Fumigation with Ozone to Extend the Storage Life of Mango Fruit cv Nam Dok Mai No. 4

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Abstract: Ozone has been used as a postharvest treatment to delay the physiological and biochemical changes in fruit during storage. In this study, mango fruit (Mangifera indica L.) cv Nam Dok Mai No. 4 were fumigated with ozone at the concentrations of 2 µL L⁻¹ for 20 min and 10 µL L⁻¹ for 10 min on arrival and after 3 days of storage compared to untreated fruit. Fruit were stored in ambient temperature (25 ºC) to determine the effects of ozone on mango quality. Ozone at 10 µL L⁻¹ significantly decreased the respiration rate at day 4 and day 6 compared with the control. After treatment, ethylene production was reduced in treated fruit, but at day 4 and day 6, the higher ethylene production was detected in ozone treatments. The lower L* and b* values in mango peel were found in 10 µL L⁻¹ ozone at day 4. In the pulp, both ozone concentrations decreased the a* values and increased the hue angle values at day 6. Ozone at 10 µL L⁻¹ reduced the weight loss of mango at day 6. However, no significant differences were detected in the fruit firmness, soluble solid content and titratable acidity among treatments. In conclusion, fumigation with ozone on mango fruit at the concentration of 10 µL L⁻¹ for 10 min at day 0 and day 3 could suppress the respiration rate, decrease the colour changes in the peel and the pulp, and reduce weight loss. These effects contributed to extend the storage life of mango fruit after harvest.

Keywords: ozone fumigation, colour change, storage life, mango.

INTRODUCTION

Mango fruit (Mangifera indica L.) is an economically important fruit in the tropics. In Thailand, ‘Nam Dok Mai’ is a well known cultivar as it is fibreless, it also has a delicious taste and sweet aroma. However, as a fleshy fruit, the losses resulting in senescence, desiccation, physiological disorders, mechanical injuries and microbial spoilage can occur at any point from harvest to utilization [1]. According to Sarkar [2] the total postharvest loss of mango was found as much as 34% due to improper handling and storage. Recently, ozone applications on postharvest commodities, including mango fruit were reported to be an effective method in delaying ripening, reducing microbial contamination and improving fruit quality during storage.

Ozone (O₃) is a molecule comprising three oxygen atoms. It has an oxidation reduction potential of 2.07 eV, which is much higher than that of chlorine, hypochlorous acid and hydrogen
peroxide [3]. Ozone is an unstable molecule; the half life of ozone in water is about 20-30 min at room temperature [4]. The half-life of ozone in air depends on temperature, humidity and the presence of reactable substrates [5]. Nagy [6] reported that the half life of ozone in air at 20 °C is about three minutes, but increase to six minutes at 4°C. The use of ozone in packinghouses and storage rooms includes the control of postharvest diseases on fruit, retarding the production of spores from decaying fruit, sanitation of surfaces, odor elimination for mix storage and ethylene removal [7-8]. Ozone is very effective in removing ethylene through a chemical reaction to extend the storage life of many fruits and vegetables [9]. Treatment with ozone can extend the shelf life of apples, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and by oxidizing ethylene to retard ripening [10]. Ozone is considered a potential tool to extend storage life (i.e., fruit ripening delay/firmness retention) with the added advantage of controlling disease proliferation [11-14]. Gaseous ozone treatment at the concentration of 4 µL L⁻¹ for 30 min every 3 hours for 15 days on whole and sliced tomatoes retained good appearance, overall quality and reduced microbial counts [12]. Tzortzakis [14] reported that gaseous ozone at 0.005-0.1 µmol mol⁻¹ maintained soluble sugars, increased carotene, lutein and lycopene, and maintained firmness of tomatoes. Fumigation with gaseous ozone at 200 µL L⁻¹ for 2, 4 and 6 hours reduced the growth of green mold on the tangerine fruit peel and decreased superoxide dismutases, catalase, and ascorbate peroxidase in the peel [15]. Rodoni [16] proved that the application ozone gas at 10 µL L⁻¹ in 10 min on tomatoes delayed softening, and reduced fruit damage and weight loss. Because of its strong oxidizing activity, ozone may cause physiological injuries to produce [17]; bananas developed black spots after 8 days of exposure to 25 and 30 ppm gaseous ozone. Carrots exposed to ozone gas during storage had a lighter, less intense colour than the control [18]. The objective of this study was to investigate the effects of gaseous ozone at low and high concentrations on the storage life of mango fruit cv Nam Dok Mai No. 4.

MATERIALS AND METHODS

Fruit materials and ozone fumigation. Mango fruit cv Nam Dok Mai No. 4 at the mature stage were purchased at the wholesale market (Pak Khlong Talat) in Bangkok, Thailand and transported to the laboratory of Postharvest Technology Division, King Mongkut’s University of Technology, Thonburi. Mangos with blemish and other defects were eliminated. The selected fruit were washed with clean water and disinfected with 100 ppm Clorox (NaOCl), and then air dried for 30 min. Fruit were placed in a hermetic transparent glass chamber (50 x 30 x 30 cm) for ozone fumigation at 25 °C. In the treatment of the high concentration (10 µL L⁻¹), ozone was applied by the ozonizer - model B6ATP, Euro Entech Co., LTD - Thailand, with a capacity of 2,500 mg h⁻¹. In this system, ozone was generated by electric discharge from pure oxygen; the concentration of ozone was measured by an ozone gas sampling pump with detector tube (GASTEC Model GV-100 – Japan). When the ozone release reached the expected concentration, the generator was switched off and the fruit were continued to keep in the chamber for 10 min. In the treatment of the low concentration (2 µL L⁻¹), ozone was applied by the ozonizer with a capacity of 500 mg h⁻¹. After reaching the concentration, fruit were continued to keep in the chamber for 20 min. In both ozone treatments, ozone was applied twice; one was on arrival (day 0) and the other was after 3 days of storage. Fruit with non-ozone fumigation were the control
fruit. After treatment, all fruit were stored at 25 °C and randomly taken to determine parameters at day 0 and 2 day intervals during storage (three replicates of eighteen fruit per treatment).

**Respiration rate and ethylene production.** In order to investigate the effect of ozone on the physiological changes immediately after treatment, the respiration rate and ethylene production were measured after ozone fumigation at day 0. After that, these parameters were determined at 2 day intervals during storage. Samples of fruit were weighed and put in a closed plastic box, and then incubated at 25 °C for 1 hour. One mL gas sample from the headspace of each box was injected into the gas chromatograph (Shimadzu GC-2014 ATF, Bara Scientific Co., Ltd) to measure CO\(_2\) and C\(_2\)H\(_4\) concentrations. The fruit respiration rate was expressed as mg CO\(_2\) kg\(^{-1}\) h\(^{-1}\) and ethylene production was expressed as µL C\(_2\)H\(_4\) kg\(^{-1}\) h\(^{-1}\).

**Fruit colour.** The colour of the peel and pulp of fruit was recorded from 3 positions of each mango by the colourimeter (Konica Minolta CR-400, Japan). The colour was expressed as the values of L*, a*, b* and hue angle (h).

**Firmness.** Fruit firmness was measured by using the texture analyzer (TA.XT plus, Charpa Techcenter Co.LTD) with the non-destructive method (limited distance compression), fitted with an 3.5 cm flat probe, return distance: 45 mm, return speed: 20 mm sec\(^{-1}\), and contact force: 10 g. Results were expressed in Newtons (N).

**Weight loss.** Individual fruit were weighed at day 0 and 2 day intervals to detect the decrease of fruit weight during storage. Results were expressed as a percentage of weight loss relative to the initial weight.

**Soluble solid contents** (SSC) were measured by the refractometer (Atago, PAL-1, Japan) and expressed as °Brix.

**Titratable acidity** (TA) was determined by titration with 0.1 M NaOH and expressed as a percentage of citric acid.

**Statistical analysis.** The Statistical Package for the Social Science (SPSS) software for Windows was used for analysis of variance (ANOVA) and least-significant difference (LSD) at the 95% confidence level of each variable value under the completely randomized design (CRD).

**RESULTS AND DISCUSSION**

**Effect of ozone treatments on ethylene production**

Ozone treatments in both concentrations (2 µL L\(^{-1}\) for 20 min and 10 µL L\(^{-1}\) for 10 min) significantly \((p \leq 0.05)\) reduced ethylene production immediately after treatment. The results showed that ethylene production of untreated fruit were about 1.5-fold higher than treated fruit after ozone fumigation. However, ethylene production in treated fruit increased at day 2 and remained significantly higher than untreated fruit at day 4 and day 6 (Fig 1A). At day 6, ethylene emission in ozone treatment at 10 µL L\(^{-1}\) was significantly lower than the ozone treatment of 2 µL L\(^{-1}\), but significantly higher than the control.

Ozone reacting with ethylene was found in many kinds of fruit, Skog and Chu [19] reported that ozone at 0.4 ppm was attributed to the oxidation of ethylene in the cool storage
room and increased the shelf-life of apples and oranges. Similarly, ozone at 0.4 ppm applied in the storage room could effectively prevent ethylene accumulation in apples and pears [20]. Wild [21] found that ozone at very low levels could also reduce ethylene in citrus fruits during storage. In this research, ozone may react rapidly with ethylene in fruit, so the concentrations of ethylene in treated fruit after ozone fumigation at day 0 decreased immediately. However, ozone may induce oxidative stress in fresh fruit which promotes various physiological responses, including synthesis of ethylene [5]. The increase of ethylene emissions at day 2 and day 4 may be due to the trigger of ethylene synthesis after stress by ozone treatments at day 0 and day 3.

![Image](image.jpg)

**Fig 1.** Effect of ozone treatments on ethylene production (A) and respiration rate (B) in mango fruit. Vertical bars represent S.E of means and are invisible when the values are smaller than the symbol. Asterisks stand for significant differences among treatments at $p \leq 0.05$ (LSD test). AT = after treatment at day 0.

**Effect of ozone treatments on respiration rate**

Respiration rates in all treatments increased from the early stage of storage to day 4 and then decreased slightly. In this research, ozone treatments revealed a lower respiration rates during storage. However, only the treatment of high ozone concentration showed a significant difference in comparison to the control (at day 4 and day 6) (Fig 1B). Many research proved that the efficacy of ozone inhibiting the respiration rate depends on the type of produce and the ozone concentration. Ozone treatment prolonged the shelf life in tomatoes due to a lower respiration rate and $C_2H_4$ emission [11]. Gane [22] observed that the exposure of ripe bananas to 1.5-1.7 ppm ozone did not change the respiration rate and extended fruit shelf life. Palou [23] detected a similar $CO_2$ and $C_2H_4$ emission in O’Henry peaches under ozone or air treatment. In particular, Rodoni [16] reported that the respiration rates increased immediately after treatment, but no differences were found afterward between controlled tomatoes and treated fruit.

**Effect of ozone treatments on mango colour**

**Mango peel colour**

There was a slight effect of ozone treatment on the change of mango peel colour. The treatment of 10 $\mu L \cdot L^{-1}$ ozone significantly lowered the $L^*$ value and the $b^*$ value at the 4$^{th}$ day of
storage compare with control treatment (Table 1). No significant differences in the a* values and hue angle values of mango peel were detected among treated and untreated fruit.

Table 1. The changes of colour in mango peel as influenced by ozone treatments

<table>
<thead>
<tr>
<th>Peel colour parameters</th>
<th>Treatments</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>L* values</td>
<td>Control</td>
<td>62.85 ± 0.30</td>
<td>64.88 ± 0.11</td>
<td>67.93 ± 0.87</td>
<td>65.11 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>62.85 ± 0.30</td>
<td>63.35 ± 1.92</td>
<td>65.02 ± 1.06</td>
<td>61.57 ± 3.34</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>62.85 ± 0.30</td>
<td>62.14 ± 1.49</td>
<td>63.50 ± 1.16</td>
<td>66.03 ± 0.85</td>
</tr>
<tr>
<td>a* values</td>
<td>Control</td>
<td>-11.35 ± 0.25</td>
<td>-11.20 ± 0.58</td>
<td>-8.33 ± 0.97</td>
<td>-2.35 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>-11.35 ± 0.25</td>
<td>-11.24 ± 0.17</td>
<td>-10.51 ± 1.10</td>
<td>-2.51 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>-11.35 ± 0.25</td>
<td>-10.87 ± 0.67</td>
<td>-9.23 ± 0.46</td>
<td>-2.74 ± 0.27</td>
</tr>
<tr>
<td>b* values</td>
<td>Control</td>
<td>19.15 ± 0.47</td>
<td>25.15 ± 1.09</td>
<td>31.22 ± 1.68</td>
<td>29.23 ± 2.32</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>19.15 ± 0.47</td>
<td>24.42 ± 0.43</td>
<td>27.02 ± 0.97</td>
<td>24.76 ± 2.69</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>19.15 ± 0.47</td>
<td>24.09 ± 2.58</td>
<td>24.12 ± 0.88</td>
<td>29.61 ± 1.88</td>
</tr>
<tr>
<td>h values</td>
<td>Control</td>
<td>120.3 ± 0.5</td>
<td>114.7 ± 2.0</td>
<td>105.0 ± 1.5</td>
<td>96.1 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>120.3 ± 0.5</td>
<td>116.0 ± 0.5</td>
<td>11.3 ± 2.7</td>
<td>94.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>120.3 ± 0.5</td>
<td>114.8 ± 3.9</td>
<td>111.0 ± 1.5</td>
<td>96.7 ± 1.0</td>
</tr>
</tbody>
</table>

Values represent mean (± S.E.) of measurements recorded on three replicates. Values followed by the different letters within the same column indicate significant differences among treatments at p ≤ 0.05 (LSD test).

In this research, the lightness (L*) values in mango peel increased when the fruit started to ripen and reduced when the fruit turn overripe. The peak of L* values of 10 µL L⁻¹ ozone treatment slowed down by 2 days compare to control treatment. It is suggested that ozone treatment at 10 µL L⁻¹ may delay the change of fruit peel colour during fruit storage.

Mango pulp colour

Ozone treatments in both low and high concentrations lowered the colour change in mango pulp at the later stage of storage. The results showed the significantly lower a* values and the significantly higher h values in ozone treated fruit at the 6th day of storage compared with untreated fruit (Table 2). However, no significant differences in the L* and b* values of mango pulp were observed among treatments during storage.

According to El-Saedy [24], the skin color of mango did not change significantly during storage and remained green in all ozone treated and untreated fruit, but ozone treatment significantly slowed the colour changes in mango flesh. Sharpe [25] reported that ozone treatment at 450 ppb did not significantly affect the chlorophyll fluorescence of apples and grapes over a period of 12 days. In addition, there were no significant colour changes over time in lightness, chroma and hue parameters in control or ozone treated apples. The same results were found by the research of Rodoni [16]; no difference in fruit colour was observed between the control and ozone treated tomatoes. In this research, ozone treatments suppressed the colour change of mango peel and pulp at the later period of storage due to the decrease of the changes in
the lightness and hue parameters. Only the high concentration of ozone significantly lowered the change of mango peel, while both low and high ozone concentrations affected the change of mango pulp.

**Table 2.** The changes of colour in mango pulp as influenced by ozone treatments

<table>
<thead>
<tr>
<th>Pulp colour parameters</th>
<th>Treatments</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>L* values</td>
<td>Control</td>
<td>87.10 ± 0.19</td>
<td>80.07 ± 1.23</td>
<td>77.88 ± 0.69</td>
<td>72.56 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>87.10 ± 0.19</td>
<td>82.17 ± 0.66</td>
<td>78.64 ± 0.39</td>
<td>72.93 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>87.10 ± 0.19</td>
<td>81.09 ± 0.57</td>
<td>78.93 ± 0.59</td>
<td>73.86 ± 0.72</td>
</tr>
<tr>
<td>a* values</td>
<td>Control</td>
<td>-2.38 ± 0.26</td>
<td>-2.06 ± 0.50</td>
<td>1.56 ± 0.47</td>
<td>8.11 ± 0.34 a</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>-2.38 ± 0.26</td>
<td>-2.85 ± 0.78</td>
<td>-0.38 ± 0.97</td>
<td>6.67 ± 0.23 b</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>-2.38 ± 0.26</td>
<td>-2.34 ± 0.71</td>
<td>0.44 ± 0.88</td>
<td>7.06 ± 0.28 b</td>
</tr>
<tr>
<td>b* values</td>
<td>Control</td>
<td>24.64 ± 0.45</td>
<td>42.40 ± 1.47</td>
<td>48.48 ± 1.71</td>
<td>52.01 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>24.64 ± 0.45</td>
<td>42.65 ± 0.98</td>
<td>47.33 ± 1.20</td>
<td>52.42 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>24.64 ± 0.45</td>
<td>43.85 ± 1.89</td>
<td>50.78 ± 1.33</td>
<td>52.19 ± 0.41</td>
</tr>
<tr>
<td>h values</td>
<td>Control</td>
<td>95.10 ± 0.87</td>
<td>92.83 ± 0.78</td>
<td>88.13 ± 0.61</td>
<td>81.14 ± 0.35 b</td>
</tr>
<tr>
<td></td>
<td>2 µLL⁻¹/20 min</td>
<td>95.10 ± 0.87</td>
<td>93.85 ± 1.10</td>
<td>90.51 ± 1.19</td>
<td>82.75 ± 0.27 a</td>
</tr>
<tr>
<td></td>
<td>10 µLL⁻¹/10 min</td>
<td>95.10 ± 0.87</td>
<td>93.15 ± 1.06</td>
<td>89.56 ± 1.00</td>
<td>82.30 ± 0.34 a</td>
</tr>
</tbody>
</table>

Values represent mean (± S.E.) of measurements recorded on three replicates. Values followed by the different letters within the same column indicate significant differences among treatments at p ≤ 0.05 (LSD test).

**Effect of ozone treatments on fruit weight loss**

During storage, the weight loss of fruit treated with ozone at 10 µL L⁻¹ showed the lowest values. At day 6, the weight loss of this treatment was reduced significantly compared to the ozone treatment at low concentration as well as the control treatment (Fig 2A). The maintenance of fruit weight by the ozone treatment was reported by Rodoni [16]; the weight loss of tomato fruit increased during storage in both the control and ozone treatments; then, after 9 days at 20 °C, ozone exposed tomatoes at 10 µL L⁻¹ showed reduced weight loss. Ozone exposed papaya fruit from 1.5 to 5.0 ppm had reduced weight loss [26]. In contrast, Tzortzakis [14] reported that weight loss of tomato fruit was found to be unaffected by low level ozone treatment, but was increased by exposure to higher concentrations of ozone. Ozone treatment at 150 ppb on mango showed the higher weight loss [24]. This finding was also observed in a range of other fresh commodities including ‘Howes’ cranberries [27], ‘Casselman’ plums [28], ‘Zee Lady’ peaches [23, 29], and tomatoes [30]. It has been suggested that higher levels of ozone may result in damage to the cuticle and/or epidermal tissues [23] and higher weight loss. Ali [26] suggested that the greater thickness of papaya cuticle compared to other fruit such as tomatoes and cranberries could be the reason for the reduced weight loss in ozone treated papaya. In this
research, the decrease of weight loss in the treatment of 10 µL L⁻¹ ozone may be due to the suppression of respiration rate and the fruit skin structure.

**Effect of ozone treatments on fruit firmness**

Ozone treatments in low and high concentrations did not affect fruit firmness. Although the highest firmness of mango fruit was detected in ozone treatment at 10 µL L⁻¹ during storage, but no significant differences were detected among ozone treated and untreated fruit (Fig 2B). The same observation was found by Sharpe [25]; the firmness of apples, carrots and grapes were not significantly affected by ozone treatment at 450 ppb. Ozone treatments did not alter the activity of polygalacturonase and galactosidase [16]. In contrast, Tzortzakis [14] showed that ozone treatment maintained the firmness of tomatoes during storage. Similar finding in mango, ozone treated fruit at 150 ppb after 48 hours of storage were firmer than untreated fruit [24].

![Fig 2. Effect of ozone treatments on weight loss (A) and firmness (B) in mango fruit. Vertical bars represent S.E of means and are invisible when the values are smaller than the symbol. Values followed by the different letters indicate significant differences among treatments at $p \leq 0.05$ (LSD test).](image)

**Effect of ozone treatments on soluble solid content (SSC) and titratable acidity (TA)**

The result showed that the SSC of ozone treated fruit were lower than control fruit during storage (Fig 3A). However, no significant differences of SSC were detected among treatments. According to Ali [26], ozone treated papaya from 1.5 to 5.0 ppm for 96 hours had higher SSC in comparison with non-treated fruit. In mango, exposure to 150 ppb ozone did not affect fruit SSC. However, dipping in hot water at 55°C for 5 min then fumigation with 150 ppb ozone before storage slowed the increase in SSC and the decline in citric acid [24].
Fig 3. Effect of ozone treatments on soluble solid content (A) and titratable acidity (B) in mango fruit. Vertical bars represent S.E of means and are invisible when the values are smaller than the symbol.

The TA of ozone treated fruit were higher than control fruit during storage (Fig 3B). The same result was reported that no significant effect of ozone treatment on TA in strawberries was found during storage [31]. In contrast, Ali [26] found the lower reduction of TA in ozone treated papaya fruit compare to control fruit.

**CONCLUSION**

Postharvest treatment of ozone gas could maintain the shelf life of mango fruit cv Nam Dok Mai No. 4. Ozone treatments at day 0 and after 3 days of storage with a high concentration and short exposed period (10 µL L⁻¹ for 10 min) was more effective than the treatment of a low concentration and long exposed period (2 µL L⁻¹ for 20 min). This research suggests that ozone fumigation at 10 µL L⁻¹ for 10 min at day 0 and day 3 suppresses the respiration rate, inhibits the colour change in fruit skin and flesh, and decreases the weight loss of mango fruit during storage.

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