

Development of Ozonated Water Technology to Inhibit Enzymatic Browning of “Mang-Koot-Cut”

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Abstract

This work aim to produce high-concentration ozonated water using a dielectric barrier discharge for inhibiting the enzymatic browning of Mang-Koot-Cut. The ozonizer was composed of radio frequency (RF) powered mesh electrode and grounded water electrode that sandwiched a Pyrex glass tube as the dielectric material. The discharge of air flowing through the ozonizer generated ozone gas. The optimal flow rate was 2 L/min. A venturi injector caused an effective mixing between ozone gas and water to obtain ozonated water. The RF power, discharge time and airflow rate controlled the dissolved ozone concentration. Ozonated water with dissolved ozone concentrations greater than 0.2 ppm could reduce the browning reaction of Mang-Koot-Cut. Measurement of the color values L*, a* and b* showed that ozonated water could delay the browning of Mang-Koot-Cut. The results exhibited the possibility of using ozonated water to reduce the browning activity in Mang-Koot-Cut as a substitute for chemicals that are harmful to health. Ozonated water is green chemistry with no toxic residues.

Keywords: Ozonizer, Ozonated water, Enzymatic browning reaction, Mang-Koot-Cut, Color values

Introduction

Ozone (O₃) is a powerful oxidizing agent. It has 1.5 times of the oxidizing properties of chlorine, and there are various applications for gaseous and dissolved ozone or ozonated water in disinfection and food processing [1,2]. Because ozone is highly unstable and decomposes rapidly, it will decompose into oxygen within a few min or a few h depending on temperature, so there is no harmful chemical residue. The U.S. Food and Drug Administration (USFDA) has declared ozone as a substance that is safe for use in the food industry. Thus, it can be applied directly to food products as a GRAS (Generally Recognized as Safe) substance [3]. In general, ozone can be produced by 4 methods: Corona discharge, dielectric barrier discharge (DBD), UV radiation and electrolysis. Each method gives high energy to break the bonds of oxygen molecules to get the oxygen atom, and then 3 oxygen atoms combine to form ozone gas. Electrical ozone generators consist of an ozone tube and a high voltage power supply. The DBD ozone tube is more efficient in producing ozone and generates higher intensity ozone than a corona tube since DBD discharges more uniform plasmas with less thermal loss [4-6]. The DBD ozone tube consists of 2 electrodes, with dielectric material attached to either or both electrodes, providing about 1 - 2 mm air gap between the dielectric and electrode or between the 2 dielectrics. When applying high voltage to the electrodes, a high-intensity electric field is created in the gap. The electric field accelerates the electron to higher energy and collides with the oxygen molecules. Ozone production was initiated by the dissociation of molecular oxygens when collided with electrons with energy greater than 6.0 or 8.4 eV. The plasma phase from the excited state oxygen atom $O(^3P)$ show in Eqs. (1) and (2). The combination of $O(^3P)$ with O₂ resulted in the formation of ozone (O₃), as shown in Eq. (3), while the collisions between O₂ with electrons of energies higher than 13.6 eV lead to ionization, providing concurrent plasma discharge and energetic free electrons. The cross-section for momentum transfer of O₂ is high for all ranges of electron energy, causing an increase in thermal energy. As a result, the combination of O₂ with O to form O₃ was becoming less effective, and O₃ decomposed more easily [7]. Moreover, when the ozone gas bubble in the water, the excited O can react with water, leading to the decomposition of the ozone, according to Eq. (4) [8].

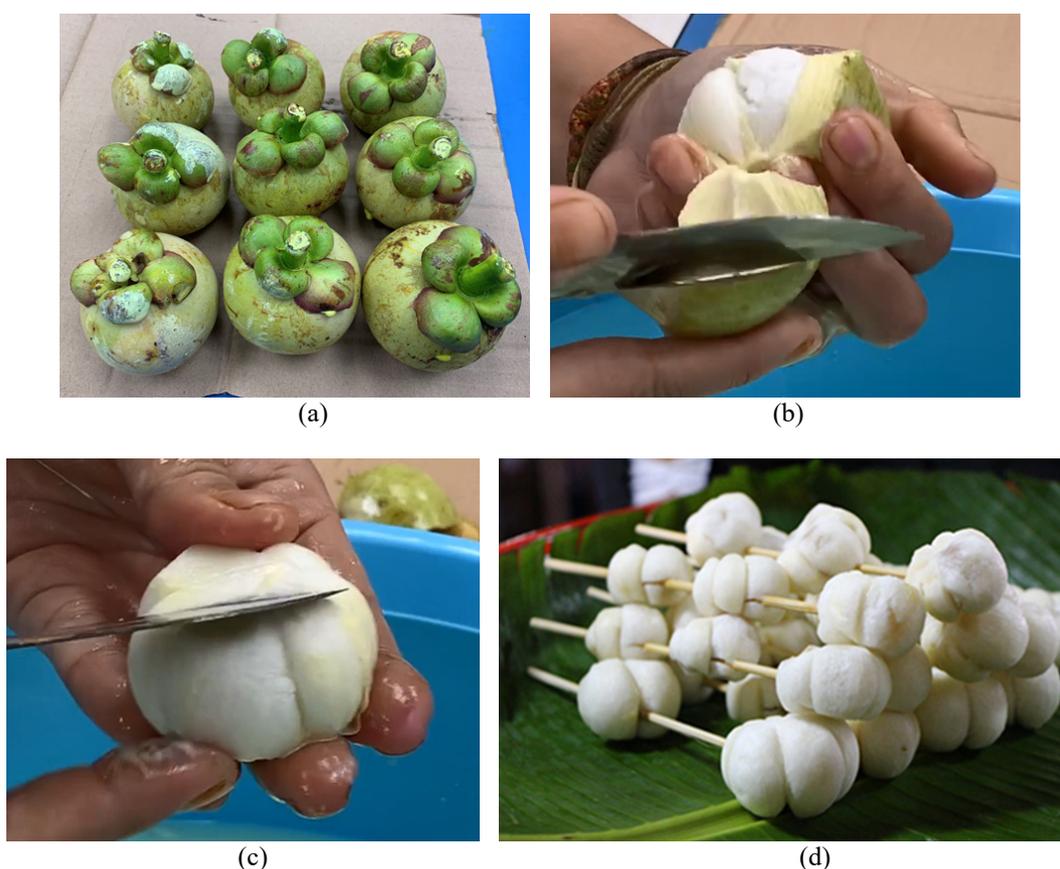


Figure 1 The processing steps for Mang-Koot-Cut showing: (a) A young mangosteen, (b) Peeling the young mangosteen, (c) Trimming off some of the peel attached to the white flesh and (d) Mang-Koot-Cut.

“Mang-Koot-Cut” or “fresh-cut unripe mangosteen” is a famous product from Nakhon Si Thammarat province. It is produced from young mangosteen fruit with a peel that is still green, as shown in **Figure 1(a)**. A knife is used to peel off the green peel, keeping only the white mangosteen pulp. Because the peel and the pulp of the young mangosteens are not yet fully separated, taking the peel off has to be done carefully, as shown in **Figure 1(b)**. It generally needs to be trimmed to peel off entirely, as in **Figure 1(c)**, to get Mang-Koot-Cut flesh, the white pulp with a rounded shape, shown in **Figure 1(d)** [9]. Mang-Koot-Cut has a sweet, crispy and delicious taste. It is popular with local people and tourists. However, it has a shelf life of only 5 - 6 h, with the surface of Mang-Koot-Cut pulp turning brown [10], which is a limitation for its market expansion.

The browning on the surface of Mang-Koot-Cut is a result of the tearing of cells during the peeling and trimming of the mangosteen pulp, which causes the cells to release polyphenol oxidase (PPO) enzyme. PPO activates the oxidizing of monophenol compounds on the Mang-Koot-Cut's surface to form o-quinone. When o-quinone is combined with amino acids, complex brown compounds occur [11], and the skin of the Mang-Koot-Cut turns brown. Typically, local manufacturers of Mang-Koot-Cut use various chemical reducing agents such as aluminum sulfate ($Al_2(SO_4)_3$) or sodium metabisulfite ($Na_2S_2O_5$) to inhibit the browning reaction.

Ozone gas and ozonated water have been used for the postharvest treatment of fruits and vegetables to increase their shelf-life. Ozone and ozonated water can effectively decontaminate fungus and inhibit browning reaction [12]. Several studies have been successful in inhibiting the activity of PPO enzymes by using ozone gas or ozonated water. Zhang *et al.* [13] showed that the PPO activity of fresh-cut celery was inhibited by ozonated water in the first 3 days of storage at 4 °C. The effect of inhibition to PPO activity increased as the ozone concentration increased in water; the best condition was dipped into 0.18 ppm ozonated water for 5 min. Rico *et al.* [14] reported that fresh-cut lettuce treated with ozone 1 ppm showed lower PPO enzymatic activities than the one treated with calcium lactate over 10 days of storage because the high oxidation potential of ozone could decrease higher of the PPO activity. Zhao *et al.* [15] found that treatment with ozone concentration of 6.42 mg/m³ at room temperature every day for 1 h on pear fruit, during 32 days of storage, the O₃ treatments caused a significant reduction in the PPO activity. Azevedo *et al.* [16] reported that sugarcane juice treated with ozone gas at concentrations of 150 mg/L showed inactivation action of the PPO enzyme. In addition, ozone and ozonated water did not affect the physical properties changes of fruits or vegetables. To the best of our knowledge, the application of ozone to inhibit PPO activity on the surface of Mang-Koot-Cut has not previously been reported.

The main objectives of this work were to fabricate a highly-efficiency dielectric barrier discharge ozone tube using mesh-type electrodes, which can produce high ozonated water concentration. Use this ozone tube to set the ozonated water system and measure the optimum conditions of the system. The optimum conditions are used to produce the ozonated water for inhibiting the enzymatic browning of Mang-Koot-Cut during storage at 5 °C.

Experimental design and setup

The development of highly-efficiency ozone tubes for the production of ozonated water included the design, fabrication, and testing of barrier discharge ozone tubes, ozone tubes as well as installation and water mixing systems using a venturi injector. In addition, ozonated water at different concentrations was used in the production of Mang-Koot-Cut from young mangosteens to investigate the efficacy of ozonated water in inhibiting the browning reaction of Mang-Koot-Cut.

Characteristic of the ozonated water production system

Design and fabrication of the ozone tube

We developed a highly efficient ozone tube based on the electric field simulations of the dielectric barrier electrode system in our previous work [17]. We found that a high-intensity and uniform electric field was generated in the gap between the dielectric and grounded electrodes when fine mesh high-voltage electrode was utilized. The electric field was approximately 2 times higher than the conventional plate electrodes, whereas the electric field uniformity was about 90 %. Therefore, discharge is initiated with lower voltage [6]. **Figure 2(a)** shows the design of the dielectric barrier ozone tube. The coaxial dielectric electrode system consists of an inner mesh stainless electrode, Pyrex glass tube and outside grounded water electrode. The glass tube acts as the dielectric material, confining gas flow and ozone production inside the glass tube. The high voltage meshes stainless electrode contacts the stainless steel tube, which is the innermost, while the outer stainless steel tube is the housing and encloses all components of the ozone tube. The cooling system uses the flow of tap water on the outer surface of the glass tube to prevent the decomposition of ozone when the gas temperature rises during gas discharge [18,19]. **Figure 2(b)** shows the assembled ozone tube, presenting the airflow inlet, airflow outlet, cooling water inlet and cooling water outlet.

The high-voltage, high-frequency power supply for the ozone tube was fabricated in-house by the Plasma and Electromagnetic Wave Laboratory (PEwave), Walailak University, Thailand. It produced a controllable frequency and sinusoidal alternating voltage output. The output voltage was controlled by using the pulse width modulation technique [20]. The maximum output voltage was about 6 kV from peak to peak. The resonant power converter could be tuned easily to the resonant frequency of the load. The operating frequency could be varied according to the load and voltage level, typically in the 10 kHz and 1 MHz range. For the ozone tube used in this work, a frequency of 139.5 kHz was used.

Figure 2(c) shows the various components and their functions in the ozone tube. As air flowed through the high-voltage mesh electrode, gas discharge occurred to form plasma (pink) on the surface of the glass tube, covering the mesh electrode. The ozone (O₃) produced in the plasma flowed out of the ozone tube to the venturi injector for O₃-water mixing to obtain ozonated water. The water (blue) worked as a grounded electrode and dissipated heat from the plasma and glass surface.

The ozone generator system uses the developed ozone tube obtained from this work; the system has an efficiency of 2.7 g/W, which is more than household and industrial ozone generators of the commercial ozone generators. This efficiency is near to the efficiency of some large commercial ozone generators [21].

Experimental setup for the production of ozonated water

Figure 3 shows an experimental setup to investigate the parameters affecting the concentration of ozonated water, such as airflow rate, radio frequency power, and discharge time. An oil-free compressor supplied air, and the airflow rate was adjusted by a gas flow meter. Tap water at 27 °C was used for the cooling. The venturi injector generated a pressure gradient by water flow from a pump, which caused the ozone gas to become very tiny bubbles inside the water and dissolve ozone gas into the water effectively. The closed-loop system of ozonated water production caused the concentration of ozonated water to increase over time. The ozonated water was stored in a polyethylene terephthalate (PET) bottle, whereas its concentration was measured by Ozone vacu-vials kit K-7423 and CHEMetrics I 2019 photometers. The K-7423 uses the DPD (N, N-diethyl-p-phenylenediamine) Method. Potassium iodide is added to the sample before analysis. Ozone reacts with the iodide to release iodine, and this iodine reacts with DPD to form a pink color. The pink color detected by the I 2019 spectrophotometer capable and expressed the results as ppm (mg/L) O₃ [22]. The experimental procedure for each parameter was as follows:

Air flow rate

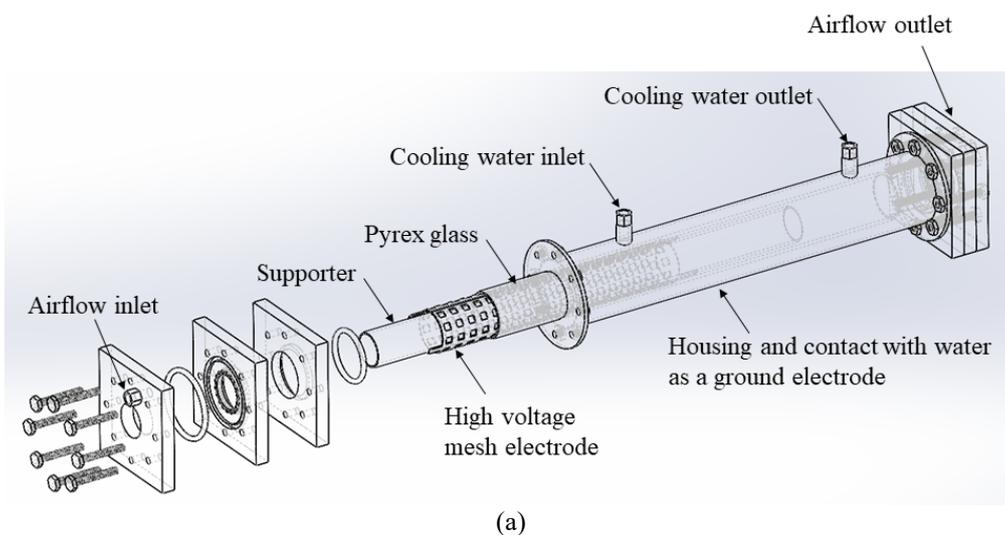
Fix the RF power at 30 W, the airflow rate at 0.2 L/min, and 3 L of the ozonated water store in the PET bottle. Measure the concentration of ozonated water after 20 min, then change the airflow rate to 0.4, 0.6, 0.8, 1, 2, 3, 4 and 5 L/min.

RF power

Set the airflow rate at 2 L/min, the RF power at 12 W and 3 L of the ozonated water store in the PET bottle. Measure the concentration of ozonated water after 20 min, then change the RF power to 15, 20, 22.5, 30, 37 and 46.5 W.

Discharge time

Set the airflow rate at 2 L/min, the RF at 14 W and 3 L of the ozonated water store in the PET bottle. Measure the concentration of ozonated water every 10 min for 60 min, then change the RF power to 17 and 21 W.



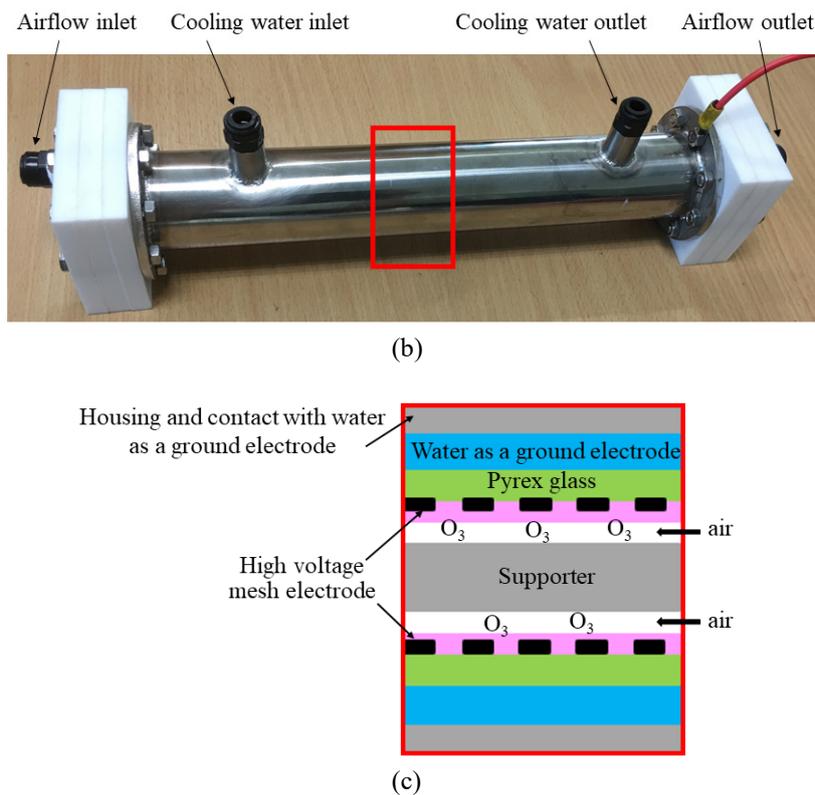


Figure 2 (a) The design of the dielectric barrier ozone tube, (b) The assembled ozone tube and (c) Scheme of the various components and their functions in the ozone tube.

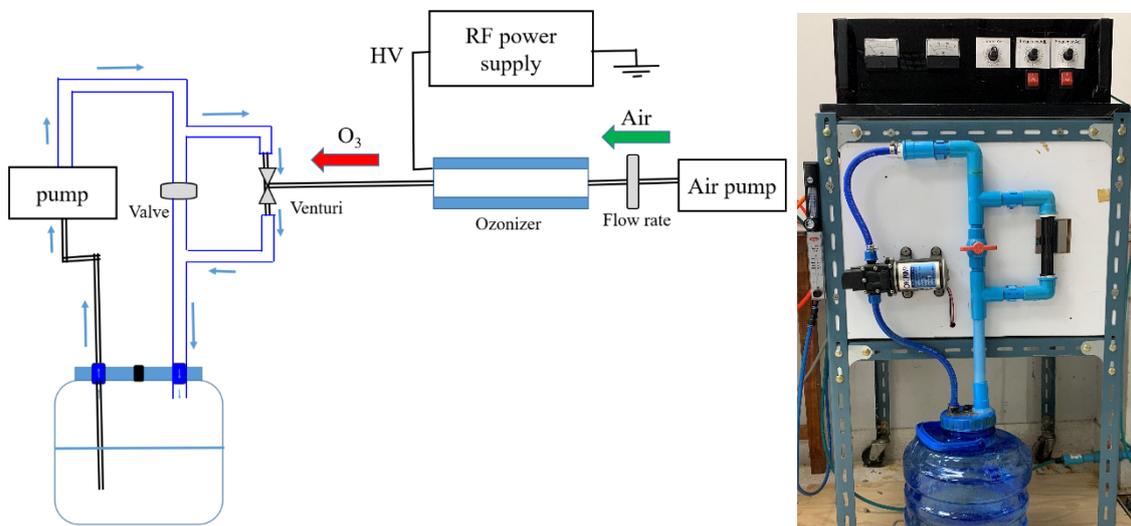


Figure 3 Experimental setup to investigate the parameters affecting the concentration of ozonated water.

Effect of ozonated water on the browning reaction of Mang-Koot-Cut

Ozonated water was integrated into the production procedure of Mang-Koot-Cut from young mangosteens to investigate the efficacy of the ozonated water for inhibiting the browning reaction of Mang-Koot-Cut. Young mangosteens of light yellow color with partially distributed pink dots, classifying as color level 1 according to the Mangosteen Color Level Index [23], as shown in **Figure 4(a)**, were used in the experiments. The 3 different procedures using ozonated water, called Treatment 1, Treatment 2 and Treatment 3, were as follows:

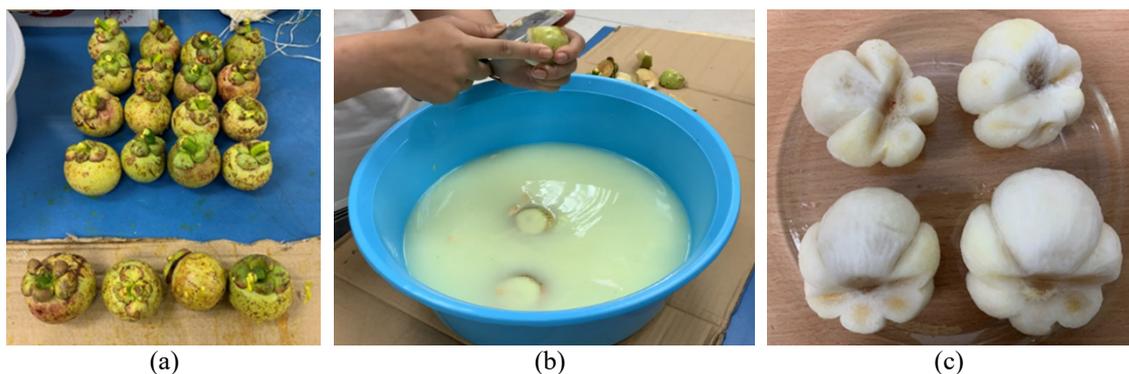


Figure 4 (a) Young mangosteens of color level 1, according to the Mangosteen Color Level Index, (b) Peeling and trimming process for the young mangosteens before soaking them and (c) Mang-Koot-Cut obtained by each treatment.

1) Treatment 1: Peel the young mangosteens and soak them in water as a control treatment, as shown in **Figure 4(b)**.

2) Treatment 2: Peel the young mangosteens in water and then soak them in the ozonated water at 0.1, 0.2 and 0.3 ppm for another 30 and 60 min; each condition uses 3 samples.

3) Treatment 3: Peel the young mangosteens in ozonated water at 0.1, 0.2, and 0.3 ppm concentration and then soak them in those ozonated water for another 30 and 60 min; each condition uses 3 samples.

Mang-Koot-Cut obtained by each treatment, as shown in **Figure 4(c)**, was stored under atmospheric pressure at a temperature of 5 °C. The color was measured using the 3 nh Digital Colorimeter NH310 (Shenzhen ThreeNH Technology Co.,Ltd., China) with a port size of 8 mm, the color display set to D65 illuminant and observer of 10 °C. Three Mang-Koot-Cut per condition, each Mang-Koot-Cut has measured 3 areas around of fruit, and L^* , a^* , b^* values were based on an average of 3 Mang-Koot-Cut. The colorimetric analysis was measured every day of refrigerated storage. Compare the data of all treatments.

Results and discussion

Characteristics of the ozonated water production system

Figure 5(a) shows the dependence of ozonated water concentration on airflow rates. By increasing the airflow rate, ozone concentration increased linearly in the range of 0 - 2 L/min and decreased slowly when the airflow rate was greater than 2 L/min. Thus, ozonated water with a maximum concentration about of 0.30 ppm was obtained at the optimum airflow rate of 2 L/min. While the airflow rate was low, increasing the airflow rate produced more ozone gas and made the ozonated water more concentrated. Because there was more oxygen gas entering the ozone tube, which increased the production rate of O atoms, ozone generation was increased as a result. However, the production rate of ozone gas decreased when increasing the airflow rate over 2 L/min, causing the reduction of ozonated water concentration. Due to increasing the airflow rate while the RF power was constant, oxygen gas molecules rapidly pass through the discharging region, or the gas residence time decreased. Hence, the probability of collision of oxygen molecules with electrons decreases, causing a decrease in a plasma discharge. Therefore, when the plasma density decreased, O atoms and ozone gas production rate lowered [24-26]. **Figure 5(b)** shows the dependence of ozonated water on RF power when the airflow rate was constant at 2 L/min. More ozone gas was produced by increasing the RF power from 10 to 30 W, which made the ozonated water more concentrated. However, when the RF power was greater than 30 W, the increase of RF power decreased the concentration of ozonated water. When the RF power was greater than 30 W, the collision rate between the electrons, neutral gas atoms and molecules increased, which caused the gas temperature to increase and reduced the ozone gas generation rate. At high temperatures, the chance of combining O atoms into O_3 decreased, and the decomposition rate of O_3 increased [27].

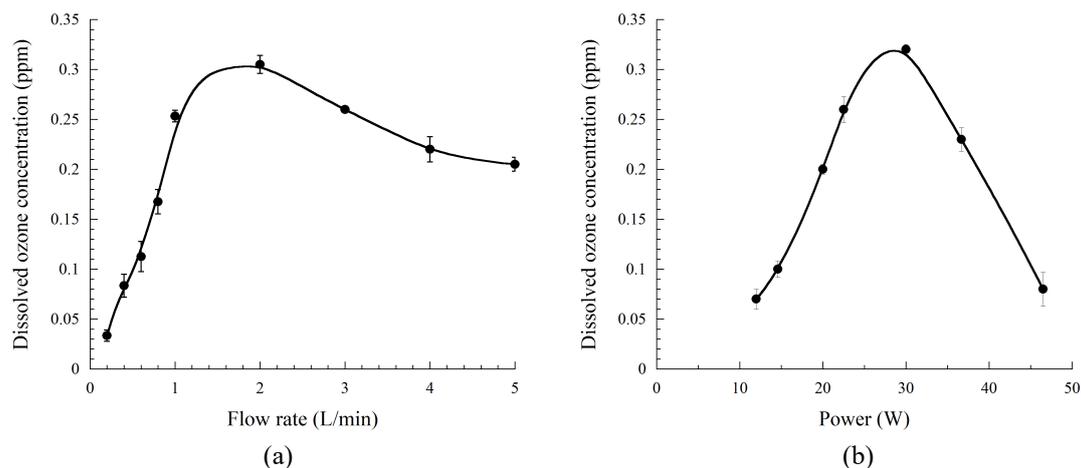


Figure 5 (a) The dependence of ozonated water's concentration on airflow rates at the power 30 W. (b) The dependence of ozonated water on RF power when the airflow rate was constant at 2 L/min.

An increase in ozonated water concentration as the discharge time increased, while the RF power of 14, 17 and 21 W was characterized, as shown in **Figure 6**. During the first 10 min, the concentration of ozonated water increased rapidly and gradually became saturated. The higher the RF power, the higher the increasing rate of ozonated water's concentration. At a constant water temperature of 25 °C, the dissolution rate of ozone gas into water depended on the concentration of ozone gas as well as the density gradient of ozone gas and ozonated water. During the first 10 min, the concentration of ozonated water was low and the density gradient was high, resulting in a high dissolution rate. Therefore, the concentration of ozonated water increased rapidly [28]. After more than 10 min, a saturation of the dissolved ozone in the water occurred; no more ozone gas could dissolve into the water. The concentration of ozonated water, therefore, became constant [29].

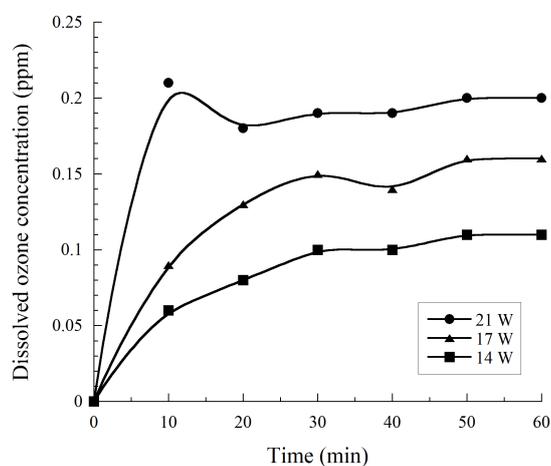


Figure 6 The dependence of ozonated water's concentration on the discharge time, while the airflow rate 2 L/min and the RF power of 14, 17 and 21 W.

Effect of ozonated water on the browning reaction

Figure 7 shows the color comparison of the Mang-Koot-Cut of different treatments after 17 days of storage. The color characteristics of the control treatments on Day 1 and Day 17 are shown in **Figures 7(a)** and **7(b)**, respectively. It was found that Mang-Koot-Cut developed a distinct brown appearance after 17 days. **Figures 7(c)** and **7(d)** show the characteristics of Mang-Koot-Cut of Treatment 2 after 17 days of storage, showing the effect of inhibiting the browning reaction when soaked in ozonated water at a concentration of 0.3 ppm for 30 and 60 min, respectively. As a result, 30 min soaking was less brown

color development than that of 60 min. For Treatment 3 using ozonated water at a concentration of 0.2 ppm for peeling then soaking for 30 and 60 min, the color appearance of Mang-Koot-cut is shown in **Figures 7(e) and 7(f)**, respectively. In Treatment 2, 30 min soaking was less brown color development than that of 60 min. From the results of Treatment 2 and Treatment 3, it was found that 30 min of soaking Mang-Koot-Cut was optimum to inhibit the browning reaction. However, Mang-Koot-Cut became browner when soaking for 60 min. This was probably caused by the oxidation of the complex molecules of Mang-Koot-Cut by ozonated water [30,31].

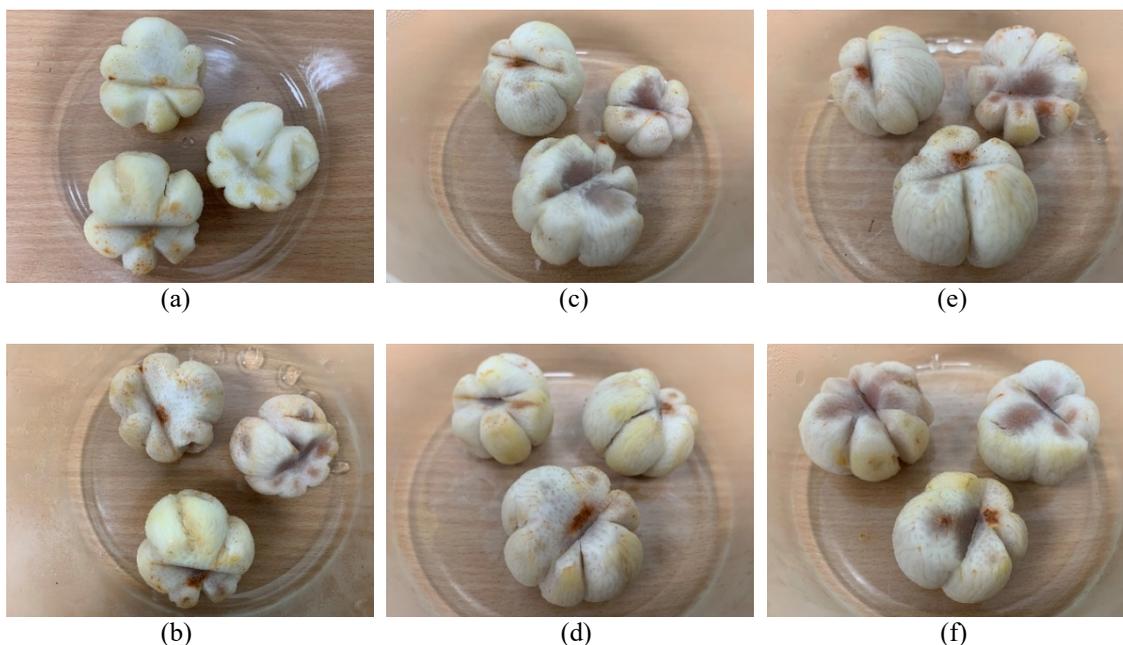


Figure 7 Color of Mang-Koot-Cut with different treatments: (a) and (b) show color characteristics of the control treatments before and after 17 days of storage. (c) and (d) show characteristics of Mang-Koot-Cut of Treatment 2 with concentration 0.3 ppm 30 and 60 min, respectively. (e) and (f) show the characteristics of Mang-Koot-Cut of Treatment 3 with a concentration of 0.2 ppm at 30 and 60 min, respectively.

The colors of Mang-Koot-Cut were measured using L^* , a^* and b^* units, as shown in **Figures 8 - 10**. The brightness L^* of the Mang-Koot-Cut for all treatments decreased with longer storage time due to the browning reaction [10], as shown in **Figure 8**. The L^* value are a color scale measuring light to dark colors in food samples with the higher L^* value ($L^* = 100$) signifying the lightest end of the range compared to $L^* = 0$, which indicates more black color [32], therefore the lower L^* value indicates that Mang-Koot-Cut has more browning. For Treatment 2, Mang-Koot-Cut had the highest L^* for both soaking times of 30 and 60 min by soaking in 0.3 ppm of ozonated water, as shown in **Figures 8(a) and 8(b)**, respectively. As processed by Treatment 3, peeling the young mangosteen in 0.2 ppm of ozonated water for 30 min then soaking the Mang-Koot-Cut in 0.2 ppm of ozonated water for another 30 min gave the highest L^* , as shown in **Figure 8(c)**. However, the L^* value decreased until it was close to the L^* value of the control treatment when the times were increased to 60 min, as in **Figure 8(d)**.

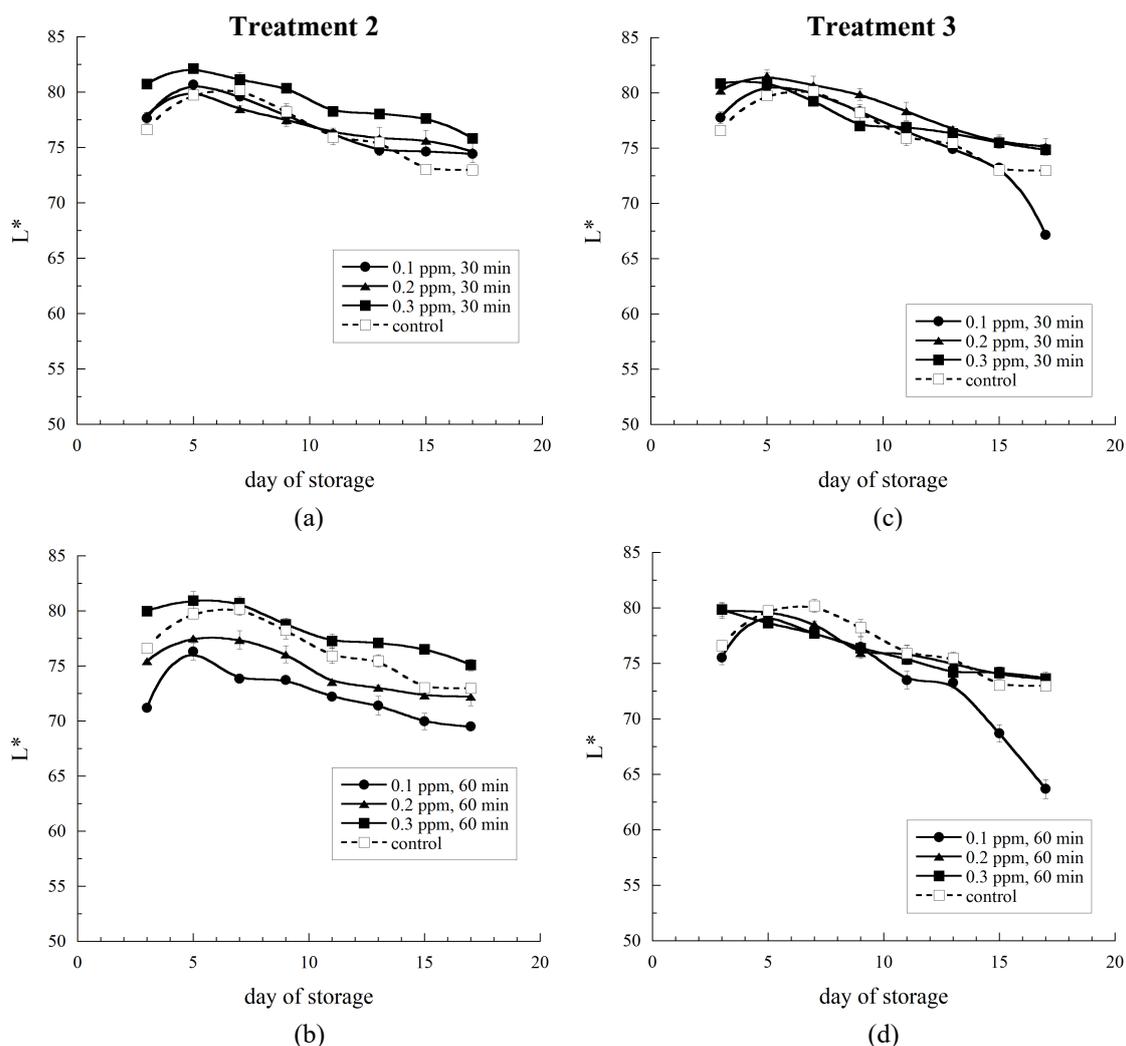


Figure 8 The brightness L^* of Mang-Koot-Cut for all treatments: (a), (b) Treatment 2 for 30 and 60 min, respectively and (c), (d) Treatment 3 for 30 and 60 min, respectively.

Figure 9 shows the redness a^* of all treatments, which tended to increase when the storage times of Mang-Koot-Cut was longer. The a^* value is a measure of green ($-a^*$) to red ($+a^*$) color in food samples [32], therefore the higher a^* value indicates that Mang-Koot-Cut has more browning. In **Figures 9(a)** and **9(b)**, it is clear that the soaking of Mang-Koot-Cut in 0.3 ppm of ozonated water for 30 and 60 min for Treatment 2 resulted in the lowest a^* value, indicating that minimal browning reaction occurred. In addition, the a^* for 30 min soaking was lower than that of 60 min. With Treatment 3, Mang-Koot-Cut had more red (higher a^*) than that of control treatment during the storage period of day 1 to day 10. After day 8, however, the increasing rate of the a^* of the control treatment became higher, and the a^* of Treatment 3 was lower after day 10. By using 0.2 ppm of ozonated water for peeling and soaking, the a^* value was the lowest, as shown in **Figures 9(c)** and **9(d)**.

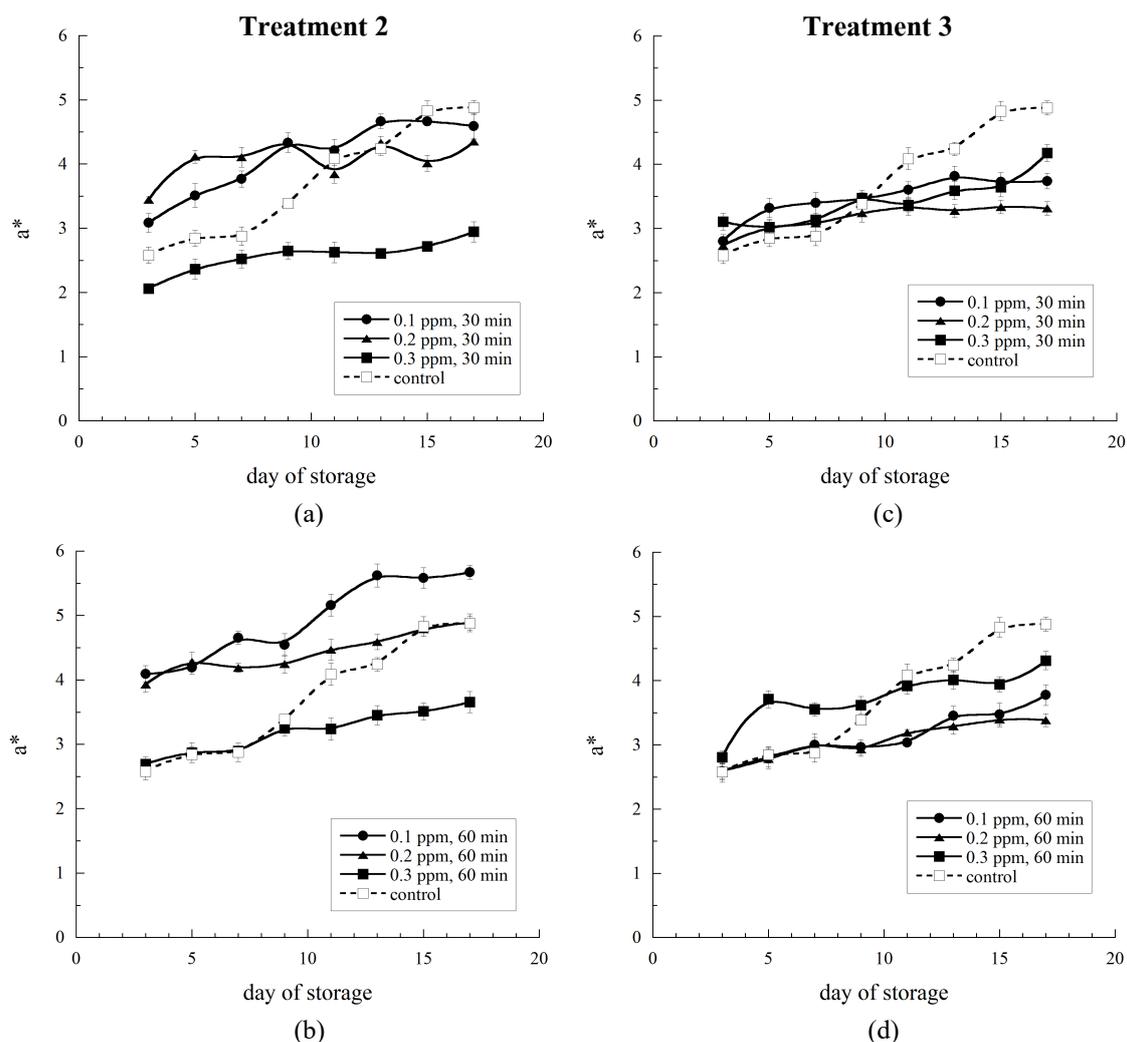


Figure 9 The redness a^* of Mang-Koot-Cut for all treatments: (a), (b) Treatment 2 for 30 and 60 min, respectively and (c), (d) Treatment 3 for 30 and 60 min, respectively.

The yellowness b^* of Mang-Koot-Cut of each treatment and each condition was different since the 1st day of the storage. The b^* value is a measure of blue ($-b^*$) to yellow ($+b^*$) color in food samples [32], therefore the higher b^* value indicates that Mang-Koot-Cut has more browning. It is constant throughout storage, then slightly increases after 17 days (**Figure 10**) because the tone of brown is based on a reddish color more than yellow color. In the case of Treatment 2, using ozonated water at a concentration of 0.3 ppm had lower b^* than other treatments, including the control sample, as shown in **Figures 10(a)** and **10(b)**: Increasing soaking time from 30 to 60 min increased b^* . **Figures 10(c)** and **10(d)** show the b^* of Mang-Koot-Cut when using Treatment 3. It was found that using 0.2 ppm ozonated water for peeling and soaking resulted in the lowest b^* values. However, the use of 0.3 ppm ozonated water resulted in higher b^* than that of the control treatment when the time was increased from 30 to 60 min.

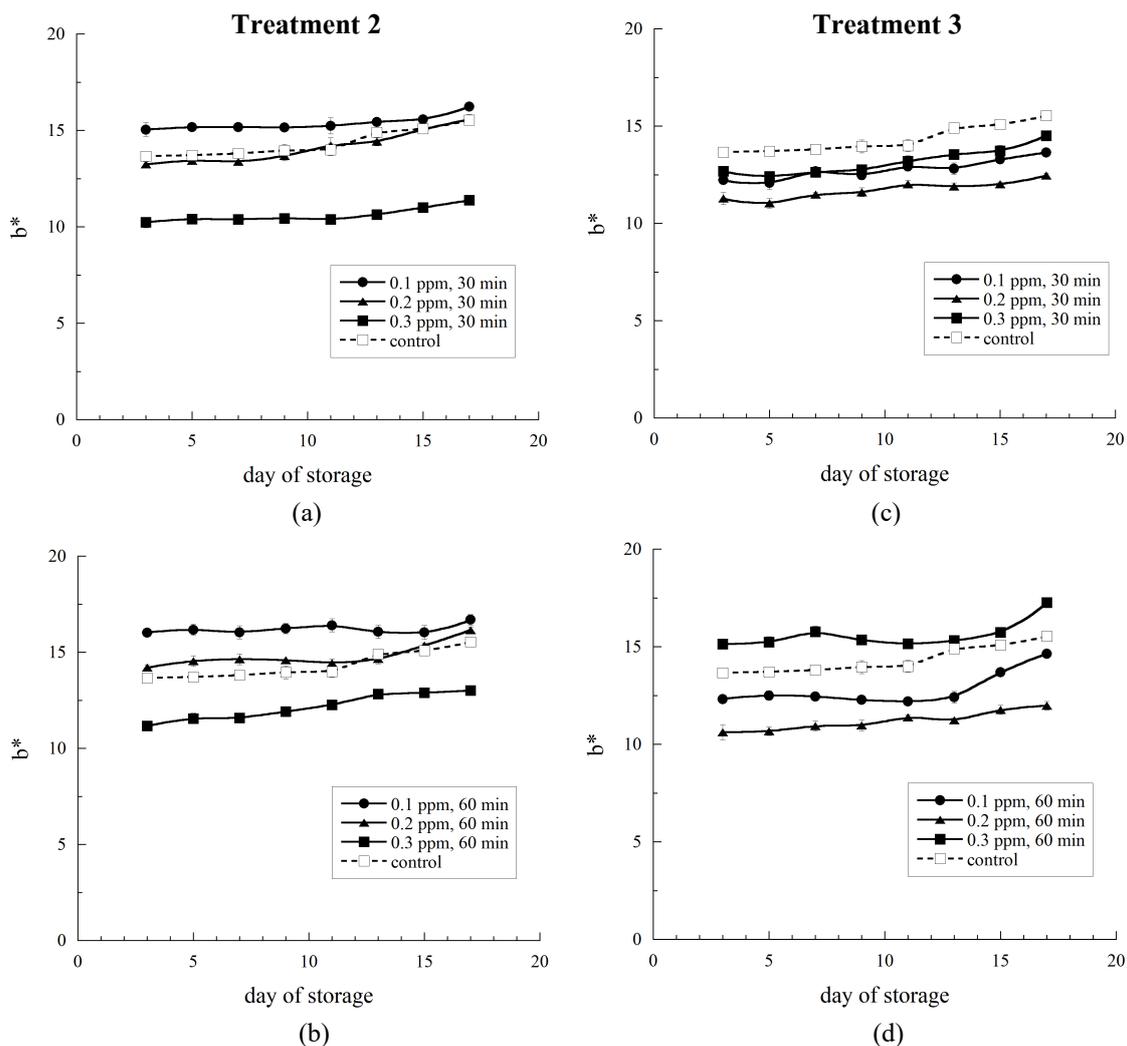


Figure 10 The yellowness b^* of Mang-Koot-Cut for all treatments: (a), (b) Treatment 2 for 30 and 60 min, respectively and (c), (d) Treatment 3 for 30 and 60 min, respectively.

The experimental results in **Figures 8 - 10** show that the optimum condition to inhibit the browning reaction of Treatment 2 was to soak Mang-Koot-Cut in 0.3 ppm ozonated water. For Treatment 3, however, the young mangosteen had to be peeled in ozonated water and then soaked for 30 min in ozonated water at a concentration of 0.2 ppm.

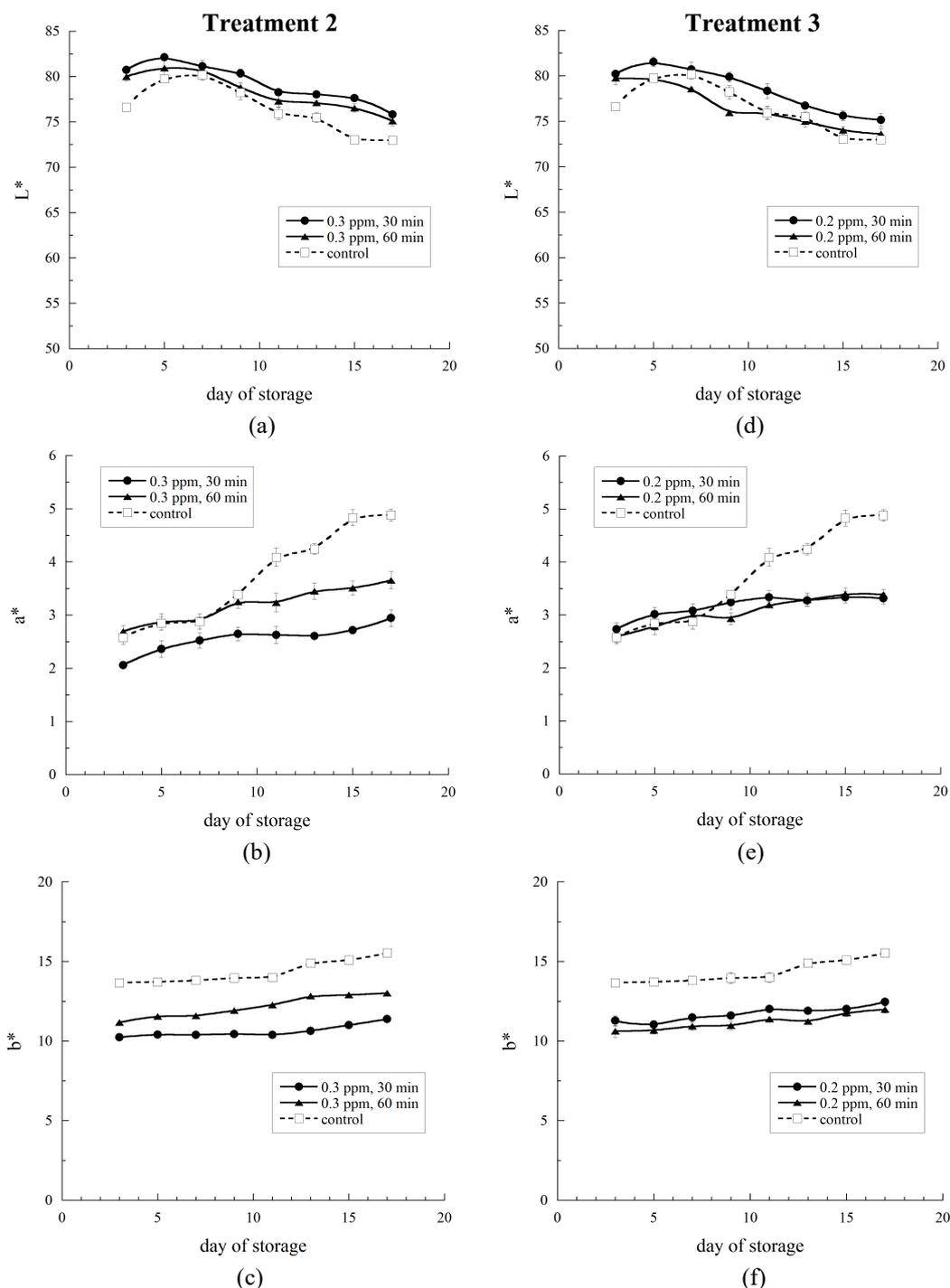


Figure 11 Differences in L^* , a^* and b^* between Treatment 2 and Treatment 3 at different times, with (a), (b) and (c) showing Treatment 2 with a concentrations of 0.3 ppm for 30 and 60 min, and (d), (e) and (f) showing Treatment 3 with a concentrations of 0.2 ppm for 30 and 60 min.

The differences in L^* , a^* and b^* for Treatment 2 and Treatment 3 at different times are shown in **Figure 11**. In Treatment 2, increasing the time of soaking Mang-Koot-Cut from 30 to 60 min would decrease L^* while a^* and b^* increased. Using Treatment 3, increasing the peeling time for young mangosteen and soaking Mang-Koot-Cut from 30 to 60 min reduced L^* , while a^* and b^* values were not much different. These results suggested that ozonated water could inhibit the enzymatic browning reaction. In case longer soaking time will result in lower color quality, maybe because in water, the half-life of ozone is much shorter than in air (half-life between 20 to 30 min) [32]. That mean ozone

decomposes faster in water; therefore, oxygen molecule from decomposition is the reason for more reddish color. The use of ozone can reduce the browning content of Mang-Koot-Cut because ozone can inhibit the activity of the enzyme PPO [14], corresponding to Pongprasert *et al.* [30], Jandric *et al.*, [34] and Najafi and Shaban [35]. Further, ozone can destroy color-causing precursors, including phenol compounds [36].

Conclusions

The developed ozonated water production system consists of a dielectric barrier discharge ozone tube, which uses a fine mesh electrode and grounded water electrode that sandwiched a Pyrex glass tube. The discharge in the air was produced by a high-voltage and high-frequency RF power supply to produce ozone. Ozonated water of 0.3 ppm, obtained through a venturi injector, was produced with an airflow rate of 2 L/min, which used only 30 W of RF power, and took a discharge time of about 10 min. Using 0.3 ppm ozonated water to soak Mang-Koot-Cut for 30 min could inhibit the enzymatic browning reaction caused by the PPO enzyme and phenol compounds. When peeling young mangosteen and soaking the Mang-Koot-Cut in ozonated water, its concentration could be reduced to 0.2 ppm to inhibit the browning reaction. The use of ozonated water, a non-residue process, will be a safe alternative to using chemicals that are harmful to consumers. For the practical use of ozone water to inhibit the browning reaction of Mang-Koot-Cut, more studies are needed to investigate the effect of ozonated water on taste, texture and acceptance by customers.

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