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THE INFLUENCE OF OZONE EXPOSURE ON ORGANOLEPTIC AND FLAVONOID LEVELS OF RED BETEL (*PIPER CROCATUM RUIZ & PAV.*) LEAVES

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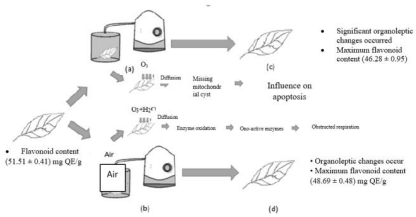
Graphical abstract



Abstract

This research aims to determine the levels of flavonoids found in red betel (*Piper crocatum Ruiz & Pav.*) Leaves and the effectiveness of ozone in reducing cell damage. The ozone was created using a corona discharge ozone generator, which creates an electrical discharge by applying high voltage to ambient air. An ozone analyzer was used to track the ozone concentration to maintain uniformity throughout the tests. Using air and water exposure methods, ozone was applied to red betel leaves (*Piper crocatum Ruiz & Pav.*) at various durations: 120, 240, 360, and 480 seconds. Organoleptic testing on red betel leaf cells revealed damage, an evaporation mechanism, and variations in flavonoid content. Results showed distinct organoleptics produced by ozone flow and exposure approaches. The exposure method through water produced the greatest organoleptic results with an ozone flow time of 240 seconds. The amounts of flavonoids in red betel leaves showed variations in ozone concentration and exposure method. The maximum concentrations of red betel leaf flavonoids (48.69 + 0.48 mg QE/g) were detected at an ozone flow time of 240 seconds using the exposure method through water. Significant differences ($p < 0.05$) in flavonoid levels and organoleptic qualities were found in a one-way ANOVA. The water exposure method at 240 seconds resulted in the highest flavonoid concentration (48.69 ± 0.48 mg QE/g) and optimal organoleptic quality, with significant differences observed ($p< 0.05$).

Keywords: Red betel (*Piper crocatum Ruiz & Pav.*), ozone, organoleptic, levels of flavonoids

1.0 INTRODUCTION

The Indonesian people have employed medicinal plants as an alternative type of medicine for a very long time to encourage health, prevent sickness, cure illness, and restore health [1]. Numerous vital substances that are effective for enhancing health are found in medicinal plants. These vital substances are classified as phytochemicals, a class of organic substances that can be used to maintain and enhance health as well as treat disease. One of the many benefits of red betel (*Piper crocatum iRuiz & Pav*.) is that it is a medicinal herb.

The community uses red betel to treat a variety of illnesses, such as hypertension, ulcers, vaginal discharge, breast cancer, liver, eye, gingivitis, prostate, and joint pain, to lower and control blood sugar, as cosmetics, and as an antiseptic to clear microorganisms from wounds [2]. Red betel contains flavonoid components, essential oils, alkaloids, stereoid, tannins-polyphenols, and neolignan compounds. Polyphenolic substances known as flavonoids are present in almost all plants. Antioxidants, anti-atherosclerotic, anti-thrombogenic, anti-inflammatory, anti-tumor, antiviral, and anti-osteoporosis agents are all functions of flavonoids [3]. According to research, red betel contains flavones and flavonols, both kinds of flavonoids. Red betel leaves contain 36.3778 g/ml of flavonoids [4]. Especially red betel leaves, traditional medicinal substances can be used in fresh or dry form [5]. Fresh ingredients used in conventional medicines must first undergo a washing procedure before being converted into dosage forms. The medicinal plants must be used right away if the compounds are to be used in fresh form. This is due to the fact that if the therapeutic substances are not employed right away, their quality will decline. This decrease in quality is anticipated to lead to a reduction in the chemical content of medicinal plants. It manifests as cell damage to fresh medicinal ingredients, which is characterized by wilting, discolored leaves, and the weakening of stiff stems. Additionally, if the postharvest processing of traditional medicinal substances is done properly, they can produce results of the highest calibre, have high-quality, stable, and effective substance levels, and have a pleasing physical appearance.

After harvesting, medicinal herbs are dried into simplicia. *Simplicia* is a natural product that has only been dried for use in medicine and has not been subjected to any further treatments [6]. Farmers still produce *Simplicia* in a traditional manner that occasionally deviates from good and proper handling techniques, making it challenging to produce simplicia of the highest quality. *Sambiloto* leaves, *Mahkota dewa*, simplicia rhizome of turmeric, temureng rhizome, and 16 species of *Aspergillus mold*, nine of the molds have the ability to create mycotoxins that are detrimental to human health. The simplicity might be harmed during storage. Therefore, if postharvest management involves drying medicinal plants improperly and incorrectly, it is less than ideal for preserving quality [7].

Ozone technology can be used as a post-harvest handling substitute. Ozone postharvest handling of produce, fruit, and fishery items has promising futures as it is regarded as safe and efficient [8]. 15 minutes of ozone exposure was the ideal amount of time to keep chili fresh [9]. As ozone will turn into oxygen when exposed to sunlight, using it to handle plants after harvest won't alter or harm their nutritional content [10]. According to a study on chilies exposed to ozone, they were able to maintain their freshness and water content which is Typically, they stay fresh for 2–3 days at room temperature (27 °C) [11].

Strongly oxidizing and odorous, ozone (O_3) is an unstable form of oxygen containing three oxygen atoms [12]. Ozone has the ability to act as a disinfectant that can kill bacteria, it does so 3250 times more quickly than chlorine does. Ozone can break down into atomic oxygen (O*) and molecular oxygen gas (O_2) when exposed to ultraviolet radiation with a wavelength between 240 and 340 nanometers. Ozone is therefore safe to use. Red betel leaves (Piper crocatum Ruiz & Pav.) were collected, identified based on morphology, and a voucher specimen was preserved in the herbarium for reference [13].

The absorption of light and the collision process are the two mechanisms that contribute to the production of ozone [14]. After oxygen gas (O_2) absorbs ultraviolet light with a wavelength of less than 240 nm, a dissociation event which is the breakup of oxygen gas (O_2) into two oxygen atoms (O) occurs in the light absorption process. Each newly created oxygen atom becomes extremely reactive and can combine with oxygen gas (O_2) to create ozone (O_3). By flowing oxygen gas through an area that is given a high voltage, which causes oxygen to ionize, the collision process that leads to the creation of ozone is started. Plasma conditions refer to oxygen molecules that have been ionized. Ozone can be created when the resultant oxygen molecules are combined. When used for post-harvest processing, ozone is safe since it evaporates into oxygen gas (O_2) and yields oxygen atoms (O) [15].

Ozone plasma can be produced using a dielectric-impeded discharge by employing an alternating high voltage source to flow oxygen in a narrow gap between two electrodes and a glass-based dielectric material. In this scenario, the dielectric material serves as an energetic electron filament (1–10 eV) for the current. Electrons are driven toward the anode, and positive particles are accelerated toward the cathode as a result of the high voltage placed between the electrodes. When oxygen enters the gap and bumps into electrons, ozone is created [16]. In this situation, the voltage starts to influence the ozone concentration. The electric field inside the reactor increases in proportion to the voltage supplied between the electrodes. This raises the energy of electrons, which causes additional ionization [17]. Voltage and ozone flow

duration both influence ozone concentration. With an increase in ozone flow at a particular voltage, ozone concentrations will rise [18].

Two ozone exposure methods were used through water and air with varying ozone flow durations, to compare their effects on microorganisms [19]. This study intends to ascertain the impact of ozone exposure on cell damage and levels of red betel leaf (*Piper crocatum Ruiz & Pav*.) flavonoids based on background data and other prior studiesp [20, 21].

2.0 METHODOLOGY

This The study was conducted at two different locations: The Biophysics Laboratory, Physics Department, Faculty of Science and Technology, Airlangga University; here, red betel leaves (Piper crocatum Ruiz & Pav.) were exposed to ozone and their flavonoid levels were measured. The Analytical Chemistry Laboratory, Chemistry Department, Faculty of Science and Technology, Airlangga University, was used to characterize ozone concentrations.

Sample Treatment

The control group and the treatment group were the two groups in this study that were divided into a completely randomized design (CRD) system. Ozone exposure was not given to the control group. The treatment group was split into two groups: one for aqueous ozone exposure and the other for airborne ozone exposure. Four different ozone flow times 120 seconds, 240 seconds, 360 seconds, and 480 seconds were used for each treatment group. Three times were used to complete the research.

Each sample underwent ozone exposure through water by being cleansed and submerged for 6 minutes each time ozone flowed. The sample was made up of fresh red betel leaves from the red betel plant (*Piper crocatum Ruiz & Pav*.) in Surabaya's Rungkut area located at coordinates Latitude: -7.3541, Longitude: 112.7871. The 48-hour duration allows sufficient time to observe changes in plant characteristics while minimizing variability. The final findings show a correlation between ozone flow time, cell damage, and levels of flavonoids in red betel leaf (*Piper crocatum Ruiz & Pav*.).

Organoleptic and Flavonoid Level Observation

Red betel leaves' organoleptic properties were observed for 48 hours (*Piper crocatum Ruiz & Pav*). A double-beam UV-Vis spectrophotometert was used to assess the flavonoid concentration after 48 hours.

By chopping up the red betel leaves into tiny pieces and then mixing them to create fresh red betel leaf powder, it is possible to measure the flavonoid content of red betel leaves. Additionally, the production of extraction by maceration entails weighing up to 5 grams of sample powder, macerating it in a 96% ethanol solvent for 48 hours while stirring it often, and filtering the mixture through filter paper to separate the filtrate and dregs. Using a rotary evaporator, the dregs that have been removed from the filtrate are subsequently turned into a thick extract. Furthermore, the quantities of flavonoid components were determined using UV-Vis spectrophotometry. By adding 0.5 mL of 2% $AlCl_3$ and 5% CH_3COOH that had been dissolved in ethanol to 1 mL of the extract solution at a concentration of 1000 mg/L and vortexing for 20 minutes, the amount of flavonoids present can be determined. Create a quercetin standard curve next, and use the solution of the quercetin standard curve equation to calculate the concentrations of flavonoids. The absorbance value was entered into the linear equation of the quercetin standard curve to determine the flavonoid concentration [20].

The formula used to determine total flavonoid levels is as follows:

$$x\,(concentration) = \frac{y\,(absorbance) - intercepts}{gradient}$$

$$total\ flavonoid\ levels = \frac{(x)\ x\ (extract\ volume)}{(extract\ mass)}$$

Statistical Analysis

To examine the flavonoid content of red betel leaf (*Piper crocatum Ruiz & Pav*), the factorial Anova test (Two-Way Anova) and the Statistical Package for Social Science (SPSS) were used. The purpose of this experiment is to determine if the treatment has an impact on the amounts of flavonoids in red betel leaves (*Piper crocatum Ruiz & Pav*).

3.0 RESULTS AND DISCUSSION

The quantity of red betel leaf (*Piper crocatum Ruiz & Pav*.) flavonoids was significantly affected by the ozone exposure technique (by air and water), as shown by the results of the two-way ANOVA test. At $p = 0.002 < 0.05$, ozone concentration significantly affected flavonol levels; at $p = 0.000 < 0.05$, it had no effect; and at $p = 0.180 > 0.05$, the interaction between the two components had no effect.

The Effect of Ozone Flow Time on Ozone Concentration

Figure 1 depicts the value of the ozone concentration (O_3) at each ozone flow period. According to the graph, the value of the concentration will also increase the longer the ozone is allowed to flow.

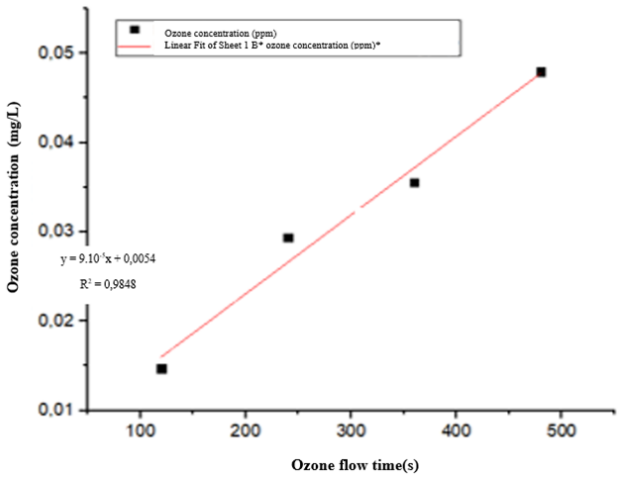


Figure 1 Graph of ozone concentration against ozone flow time

Organoleptic

Data on morphological alterations and mass loss of red betel leaves (*Piper crocatum Ruiz & Pav*.) after exposure to ozone through the air were collected. Figure 2 displays modifications in red betel leaves in both control samples and samples kept in the fridge. Figure 3 depicts how red betel leaves react to exposure to ozone through water at various concentrations. Figure 4 depicts how red betel leaves react to exposure to ozone in the air at different amounts.

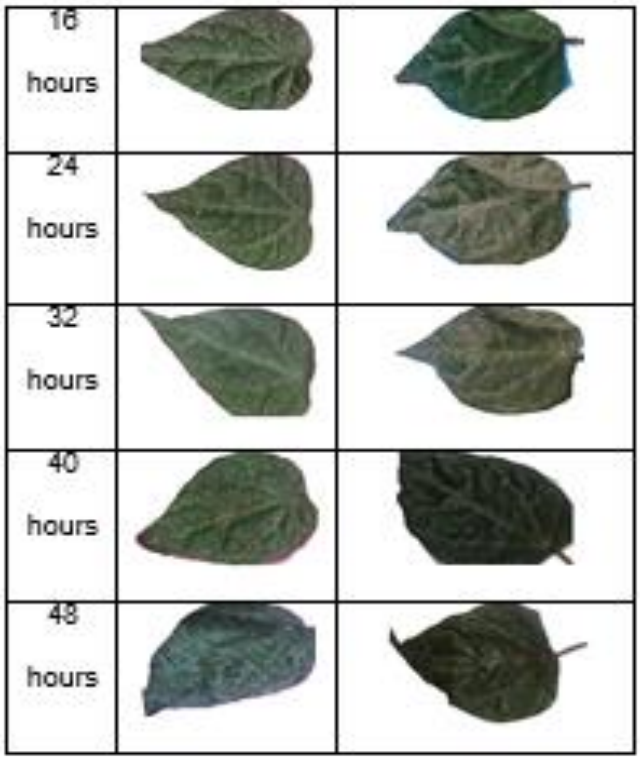


Figure 2 Image of red betel leaves (*Piper crocatum Ruiz & Pav*.) subjected to ozone exposure at various flow times

Results indicate that morphological changes in red betel leaves (*Piper crocatum Ruiz & Pav*.) subjected to ozone via the air occur more rapidly than in red betel leaves exposed to ozone through water or in the absence of ozone treatment. Originally sparkling and bright green, the leaves have changed from that color to a dark, ominous black. With longer ozone flow times, this shift becomes more pronounced. Quantitative color analysis using RGB values showed a shift from green to black in red betel leaves with increasing ozone exposure time.

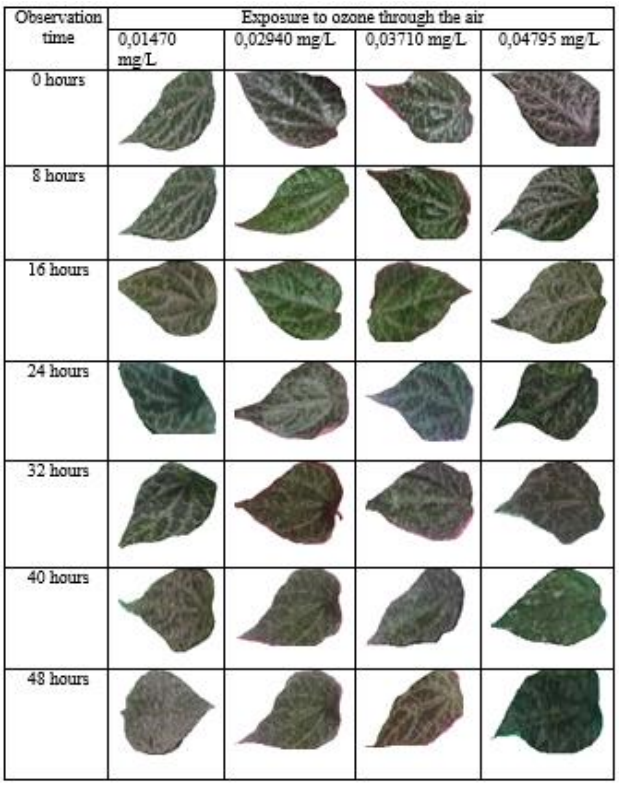


Figure 3 Organoleptic red betel leaves (*Piper crocatum Ruiz & Pav*.) with exposure to ozone through water at various concentrations

According to organoleptic studies, red betel leaves (*Piper crocatum Ruiz & Pav*.) exposed to ozone through water do not degrade quickly. In comparison to the control treatment, the treatment of red betel leaves (*Piper crocatum Ruiz & Pav*.) in the refrigerator, and the treatment of exposure to ozone through the air, this treatment was able to retain the freshness of the red betel leaves (*Piper crocatum Ruiz & Pav*.) better. Red betel leaves (*Piper crocatum Ruiz & Pav*.) exposed to ozone through water underwent the least amount of morphological changes, including changes in the colour and texture of the leaves and leaf stems as well as minimal changes in mass (mass loss), which occurred during the ozone flow time of 240 seconds.

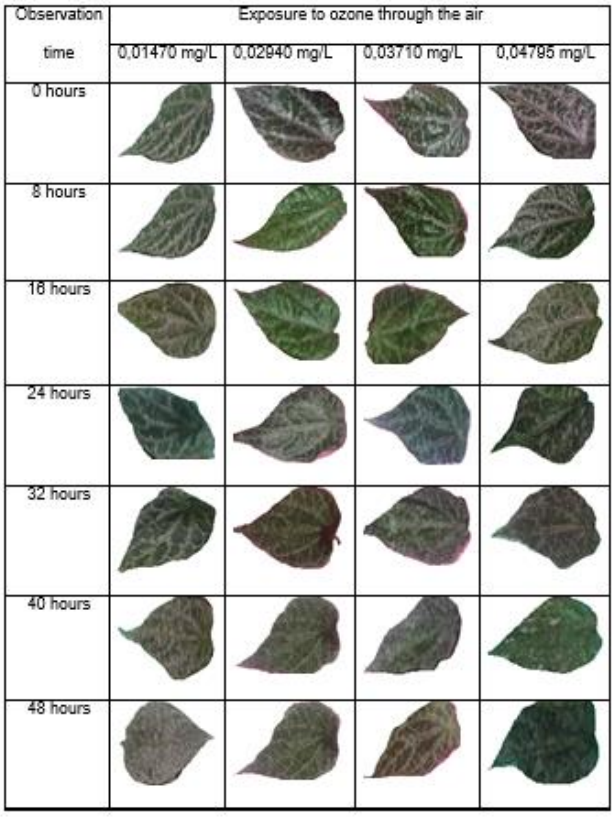


Figure 4 Organoleptic red betel leaves (*Piper crocatum Ruiz & Pav.*) with exposure to ozone through the air at various concentrations

Figure 4 illustrates that red betel leaves (Piper crocatum Ruiz & Pav.) exposed to ozone via the air develop morphological changes more quickly than those exposed to ozone via water and without ozone treatment. Originally sparkling and bright green, the leaves have changed from that colour to a dark, ominous black.

Mass Loss of Red Bethel (*Piper crocatum Ruiz & Pav.*)

Figure 5 displays the trend of the mass loss of red betel leaves (*Piper crocatum Ruiz & Pav.*) under control and treatment following exposure to ozone through air and water.

According to measurements of mass loss, red betel leaf (*Piper crocatum Ruiz & Pav.*) exposed to ozone for longer periods of time lost more mass, as seen in Figure 5. Red betel leaf (*Piper crocatum Ruiz & Pav.*) lost very little bulk as a result of this treatment for ozone exposure through the air. At a 120-second ozone flow duration, the average mass loss of red betel leaf (Piper crocatum Ruiz & Pav.) was 57.36 ± 0.47%. When exposed to ozone via water, red betel leaf (Piper crocatum Ruiz & Pav.) had a little mass loss at an ozone flow duration of 120 seconds, or (41.59 ± 0.64%). The best concentration used in this treatment is to keep red betel leaves (Piper crocatum) fresh.

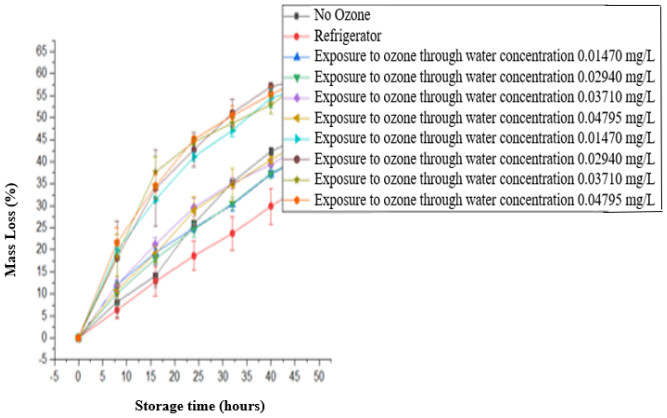


Figure 5 Mass loss of red betel leaves (Piper crocatum Ruiz & Pav.) under different conditions: control (no ozone), ozone via air, and ozone via water, stored in the refrigerator over time. The effect of ozone concentration (0–5 ppm) on mass loss is illustrated. Images provide a visual timeline of leaf changes during storage

Flavonoid content of red betel (Piper crocatum Ruiz & Pav.) Figure 6 displays the amounts of flavonoids in red betel leaf (Piper crocatum Ruiz & Pav.) after exposure to ozone via air and water.

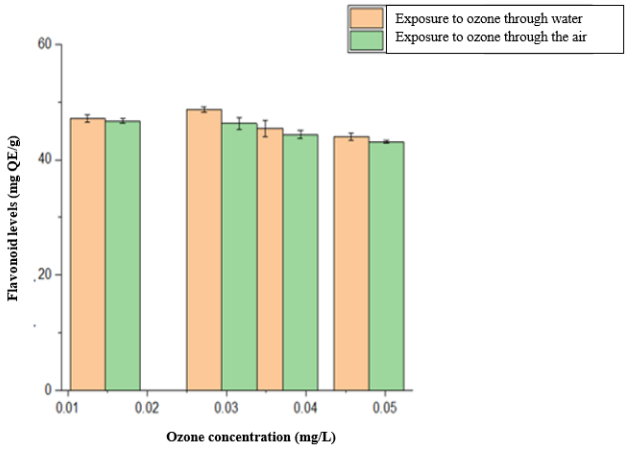


Figure 6 Graph of red betel leaf (*Piper crocatum Ruiz & Pav.*) flavonoid levels after exposure to ozone through air and water to ozone concentrations

The graph shows that the greatest concentrations of flavonoids were found in red betel leaves (*Piper crocatum Ruiz & Pav.*) exposed to ozone via water at a concentration of 0.02940 mg/L, or 48.69 ± 0.48% QE/g. Through exposure to ozone through water, the ideal concentration to sustain the levels of flavonoids in red betel leaves (*Piper crocatum Ruiz & Pav.*) is 0.02940 mg/L. exposure to ozone through water is more beneficial than exposure to ozone through the air [19]. Figure 7 depicts the method of ozone exposure through red betel leaves (*Piper crocatum Ruiz & Pav.*).

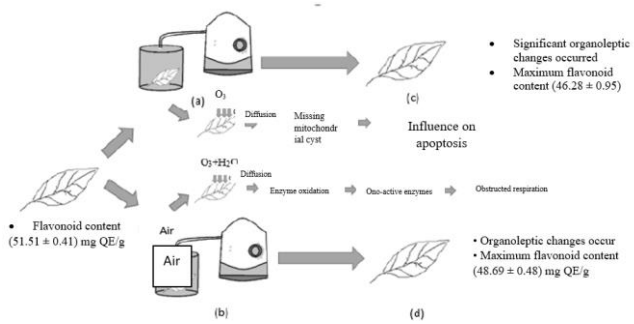


Figure 7 Mechanism of ozone exposure to red betel leaves (*piper crocatum Ruiz & Pav*.), (a) exposure to ozone through the air on red betel leaves, (b)) exposure to ozone through water on red betel leaves (c) ozone causes changes in organoleptic conditions and levels of flavonoids

When ozone is added to water, the process of breakdown results in the production of OH free radicals [21]. The presence of various substances with various functions affects the conversion of ozone into OH radicals. The initiator, inhibitor, and promoter roles are involved here. Superoxide radical ions are created when the initiator, a hydroperoxide ion, reacts directly with ozone. This superoxide radical ion can react with ozone quickly to create free radicals like the ozonide radical ion, which subsequently combines with oxygen to create OH radicals. When substances interact with OH radicals, crucial free radicals known as promoters in this case, superoxide radical ions are created. In addition to serving as an initiating agent and a promoter, hydrogen peroxide has a special place in the decomposition of ozone. Despite being extremely hot, hydrogen peroxide remains stable under certain conditions and can be safely handled with proper precautions.

4.0 DISCUSSION

The quercetin standard solution's maximum wavelength in this study was 412 nm; this maximum wavelength is nearly identical to the quercetin wavelength in earlier studies, which was 415 nm [22]. To estimate the absorption area that the quercetin solution creates, the maximum wavelength must be determined. There was a small variation between the research that was conducted and earlier research. It is possible to say that the research has seen a shift in the maximum wavelength in comparison to previous study [23]. This change took place from 415 to 412 nm. Hypochromic refers to this shift in absorbance toward shorter wavelengths. Solvents' effects or the absence of substituents or aucrosomes on a chromophore cause hypsochromicity.

A linear relationship exists between the concentration of the solution and the absorbance value. This implies that the absorbance value will increase along with the concentration value. The study found that there was a partially linear relationship between concentration and absorbance. Infractions of the Lambert-Beer legislation may be the reason for this. Discrepancies can result from chemical instability, instrumental errors, natural variations in biological samples, and human error or contamination, all affecting the accuracy and reliability of results. Because the term "instrumental" refers to the tool used, chemistry is related to chemical alterations that take place in the substance being measured, such as ionization and hydrolysis.

The absorbance of red betel leaf extract at 358–360 nm and 367 nm indicate the presence of flavonoids or polyphenols, which absorb UV light due to their conjugated double bonds. These compounds, known for their antioxidant and anti-inflammatory properties, are characterized by strong absorbance in this range [24]. The red betel leaves contain flavonols, according to the absorbance value at that wavelength. The red betel leaf extract generates a deep red hue, which can be used to qualitatively demonstrate this conclusion [25]. More flavonoids are present in a leaf when it is redder in hue. This is due to the fact that as the number of molecules in medicinal plant leaves increases, so will the number of molecules that absorb light at particular wavelengths. The absorption value will therefore be higher. The number of flavonoids is inversely correlated with the absorbance value.

The concentrations of red betel leaf flavonoids varied in this investigation for each ozone flow period and ozone exposure method. Flavonoids from red betel leaf (*Piper crocatum Ruiz & Pav*.) produced the highest yield at (48.69 ± 0.48 mg QE/g). These findings pertain to the amounts of red betel leaf flavonoids after 240 seconds of exposure to ozone through water. These findings are not all that dissimilar from red betel leaf flavonoids in fresh form, indicating that exposure to ozone via water can prevent a drop in red betel leaf flavonoids. According to the SPSS test results, there was no significant difference between the concentrations of red betel leaf flavonoids at 0.01470 mg/L (ozone flow time of 120 seconds) and 0.02940 mg/L (ozone flow time of 240 mg/L) ; this is because the leaves evaporated nearly equally under ozone exposure with a flow time of 120 seconds to 240 seconds. Thus, it results in nearly identical organoleptic conditions. The levels of red betel leaf flavonoids on exposure to ozone with flow periods of 120 seconds and 240 seconds were not significantly different as a result of this almost equal organoleptic condition [26].

The mechanism of ozone interaction on red betel leaves starts from the interaction with contaminant microbes on the leaves. Ozone causes phospholipids, lipoproteins, and peptidoglycan to peroxide, which compromises the integrity of microbiological pollutants [27]. Enzyme inactivation, gene inhibition, and genetic material degradation follow, all of which impair the bacteria's ability to carry out its regular cell functions [28]. The water solubility, stability of interactions with both organic and inorganic substances, pH, temperature, and organic residues in

food all affect ozone's antibacterial qualities [29]. Another method of activating water is called ozonation, which is achieved by subjecting the gas to a corona discharge that excites the oxygen electrons and UV light with a wavelength of 188 nm. High energy (about 6–7 eV) are produced by electrochemical reactions in water, which break O–O bonds and produce ozone. Super peroxide, hydroxyl, and hydroperoxyl radicals dwith intense oxidizing properties can be produced when unstable ozone molecules break down (oxidation potential 2.07 mV) [30].

When these radicals come into contact with bacterial cells, they have the ability to trigger a number of chemical processes [28]. Phospholipids found in bacterial cell walls are peroxidized by ozone, which leads to cell wall breakdown and intracellular component leakage [31]. Cell death is the result of ongoing degradation processes that compromise cellular integrity. When washing leaves with ozone, the stomatal valves in the epidermis allow ozone to enter the leaf tissue [32]. Ozone also oxidizes cell walls, causing them to burst or lyse. This results in the inactivation of the enzymes in the chloroplasts' cell nuclei and membranes, which slows down respiration. By preventing respiration, the vegetable's water content will be preserved, preventing cell damage. Thus, it will make the red betel leaves fresher [33].

The role of radical oxygen species (ROS) in damaging microbial cell membranes has been studied in previous studies (34,35). ROS will damage lipids and cell membrane channels so that they are no longer semi-permeable, causing ions to enter the cell, resulting in cell lysis.

5.0 CONCLUSION

Ozone exposure through water delayed cell damage in red betel leaves (Piper crocatum Ruiz & Pav.) by 8 hours compared to the control at 27 °C, while ozone exposure through air accelerated cell damage by 8 hours under the same conditions. With a fall in levels of 2.726 mg QE/g at 27 °C, ozone exposure through water prevented the red betel leaf (*Piper crocatum Ruiz & Pav*.) flavonoids from declining, however ozone exposure through air did not. At 27 °C, the concentration decreased by 5.142 mg QE/g (Ruiz & Pav.). Using a UV-Vis spectrophotometer to test red betel leaves (*Piper crocatum Ruiz & Pav*.), the best ozone treatment for preventing cell damage and maintaining flavonoid levels is treatment with an ozone flow time of 240 seconds using the ozone exposure technique through water at 27°C. Therefore, one effective method of preventing cell damage (organoleptic) is to expose red betel leaves (*Piper crocatum IRuiz & Pav*.) to ozone via water with a flow duration of 240 seconds.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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