The Effect of Ozone on the Physical and Chemical Qualities of Red Chili Pepper (*Capsicum anuum* L.)

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Abstract Pepper is widely known to be perishable, susceptible to weight losses, and suffer quality deterioration after harvest. Meanwhile, ozone acts as an antimicrobial agent, food sanitizer, and extends shelf-life without compromising quality and causing harm to the environment. Therefore, this study aims to obtain a proper ozone concentration for maintaining the physical and chemical qualities of red chili pepper fruit during storage to extend its shelf-life. This study was carried out using a Completely Randomized Design with five replications. The treatments consisted of control (no ozonation), as well as ozone concentrations of 0.5, 1, 1.5, and 2 mg/L. Based on the results, ozone treatment had no significant effect on fruit peel color, total carotenoid, flavonoid, capsaicin, and dihydrocapsaicin content of red chili pepper fruit during storage. However, it was significantly affected to weight loss, total phenolic content, and antioxidant activity (IC_{50} and AEAC). Exposure to 2 mg/L ozone increased total phenolic content as well as antioxidant activity and reduced weight loss. Therefore, it was concluded to be the most appropriate ozone concentration for maintaining the physical and chemical qualities of red chili pepper fruit during storage.

Keywords: capsaicin, ozone, pepper, quality, secondary metabolite

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1. Introduction

Red chili pepper (*Capsicum anuum* L.) is an annual vegetable consumed as a spice or additional ingredient in various food. It contains numerous minerals and phytochemical contents that provide several benefits for human health such as carotenoids, vitamin A and C, phenolics, capsaicin, and others with antioxidant properties [1].

Red chili pepper is often distributed as raw material to manufactures and supplied to traditional or modern markets. However, it is perishable, susceptible to weight losses, and suffer quality deterioration after harvest [2]. These conditions also adversely affect freshness during storage, transportation, and final delivery to consumers [3]. Therefore, to maintain the quality of fruit, ozonation is applicable as one of the effective alternatives.

Ozone (O_3) is a gaseous molecule consisting of three oxygen atoms and is used as an antimicrobial agent, food sanitizer, and to extend shelf-life without compromising quality and causing harm to the environment [4]. Furthermore, ozone is commonly used to treat vegetables and fruits after harvesting, either in aqueous or gaseous form [5]. Red pepper treated by ozone exhibited a significant reduction of disease, weight loss, and increased firmness maintenance [6]. The application of 2 mg/L ozone to green pepper decreased weight loss and respiration rate [7]. A previous study showed that ozone treatment resulted in better flavonoid and phenolic contents, as well as the antioxidant activity and firmness of pear fruit [8].

Ozone is commonly used at low concentrations for treating postharvest products [9], while higher doses might promote oxidation and chemical quality deterioration [10]. When applied properly, ozone treatment preserves product quality by minimizing weight loss and enhancing the texture, visual, and nutritional quality [11]. However, there are only a few studies on its effect on the physical and chemical qualities of red chili pepper fruit during storage. Therefore, this study aims to obtain a proper ozone concentration for maintaining the physical and chemical qualities of red chili pepper fruit during storage to extend its shelf-life.

2. Materials and Methods

2.1. Materials and Experiment Procedure

This study was carried out using a Completely Randomized Design with five replications. The treatments consisted of
control (no ozonation), and ozone concentrations of 0.5, 1.5, and 2 mg/L. The red chili pepper fruits cultivars Tanjung were harvested by farmers in Jatinangor, West Java, Indonesia at full maturity. Furthermore, the samples were placed in a cylindrical stainless container and then immersed in ozonized water obtained by bubbling ozone gas into sterile deionized water at a maintained flow rate of 2.5 L/min. The samples were stored at room temperature (23°C) for 7 days.

2.2. Physical Quality Measurement

Physical quality including firmness, weight loss, and color parameters were measured. Firmness was measured using Texture Analyzer TAXT (Stable Micro Systems Ltd. Surrey, UK). The sample was placed on the instrument, then a cylindrical probe (d=6 mm) was used to penetrated it. Color measurement for fruit peel consisted of L*, a*, b*, and ΔE* (total color difference), and was analyzed using a reflectant spectrophotometer (Konica Minolta CR-400, Japan). L* (lightness) ranges from black (0) to white (100), a* is green (-) to red (+), and b* is blue (-) to yellow (+). ΔE* was calculated using the following equation:

\[ \Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \] (1)

ΔE* value is classified as small difference (1.5<ΔE*), distinct (1.5<ΔE*<3), and very distinct (ΔE*>3) [12].

2.4. Extract Preparation

Red chili pepper fruit was dried in an oven at 50°C. The sample was dissolved with 8 mL of methanol, incubated in a water bath at 50°C for 4 h, and then centrifuged at 4000 rpm for 15 min to obtain the supernatant. This method was applied for all chemical quality analyses, except for carotenoid measurement. The extract preparation for carotenoid content used the diluted of 20 mg of dried sample in 8 mL of acetone, then sonicated for 30 min at room temperature. The mixture was centrifuged at 4000 rpm for 15 min.

2.5. Determination of Chemical Quality

The red chili pepper extract was measured for total carotenoid, flavonoid, phenolic, antioxidant activity, capsaicin, and dihydrocapsaicin at 0 (initial) and 7 days after storage.

The carotenoid content was obtained by separating the supernatant and then the compound was re-extracted and centrifuged at 4000 rpm [13]. The absorbance was calculated at 450 nm wavelength. Determination of total flavonoid was carried out using 1 mL of the sample that was dissolved with 1 mL of methanol and then mixed with 2.8 mL of distilled water, 0.1 mL of AlCl3, and 0.1 mL of natrium acetate [14]. The absorbance was calculated at 415 nm. Total phenolic content was determined by dissolving 0.1 mL of the sample with 0.4 mL of methanol, 2.5 mL of Folin–Ciocalteau reagent and 2 mL of NaHCO3 [15]. The absorbance was read at 765 nm wavelength.

The antioxidant activity was determined by mixing 0.5 mL of the sample with 1 mL of methanol and 1.5 mL of DPPH methanolic solutions (1 mg/L) [16]. The absorbance was determined at 515 nm using a spectrophotometer. The percentage of inhibition and ascorbic acid equivalent antioxidant capacities (AEAC) were determined using the following equations:

\[ \% \text{Inhibition} = \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \times 100 \] (2)

\[ \text{AEAC} = \frac{IC_{50}^\text{ascorbic acid}}{IC_{50}^\text{sample}} \times 10^5 \] (3)

IC50 (ascorbic acid) was known to be 3.12 mg/L. Capsaicin and dihydrocapsaicin was analyzed using a high-performance liquid chromatography-ultraviolet detector (HPLC-UV) [17]. The sample or standard capsaicin solution (20 μL) was injected into nonpolar column C18 (150 mm × 4.6 mm, 5 μm), while the mobile phase consisted of 0.1% of phosphoric acid:acetonitrile (60:40 v/v). The column temperature was controlled at 30°C with the flow rate at 1 mL/min, and the eluting compounds were maintained at 281 nm wavelength.

2.6. Statistical Analysis

Data were collected and analyzed with SPSS v21 using analysis of variance (ANOVA) at 5% level followed by post hoc Duncan multiple range (DMRT).

3. Results

3.1. Effect of Ozone on Physical Quality

According to Figure 1A, Figure 1B, and Figure 1C, L*, a*, and b* of red chili pepper fruit peel were not significantly affected by ozone treatment after 7 days of storage with the values ranging from 33.66 to 33.96; 29.12 to 30.71; and 13.90 to 14.73 respectively. Based on the result, these values decreased from day 0 to 7 of storage for all treatments. Furthermore, as shown in Figure 1D, ozone treatment had no significant effect on the total color difference (ΔE*). All treatments showed ΔE*>3, suggesting that the color changes were very distinct.

There was a significant difference in weight loss after ozone treatment (Figure 2A). The control had the highest value (11.98%), but was significantly reduced by ozone exposure. However, there were no significant differences between the concentrations with the values ranging from 6.50 to 8.35%.

Ozone treatment produced a significant effect on the firmness of red chili pepper fruit (Figure 2B). Control treatment (1470.65 g) significantly showed lower firmness compared to the initial (2051.08 g). However, ozone at all concentrations were not statistically different from the initial and control. They ranged from 1470.65 to 1791.79 g.
Figure 1. (A) L*, (B) a*, (C) b* and (D) ΔE* of red chili pepper fruit to ozone treatment. Means followed by the same letters in the same days of storage do not differ according to the Duncan test at 5% probability level.

Figure 2. (A) Weight loss and (B) firmness of red chili pepper fruit to ozone treatment. Means followed by the same letters do not differ according to the Duncan test at 5% probability level.

Figure 3. (A) Carotenoid, (B) flavonoid, (C) phenolic contents of red chili pepper fruit to ozone treatment. Means followed by the same letters do not differ according to the Duncan test at 5% probability level.
3.2. Effect of Ozone on Chemical Quality

As shown in Figure 3A, the total carotenoid content of red chili pepper fruit in all treatments significantly decreased from the initial (1455.16 mg/100 g). However, ozone treatment did not significantly affect the carotenoid content after 7 days of storage, which ranged from 1000.27 to 1145.10 mg/100 g. Furthermore, ozone treatment also had no significant effect on total flavonoid content (Figure 3B), ranging from 264.49 to 274.34 mg/100 g and the difference was not significant with the initial (238.94 mg/100 g).

The total phenolic content was significantly affected by ozone treatment (Figure 3C). Ozone at 2 mg/L concentration significantly increased total phenolic content (1044.66 mg/100 g) after 7 days of storage. The minimum phenolic content was obtained in control treatment (844.73 mg/100 g) that was not significantly different from ozone at 0.5 to 1.5 mg/L concentration.

Ozone treatment had a significant effect on IC50 and AEAC (Figure 4A and Figure 4B). The 2 mg/L of concentration showed the best IC50 value (4854.52 mg/L) and significantly differ from the control (6405.89 mg/L) and initial (6803.69 mg/L). Moreover, other ozone concentration negatively affected IC50. The highest AEAC value was recorded on 1.5 and 2 mg/L of ozone (65.47 and 66.59 mg/100 g respectively) which the difference was significant from control and initial (49.46 and 47.58 mg/100 g respectively).

Capsaicin and dihydrocapsaicin in red pepper fruit showed insignificant differences after ozone treatment (Figure 5A and Figure 5B), ranging from 102.27 to 109.35 mg/100 g and 30.17 to 35.31 mg/100 g respectively.

4. Discussions

The results indicated that the red chili pepper fruit showed a darker appearance with a decrease in redness and yellowness after 7 days of storage, both in the control or ozone treatment. However, color characteristics of fruit peels were not affected by ozone treatment generally. A previous study revealed that L', a', and b' of red bell pepper fruit were not influenced by ozone exposure at a concentration of 0.1 and 0.3 μmol/mol [18]. There was no significant difference in L' and ΔE* after ozone exposure, indicating that ozone did not cause discoloration on the red bell pepper fruit surface [19]. The color alterations are influenced by changes in the natural pigments contents, such as carotenoid [20]. In our study, as shown in Figure 3A, ozone did not significantly alter carotenoid content and might cause insignificant change in peel color.

During storage, transpiration and respiration processes cause moisture loss, reducing fruit weight [21]. Ozone treatment significantly decreased weight loss of red chili pepper fruit. Lower weight loss of tomato fruit was recorded after ozone application [22]. Green pepper fruit treated with both aqueous and gaseous ozone at 2 mg/L significantly decreased weight loss [7]. It was stated that the lower weight loss caused by ozone might be due to its ability to control water loss by maintaining the structure of pepper peel and slowing down the respiration rate.

In contrary with weight loss, ozone had negative impact on fruit firmness. It is consistent with other study that fresh-cut red bell pepper fruit treated with ozone had no significant effect on firmness [5]. Furthermore, the carrot treated by ozone also showed insignificant effect on firmness [23] as well as blueberries [24] during storage periods.

Ozone treatment had no significant effect on carotenoid content. A similar result was obtained from Brassica campestris [27] and Eruca sativa [28]. However, there was a decline in carotenoid content during storage on all treatments. In a similar way, the carotenoid content of chili pepper fruit reduced during storage periods at room temperature [25]. The reduction in carotenoid during storage might be caused by oxidation [26].
No significant difference was detected in flavonoid content after ozone treatment. Our study is in agreement with previous research that ozonation at 0.9 and 2.5 mg/L for 30 min did not affect total flavonoid content of tomato fruit [29]. Similarly, there was no significant difference in the total flavonoid content of broccoli at lower and higher concentrations of ozone after 7 days of storage.

Fruit treated by 2 mg/L of ozone had a positive effect on phenolic content. Ozone application increased the total phenolic content of pear fruit because a high concentration of ozone promotes phenolic biosynthesis [8]. Ozone exposure induces the activation of phenylalanine ammonia-lyase (PAL) which is the key enzyme involved in phenolic synthesis in plant tissues [30]. In addition, it triggers the modification of cell wall which enhance the release of certain conjugated phenolic compounds, leading to a higher phenolic content [31].

The results indicated that ozone at 1.5 and 2 mg/L concentration provided better antioxidant activity. Ozone treatment activates certain intracellular enzymes involved in controlling the endogenous antioxidant defense after harvesting vegetables and fruits, leading to an increase in the antioxidant activity [32]. The application of ozone increased antioxidant capacity in strawberry fruit due to the enhancement of antioxidant enzyme activity and protein expression associated with the ascorbic acid-glutathione (ASA-GSH) cycle [33]. Furthermore, enzymes involved in antioxidant activity such as glutathione peroxidase, superoxide dismutase, and phenylalanine ammonia-lyase were found in higher concentrations in ozonated fruit after 21 days of storage [34].

Ozone treatment had no significant effect on capsaicin and dihydrocapsaicin. Similarly, ozone treatment on red pepper fruit had no significant effect on capsaicin in pericarp and dihydrocapsaicin in seed and pericarp [35]. In the chilli pepper fruit, approximately 90% of the capsaicinoids are occupied by capsaicin and dihydrocapsaicin [36]. Capsaicinoids play an important role in determining the pungency of pepper fruits and related products [37]. Capsaicin is an essential component in pepper with great benefits for pharmaceutical and antioxidant purposes [38].

5. Conclusion

Ozone treatment had no significant effect on fruit peel color, total carotenoid, flavonoid, capsaicin, and dihydrocapsaicin content of red chili pepper fruit during storage. However, it significantly affected weight loss, total phenolic content, and antioxidant activity (IC₅₀ and AEAC). Exposure to 2 ppm ozone increased total phenolic content as well as antioxidant activity and reduced weight loss. Therefore, it was concluded to be the most appropriate ozone concentration for maintaining the physical and chemical qualities of red chili pepper fruit during storage.

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