article



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Application of Non-thermal Plasma-Activated Liquid for Delay Browning in an Apple Slice

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ABSTRACT

The color of fresh-cut fruits is indicated by the appearance and quality of their products. A sliced apple is a highly putrefying fruit that is sensible to react with oxygen gas, which causes the color, taste, smell, and nutritional value to change during storage in ambient air. Non-thermal atmospheric pressure plasma jet (NTAPPJ) is one of the most crucial applications for food preservation. This study was divided into two parts. First, the emission intensities of the plasma spectrum and electrical properties were investigated. Then, the colorimetric was used to investigate plasma-treated effects on different liquids (tap water, deionized water, and saline water) to delay color changes of fresh-cut apples. The I-V characteristic curve was used to obtain an optimal power of 8.5 kHz-AC pulse-driven NTAPPJ with argon gas. Additionally, the parameter a*, L*, b*, ΔE, h*, YI, Chroma, and browning index (BI) using the colorimeter method were examined. We then demonstrated that using the Ar-NTAPPJ can be considered a novel approach to increasing fresh-cut apple' toleration and shelf life. Furthermore, plasma exposure is one of the nondestructive processes that does not have any side effects on the products and can significantly delay degradation and discoloration.

Keywords: Plasma jet, Shelf life, Colorimeter, Browning index

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INTRODUCTION

Fruit and vegetable senescence is an irreversible process that involves a series of physiological, biochemical, and metabolic changes, accompanied by a decline in color, flavor, nutrition, and a shortening of the shelf life (Abreu et al., 2003; Barreiro et al., 1997; Gorny et al., 2002). Typically, the peel of the fruit, including the apple's peel, is a shield that prevents the oxygen in the air from coming into contact with its flesh. When the peel of an apple contact oxygen in the air causes its flesh from white to brown, and make it look unappetizing by the chemical reaction (Ma et al., 2017). The process of changing from the original color in an apple to brown occurs with the destruction of the cells in the fresh apple. For example, using a knife to peel the apples out causes an enzyme called polyphenol oxidase (PPO) and peroxidase (POD) (Manzocco et al., 2000; Misra et al., 2011). The phenolic compounds separated within the apple pulp come into contact (Misra et al., 2014; Misra et al., 2019; Perni et al., 2008). The phenolic compounds react with oxygen, which catalyzes the oxidation reaction by PPO, causing phenol compounds to become Ortho-quinones or O-quinones (Abidin et al., 2018; Ramazzina et al., 2015). The quinones react and amino acids or proteins can be performed in melanin, which causes browning in an apple (Rhim et al., 1999; Sharma and Ramana Rao, 2015; Xiao et al., 2010). Likely, the type that makes the hair color or skin different color of people. Several methods delay the oxidant reaction of enzymes in apples after peeling or cutting them into pieces to resolve the browning problem. For example, soaking them in lemon juice, immersion in boiling water, or normal saline solution (Zheng et al., 2019).

Non-thermal atmospheric pressure plasma jet (NTAPPJ) is one of the most popular techniques in the plasma research field (Katsigiannis et al., 2022; Niemira, 2012). NTAPPJ is an ionized gas discharge by passing a gas through a strong electric field (Lu et al., 2016). NTAPPJ consists of two concentric electrodes (high voltage and ground electrodes) with flowing gas. There are many geometries of NTAPPJ configuration and used materials such as single electrode jets, dielectric barrier discharge (DBD) jets, DBD jets, and dielectric-free electrode jets(Yan et al., 2017). Over the past decade, NTAPPJ has shown its remarkably applied in food preservation. Moreover, NTAPPJ is able to promise surface decontamination and quality enhancement of fruits and vegetables field (Lu et al., 2021). Plasma is the fourth state of matter, which consists of approximately equal numbers of positively charged ions and negatively charged electrons (Ishijima et al., 2009; Kogoma and Okazaki, 1994; Luque A and Ebert, 2008). Plasmas are described by many characteristics such as temperature, degree of ionization, and density that may be classified in different ways. Nowadays, non-thermal plasma is increasingly under research for the food industry, especially in fresh fruits and vegetable fields (Kim et al., 2015; Li et al., 2019; Matan et al., 2015; Ramazzina et al., 2016; Sonawane et al., 2020).

In this work, we aim to develop the NTAPPJ device and characterize its electrical properties and emission spectra to use it as a tool to delay browning in an apple slice by an effective power of hydrogen (pH) and oxidation-reduction potential (ORP) by plasma-activated liquid (PAL). Additionally, the CIE 1931 color system was used to provide the different color parameters of apple slices.

MATERIALS AND METHODS

Fabrication and characterization of plasma jet device

The experimental setup consists of a discharge chamber, power supply, and measurement systems. The schematic diagram is shown in figure 1A. The plasma system was designed to generate a plasma jet to activate liquid. The discharge was operated in ambient air under atmospheric pressure. The non-thermal atmospheric pressure plasma jet (NTAPPJ) electrode consists of a copper rod (3-mm diameter), which is coaxially inserted into the dielectric of an alumina (Al_2O_3) material (6-mm diameter), and covered with an outer case of nylon material as shown in figure 1B.

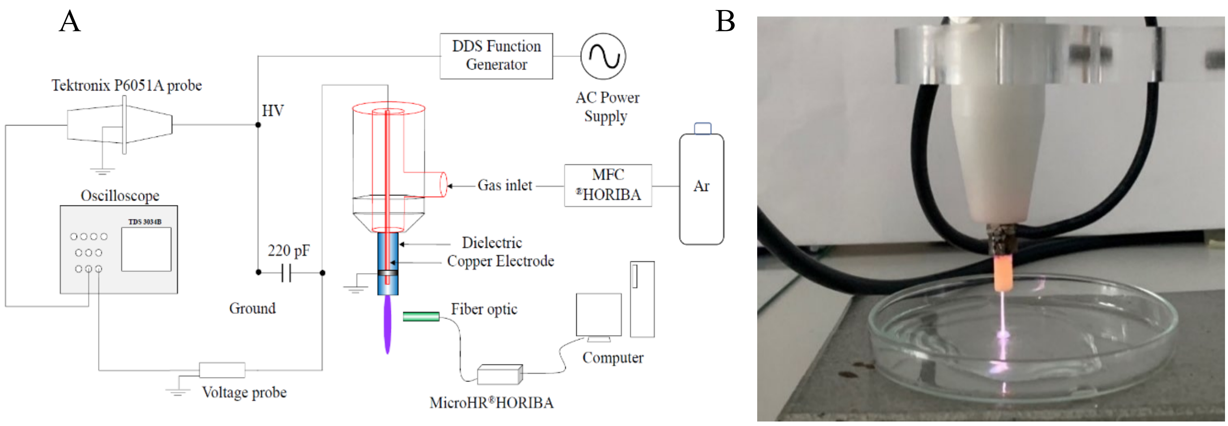

Figure 1. (A) Schematic of the experimental apparatus showing the plasma configuration operated by the AC pulse generation and (B) Illustration of the ignited Ar-plasma jet head on the liquids.

The direct digital synthesis (DDS) function generator (JUNTEK, MHS-5200A, 200 MSa/s 12Bits) powered the electrode system by kHz-AC pulse modulable. The voltage probe was placed between the high voltage electrode (HV) and the ground electrode as a diagnostic for measuring the power consumption and determining the waveforms by current and voltage characteristic method or I-V plot. The voltage across the 220 pF capacitor measured by an HV probe (Tektronix, P6015A, 75 MHz) estimated the discharge charge current. I-V characteristic waveforms can be used to determine the maximum power over the total period (T). The power and power doses consumed by NTAPPJ are expressed as follows:

$$P = \frac{1}{T}\int_0^T I(\mathrm{t})\,\mathrm{V}(\mathrm{t})\,\mathrm{dt} \tag{1}$$

$$Powerdose = \left(\frac{P}{Area}\right)\mathrm{W}/\,mm^3 \tag{2}$$

Acidity and Oxidation-reduction potential measurement

One important key to reduce browning in a sliced apple is inhibited enzymatic browning reaction. The proper acidity for the mechanism of phenolase ranges between 5-7 in the power of hydrogen (pH). Plasma-activated liquid (PAL) can produce acidity by plasma-induced liquid reactions with electrons and ions. Hence, pH is an important parameter to consider. In addition, the oxidation-reduction potential (ORP) was used to evaluate the oxidation ability in plasma solution. 1000 µl of deionized (DI) water, tap water, and saline water were treated on the Petri dish by NTAPPJ device, respectively. Then, the optimal gap distance between the plasma head, and the surface of the solutions were kept fixed at 20 mm.

To evaluate the pH values, the solutions were treated for 3, 6, 9, and 12 minutes with plasma and then immersed the pH-indicator strip (catalog number: 160347, Merck Millipore) in PAL (volume: 1000 µl) and referencing the color, as seen with the naked eye, to the corresponding pH value on the chart provided in the kit. Additionally, the pH electrode (HI1131, HANNA instrument) and ORP electrode (HI3131, HANNA instrument) were used as tools to determine pH and ORP values in the plasma solution.

Sample preparation

Fuji apples were bought from a local fruit shop and kept at room temperature for just 4 hours before plasma exposure. Then, samples of 18 cross cut slices were slashed with a penknife sterile with 90% ethanol and packaged in a plastic vacuum seal bag. After 24 hours of storage at 4° in refrigerators, three samples were used as control (untreated), and the other samples spent for different treatments. The three replicated samples were treated at varying times of 3-, 6-, 9-, and 12-minutes.

Visual quality and color measurement by colorimeter

To evaluate visual quality, the investigation of alterations in color parameters as induced by Ar-NTTAPJ plasma, the CIE 1931 color space was used to measure the color parameters. In brief, the alterations in color parameters as a result of plasma treatment on the sliced apple were reviewed. To quantify the color difference, the treated samples were scanned with a colorimeter (TES-135A, TES Electric Electronics Corp., Taiwan) using the CIE (L*a*b*) system. The color values such as L* (lightness), a* (yellowness), and b* (redness) of the sliced apple were measured before plasma exposure and 24 hours after plasma treatments.

RESULTS

Electrical properties

The electrical properties of an atmospheric pressure plasma jet (NTAPPJ) discharge for argon (99.99%) were examined. The plasma power was evaluated by an oscilloscope, and estimated by the I-V characteristic curve. Figure 2A shows that the applied voltage is sinusoidal with 8 kV as the peak-to-peak voltage. Figure 3B shows a displacement current of single short peaks appearing in the 1 mS discharge cycle. The discharge power was obtained by multiplying the area by the applied frequency as shown in equation 1. While an increase in the voltage of the DSS function generator had a significant effect on the NTTAPJ electrical parameters leading to a remarkable increase in the voltage and current. When applying an optimal condition with a voltage of 4 kV, and 8.5 kHz, the plasma power increases to 7.6 W. Moreover, the power dose was evaluated by equation 2. The results show the power dose of NTTAPJ increased when the DDS function generator rate to NTTAPJ reached the maximum at 0.26 W/mm^3.

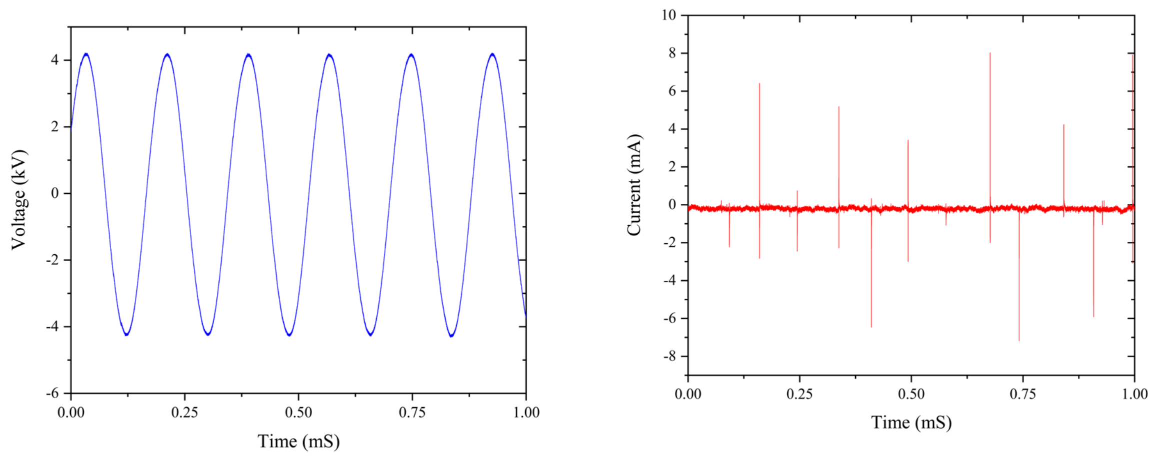

Figure 2. **(A) Three and a half complete cycles of voltage waveforms and (B) Current waveforms of NTAPPJ system based on 8.5 kHz with 5 slm of Ar gas.**

The emission spectra in the range of 300 to 1,000 nm were recorded through optical emission spectroscopy (OES). The configuration of the OES probe with plasma-dissipated power at 7.6 W was shown in figure 3. The optical emission spectra identified characteristic excited species from N_2 (from 306-380 nm) and N_2 C-B (2nd positive system) as shown in the red dot box, which was carried out by UV transmission grating with 1,200 grooves/mm, and a measured spectrum ranging from 300 to 400 nm. The strong presence of N_2 C-B has been observed due to the excitation processes like the electron impact excitation from the ground state N_2 and the first metastable state N_2. Additionally, the emission spectra of Argon I of 696.54 nm, 706.72 nm, 727.29 nm, 738.39 nm, 750.38 nm, 763.5 nm, 773.3 nm, 794.8 nm, 800.6 nm, 810.36 nm, 826.45 nm, 842.46 nm, 852.14 nm, 912.14 nm, 922.4 nm, 965.77 nm, and atomic O I (777 nm) bands were detected by UV transmission grating with 300 grooves/mm and a measured spectrum ranging from 300 to 1,000 nm.

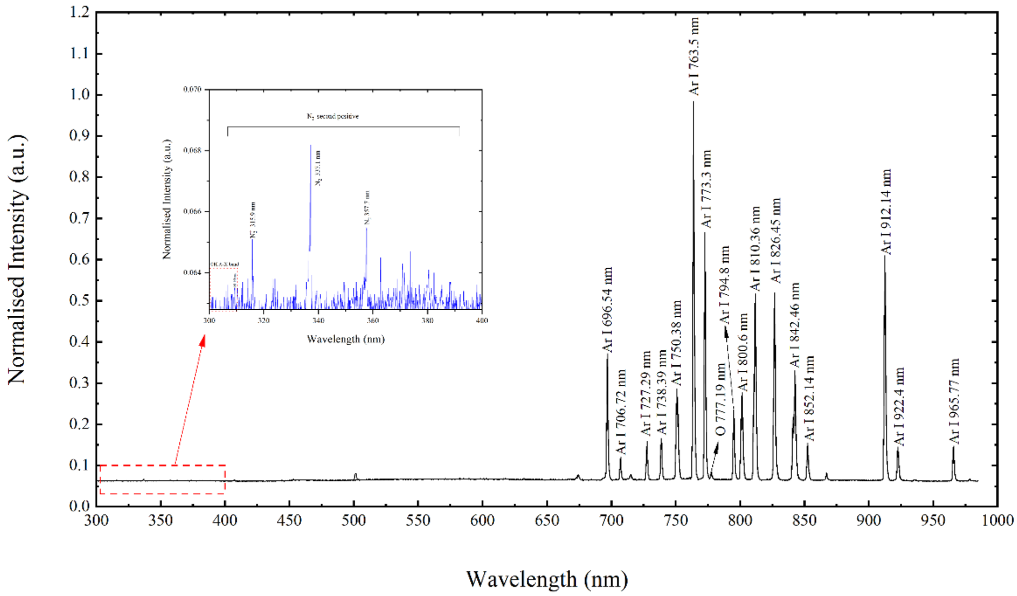

Figure 3. The optical emission spectra of plasma discharge normalized concerning Ar emission at 763.5 nm with 6 kV_{p-p} of applied voltage, 8.5 kHz of frequency, 12.6 SLPM of gas flow rate, and 3 seconds of integration time.

As the presence of OH band (A-X) transition has low intensity when compared with N_2 (from 306-380 nm) and N_2 C-B (2nd positive system) due to the content of water molecules during the interaction between plasma and solution. Typically, the amount of OH production depends on the collisions between the evaporated water molecule in the gas phase with metastable Ar (912 nm ($2p_{10} \rightarrow 1s_5$) and 696 nm ($2p_{10} \rightarrow 1s_5$)), O I (777 nm) atoms, and electrons in the plasma plume for the interaction. OH production could be explained by considering the dissociation reaction (Bruggeman and Schram, 2010; Dorn et al., 1995; Liu et al., 2010; Roy et al., 2016; Schmidt-Bleker et al., 2016) as follow:

$$Ar^{*} + H_2O \rightarrow OH + H + Ar \tag{3}$$

$$e^{-} + O_2 \rightarrow e^{-} + O + O(^1\mathrm{D}) \tag{4}$$

$$O(^1\mathrm{D}) + H_2O \rightarrow 2OH \tag{5}$$

$$e^{-} + H_2O \rightarrow OH + H + e^{-} \tag{6}$$

Color components

To study the effects of plasma treatment on sliced apple, the color components were measured on samples with varying four plasma conditions, and three replications were reviewed. Then all of the treated samples were measured for the various parameters such as lightness (L*), yellowness (a*), redness (b*), the total color difference (ΔE), hue (h*), chroma (C*), and browning index (BI) by a colorimeter. Table 1 shows the effect of plasma treatments on color components, the parameter such as L*, a*, b*, C*, ΔE, h*, and BI were increased significantly at a plasma treatment time of 3-, 12-, 6-, 3-, 9-, 12-, and 6 minutes, respectively. In contrast, the parameters decreased significantly at a plasma treatment time of 9-, 3-, 9-, 12-, 12-, 3- and 9 minutes in L*, a*, b*, C*, ΔE, h*, and BI, respectively. In the case of 3- and 9-minute treatments, have a significant effect on the browning index parameters (BI). In contrast, the other conditions have no significant effect on BI parameter.

Table 1. Analysis of variance for effect of plasma treatments on color components.

Plasma treatment time (Minute)	Plasma jet conditions			Dependent variable					
	Voltage (kV p-p)	Frequency (kHz)	Ar flow (SLPM)	*L**	*a**	*b**	*h**	*C**	*BI*
0 (Untreated)	10	8.5	12.6	61.52	-0.68	18.83	-87.94	18.84	34.73
3	10	8.5	12.6	64.95	-1.31	18.94	-86.04	18.98	32.01
6	10	8.5	12.6	61.41	-0.67	20.34	-88.12	20.35	38.29
9	10	8.5	12.6	59.73	-0.60	16.93	-87.97	16.94	31.71
12	10	8.5	12.6	60.16	-0.45	18.36	-88.60	18.36	34.88

Acidity and Oxidation-reduction potential

The most important key role in decreased browning reactions is the power of hydrogen (pH) and oxidation-reduction potential (ORP) to investigate different plasma solutions. The effect of treatment duration by plasma-dissipated power at 7.6 W is expected. The graph in figure 4A shows the results of pH existing in three different solutions (tap water, deionized water (DI), and saline water). It shows that pH dropped significantly to 4, 3, and 3, according to tap water, DI water, and saline water solutions with 12-min of treatment time. Additionally, figure 4B shows ORP values in plasma-treated DI water of 360.6, 387.1, 414, 429, and 444 mV according to 0-, 3-, 6-, 9-, and 12 minutes, respectively. It was found that 12-minutes of treatment duration in pH values of DI water were significantly higher than in tap water and saline water. Moreover, the ORP value of DI water reaches a maximum with 12-minutes of treatment.

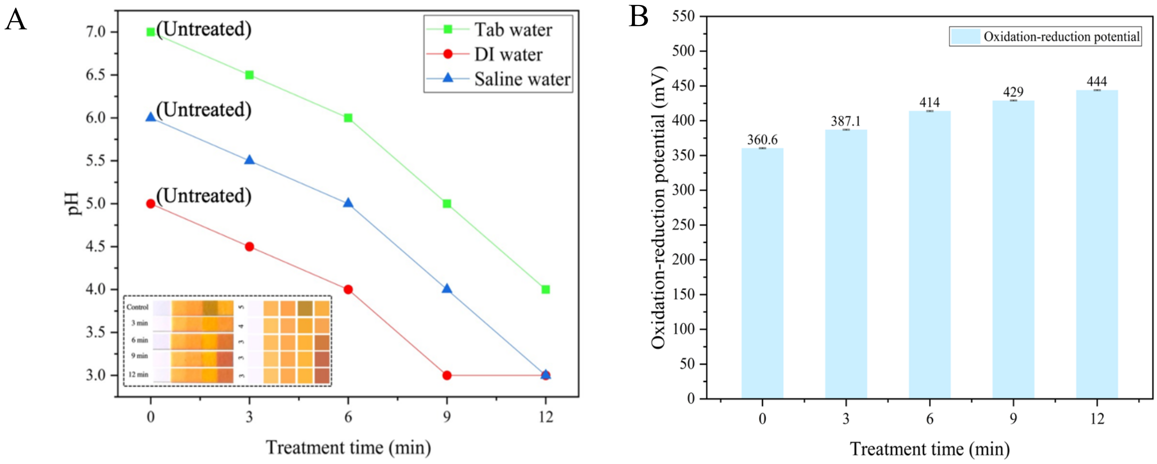

Figure 4. **(A) pH revelation in Ar-NTTAPJ-treated in three different solutions : tap water, DI water, and saline water and (B) ORP revealed in Ar-NTTAPJ-treated DI water with different time duration.**

Browning index and visual quality

The effects of treatment duration on the browning index (BI) and the visual quality of sliced apples were examined. After 24-h post-storage at 4° with a plastic vacuum seal bag. The results were shown in figure 5A, sliced apples exposed to plasma for 9-minutes showed the lowest value of BI, and slightly dropped in 3-minutes. In contrast, the value of BI significantly increase in 6-minutes of treatment when compared with the control group. To investigate the visual quality of sliced apples, the plasma treatment with a 9-minutes showed the BI value after treatment was less than before, as represented in figure 5B.

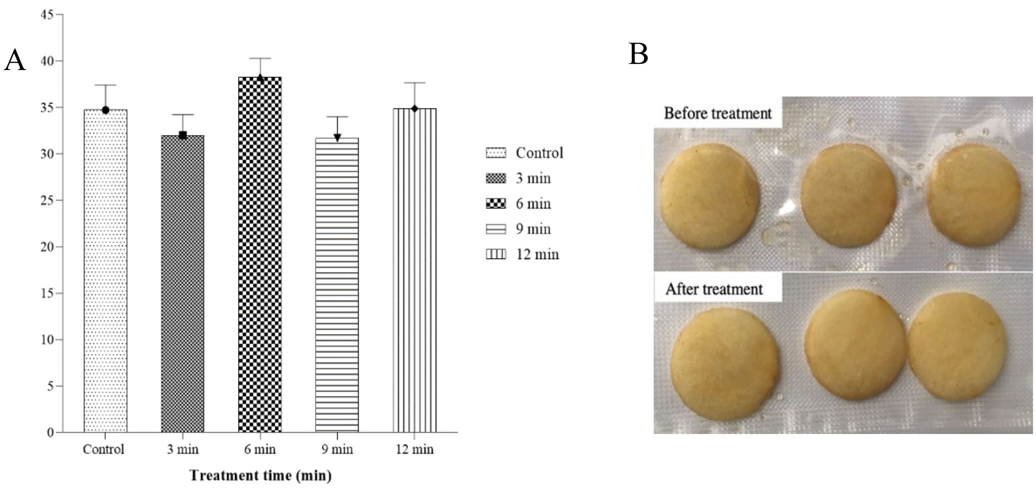

Figure 5. **(A) Browning index of fresh-cut apples under different treatments time and (B) Visual quality of fresh-cut apples under different 9-minutes post-treatments after 24 hours.**

DISCUSSION

The newly developed NTTAPJ device was characterized and its optimal condition for operation. This device consists of two important electrodes such as high-voltage and ground electrodes. The copper ring is a powered electrode, while the outer is dielectric-protected. The principle of operation of the NTTAPJ device was recently reviewed in detail (Rahman et al., 2022). It is note worthy that the NTTAPJ device is a perfectly integrated electrical circuit and is thus productive in PAL applications. Typically,the emission spectra in N2 second positive band (300-400 nm) can be used for estimate gas temperature (Bayram et al., 2015). The rorational band temperature was determined based on equation 8 with the slope of the curve on the righ-hand side term results rotational temperature. The left-hand side term is plotted against the rotation quantum numbers.

$$\ln \frac{I^{em}}{j' + j'' + 1} = -\frac{B' v h c}{K_B T} j'\left(j' + 1\right) + \ln C \tag{8}$$

The gas temperature is estimated by several parameters such as the intensity of the transition for the rotational level (I^{em}), the rotational quantum numbers of the upper and lower electronic states (j' and j''), the speed of light in vacuum, the statistical weight of the upper level, Planck's constant, and (7) Boltzmann's constant (K_B). Thus, the linear fit of plasma operated at 7.6 W was approximately 320 K. In addition, the temperature of PAL was slightly rise during plasma exposure in 12 minutes (25-28°C (ΔT)) The influence of the plasma operational parameters on the resultant RONS liquid chemistry has been investigated in previous studies. Reuter et al. reported that surrounding the plasma jet effluent with a shielding gas influences the production of RONS (Ghimire et al., 2021). However, the height of energy does can generate a higher combination of RONS such as UV, electrons, and excited molecules (Ma et al., 2015; Richmonds et al., 2011; Zhang et al., 2016; Zhao et al., 2020). Hence, higher power and longer activation generate a lower pH and cause higher ORP values, which significantly decrease enzymatic browning reaction (Kojtari et al., 2013; Guo et al., 2017; Lukes et al., 2012; Thirumdas et al., 2018). Especially, the lower pH values and higher ORP values derivatives likely play a key role in inhibiting the PPO (Liao et al., 2019; Zhai et al., 2019). ORP is the potential between the oxidation reaction occurring at the anode and the cathode reduction reaction in the electrochemical cell, which indicates the global level of ROS (Al-sharify et al., 2020; Kamgang Youbi et al., 2009).

The quality degradation of fresh-cut fruit was reported to be mainly related to the color component and browning index. Thus, we used a colorimeter to measure the change of color component, then estimated the browning index of fresh-cut sliced apples after plasma treatment. As the report from the previous study, an increase in the plasma exposure time significantly decreases the mean value of a*, these results agree with the previous study by Ali hajizadeh et al. (Hajizadeh Namin et al., 2021). According to our results, the zone of browned areas was significantly decreased in 3- and 9- minutes treatments compared to control samples. These results agree with a previous study by Ramazzina et al. (Ramazzina et al., 2016) that mentioned the effect of non-thermal plasma can prevent the browning reaction in fresh-cut kiwifruit and fresh-cut higher than controls. In contrast, the study by Pankaj et al. (Pankaj et al., 2013; Pankaj et al., 2017) reported that the browning of white grape juice was increased when compared with the control. Recently, there are several studies that focus on the mechanism of anti-browning agents for fresh fruit and vegetable products. When polyphenols mix with PPO and oxygen, they create a compound called 1,2-Benzoquinone(Almeida and Nogueira, 1995). This can also be called ortho-quinone or o-quinone (Araji et al., 2014), as shown in reaction 9. Next, the individual molecules of o-quinone connect together to make larger molecules. This process is called polymerization. It creates a compound called melanin(Land et al., 2003), which causes the apple to look brown.

$$\text{2,4-Dihydroxyphenol} \xrightarrow{\text{Oxygen}} \text{1,2-Benzoquinone} \quad (9)$$

However, the inhibition of PPO and POD enzymes was shown to depend greatly on the plasma exposure time as the results from Bjoern et al. (Surowsky et al., 2013). Consequently, samples exposed for 9 minutes were characterized by lower BI values compared to the untreated sample. These results confirm those obtained in a previous study of plasma effects on the browning inhibition (Tappi et al., 2014), as the same experimental conditions, the delay browning was observed after 9 minutes of treatment by color component analysis.

CONCLUSION

In summary, NTAPPJ was developed and characterized the basic physical to be used for activating liquids, which can be applied for delay browning in sliced apples. The optical and electrical properties were investigated. The emission spectra show the dominant Ar I and N_2 second positive bands. The complexity of the chemical parameters such as pH and ORP of plasma generated in different solutions by NTAPPJ was evaluated. Moreover, increasing the time at 3- and 9- minutes treatments with plasma-activated DI water significantly affects one of the color parameters (BI). Taken together, the developed NTAPPJ device showed potential activation in a liquid capable to delay browning in sliced apples, possibly by inhibiting the oxidation reaction. However, whether the device can equivalently function in the pre-test conditions, and still unclear in the change of taste after treatment. Thus, the device needs to be further tested on crumbliness or juiciness before the taste panel.

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