Ozone Fumigation on Sulfur Dioxide Treated Longan for Sulfur Residue Reduction and Delaying of Pericarp Browning as well as Disease Control in Longan Fruit during Storage

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Abstract:

Effect of ozone fumigation on sulfur residue reduction and postharvest qualities of sulfur dioxide treated longan fruit was investigated. The longans which were sulfur dioxide fumigated were divided into two groups. In the first group, the fruits were exposed to ozone gas (O_3) at a concentration of 200 ppm for 10 h, while the fruits of the second group were placed at room temperature as the control. The two groups of the longan fruit were randomly selected for 0 h, 2 h, 4 h, 6 h, 8 h and 10 h to determine the sulfur residue in the pericarp and the aril. The result showed that the sulfur residue was reduced by 93.50% in the pericarp and 81.54% in the aril after 10 h of ozone exposure. In the second experiment, the effect of ozone on disease incidence and pericarp browning of sulfur dioxide treated longan fruit was investigated. The results showed that all the treated fruits had lower browning index and PPO activity than the control group. None of the longans treated with ozone showed disease incidence or presence of microorganism population immediately after fumigation and for 5 days in storage at 25°C. Moreover, the ozone treatment also delayed the changes that take place in the postharvest qualities of SO_2-treated longan fruit after storage at 5°C for 35 days. It was concluded that ozonation is an alternative method for reducing sulfur residue, controlling disease incidence, and maintaining postharvest quality of SO_2-treated longan fruit.

Keywords: Ozone fumigation, Sulfur residue reduction, Sulfur dioxide, Longan fruit

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1. Introduction

Longan (*Dimocarpus longan* Linn) is a fruit that is important to Thailand's economy. The fruit has high export value, and it generates revenues worth more than 8 billion Baht to Thailand, which tends to increase every year. The important markets for the longan fruit from Thailand are China, Malaysia, Hongkong, and Singapore. However, there are problems in the exportation of the longan fruit, and these include rotting and short postharvest life. Sulfur dioxide (SO_2) fumigation is usually employed to control postharvest disease and color bleaching in the longan fruit (Tongdee, 1994). Because of the high demand for the longan fruit in the market, many producers indulge in excessive use of SO_2 for fruit fumigation to preserve fruit life, for color bleaching, and to increase the fruit value. This causes high accumulation of SO_2 in the longan fruit, which adversely affects consumer health and is a matter of concern. As a result, many countries have placed a limit on the quantity of sulfur residue in the longan fruit. This study investigated the reduction in sulfur residue and disease control in fresh longan fruit by ozone fumigation. Ozone (O_3) is a gas which has oxidizing properties. It is easy to use in chemical reactions and automatic degradation. Ozone has been used to control microorganism growth and chemical residue in various food industries as well as in the exportation of vegetable and fruit produce (Kim *et al*., 1999). Ong *et al*. (1996) reported that washing apple in water containing ozone gas (25 mg/L) reduces azinphos-methyl, captan, and fometanate hydrochloride residues by 50–100%. This is in agreement with Ku *et al*. (1998) who reported degradation of diazinon in ozone water. Ozone water oxidizes diazinon, which leads to diazinon degradation within 1 h. Sarig *et al*. (1996) reported that ozone fumigation was the preferred method instead of SO_2 fumigation to control diseases of grape peel. Fumigation with ozone at 8 mg/h for 20 min was found to produce phytoalexins, resveratrol, and pterostilbene which cause disease resistance in grapes. In addition, ozone gas (O_3) has been observed to reduce pesticide residue in lychee: exposure to ozone gas for 60 min was found most effective (Whangchai *et al.*, 2011). The objective of this study was to investigate the effect of ozone fumigation on sulfur residue reduction, reduction of pericarp browning, disease incidence, and quality maintenance of sulfur dioxide treated longan fruit during storage.

2. Materials and Methods

2.1 Ozone gas

Ozone gas was generated from a corona discharge ozone generator (Model OZONIZER) with a capacity of 1500 $mg.h^{-1}$. Ozone concentration was measured by an ozone gas sampling pump with a detector tube limit of 800 ppm (v/v; $\mu L^{-1}mL$) (Gastec Model GV-100, Japan).

2.2 Plant material

Fresh longan fruit (*Dimocarpus longan* Lour.) cv. "Daw" was harvested at the commercial mature stage (126–168 days after full bloom) and fumigated with SO_2 (SO_2 residue 2,600–2,900 ppm) from a commercial SO_2 fumigation house in Lamphun province. The fruits were uniformly selected (AAA grade), and the fruits had no disease incidence or defect.

2.3 Effect of ozone fumigation on reduction of sulfur residue in sulfur dioxide treated longan fruit

The SO_2 fumigated longans were divided into two groups. The first group was the control, which was left at 25°C. In the second group, the fruits were fumigated with ozone 200ppm of concentration for 2 h, 4 h, 6 h, 8 h and 10 h. After that, every 2 h, the fruits were randomly selected to determine the sulfur residue in the peel and the aril by the modified Monier-Williams method (AOAC, 1995).

2.4 Effect of ozone fumigation on disease incidence and microorganism population in sulfur dioxide treated longan fruit

The longans were sampled from each treatment immediately after fumigation and after 5 days of storage at 25°C. The sampled fruits were examined for microorganism population by total plate count (TPC), yeast, and mold by following the Bacteriological Analytical Manual (BAM) method. The survival of microorganisms was expressed as the mean number of the colony forming units (log $cfu.mL^{-1}$). Disease incidence was visually assessed in terms of the lesion area of fungal infection on the fruit surface. The severity of disease development on the surface was scored from 1 to 5, with 1 = incidence of disease 0–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = more than 80% of disease incidence.

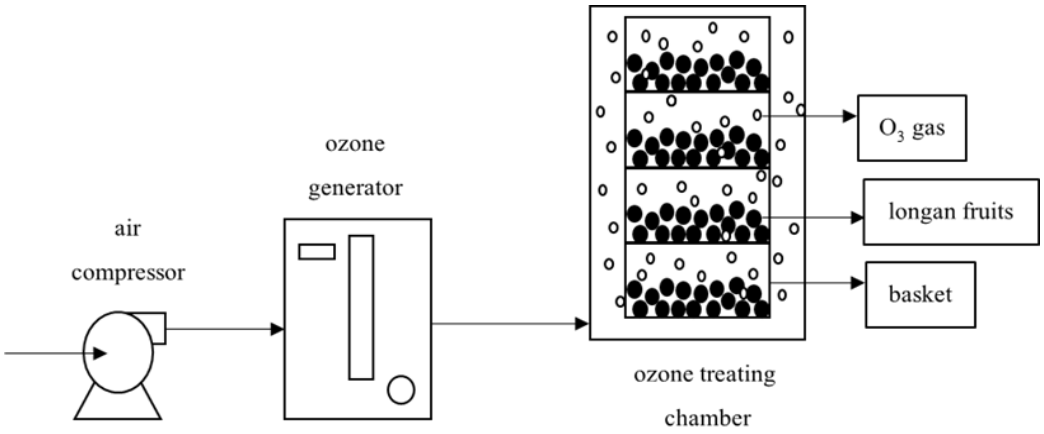

Figure 1 The schematic diagram of the experimental set-up

2.5 Effect of ozone fumigation on pericarp browning and PPO activity of sulfur dioxide treated longan fruit

The longans were sampled from each treatment for analysis of pericarp browning and PPO activity immediately after fumigation and during storage at 25°C for 5 days. The browning index was estimated by measuring the extent of the total browned area on each fruit surface on the following scale: 1 = 0–20% of browned area, 2 = 21–40% of browned area, 3 = 41–60% of browned area, 4 = 61–80% of browned area, and 5 = 81–100% of browned area. Polyphenol oxidase (PPO) was extracted by the method discussed in Huang *et al.* (1990). The longan pericarp (2 g) from 10 fruits was homogenized in 20 mL of 0.05 M potassium phosphate buffer (pH 6.2), 1 M KCl, and 2% polyvinylpyrrolidone. The solution was centrifuged at 13,500 rpm (Hermel model Z383K) for 5 min at 4°C. The supernatant was collected as the enzyme extract. The PPO activity was assayed by a modification based on the method discussed in Jiang and Fu (1998) using the reaction mixture of 0.05 M potassium phosphate buffer (pH 7.5) containing 0.2M catechol (0.8 mL) and 2 mL of crude enzyme. The tubes were incubated for 5 min at 25°C, and the absorbance was measured at 420 nm by using a visible spectrophotometer (model Thermo Spectronic). The unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.1 in the absorbance per min.

2.6 Effect of ozone fumigation on postharvest quality of sulfur dioxide treated longan fruit during storage at low temperature

By following a previous experiment, the best time duration for treatment using ozone fumigation for sulfur residue reduction was selected to determine the changes in the postharvest qualities in comparison with treatment with SO_2 alone and with non-SO_2 (control). Thereafter, all the treated samples were packed into PE bags and then stored at 5°C for 35 days. The longans from each of the treatments were randomly selected to determine the changes in the postharvest qualities every 7 days.

2.6.1 Determination of changes in qualities

Changes in the peel color were measured using a chromameter (Model Miniscan XE plus, Germany) and the degree of browning was expressed as L* and b* values (CIE 1976). L* values indicate the lightness of the exocarp, ranging from black=0 to white=100,[10] whereas b* values indicate the classification of yellow to blue, ranging from yellow (>0) to blue (<0)[23]. The total soluble solids (TSS) was measured by a digital refractometer (Atago, PAL-1 Type, Atago Co., Ltd., Japan). The result was shown as %Brix. The browning index and the disease incidence were measured by following the method from a previous experiment. The sensory evaluation was carried out by 10 students from Department of Biology, Chiang Mai University. Taste, odor, texture, and overall acceptance were scored using the method of hedonic scale on the following scale: 1 = dislike very much, 2 =dislike a little, 3 = not sure 4 = like a little and 5 = like very much.

2.6.2 Statistical analysis

Statistical analysis was carried out by using SPSS version 10, while the experimental design followed Duncan's multiple range test (p=0.05) to determine the significant differences between the various treatments.

3. Results and Discussion

3.1 Effect of ozone fumigation on reduction of sulfur residue in sulfur dioxide treated longan fruit

The longan fruit after ozone fumigation (200 ppm) for 2 h, 4 h, 6 h, 8 h, and 10 h showed a decrease in the sulfur residue in the pericarp and the aril in all the treatments (Figures 2–3). Ozone fumigation for 4 h was effective on the longan fruit with regard to reduction in the sulfur residue reduction, at 40.07% reduction in the pericarp (Figure 2) and 55.1% reduction in the aril (Figure 3) when compared with O_3-untreated fruit which had 14.22% in the pericarp and 20.12% in the aril. The greatest decrease in the sulfur residue in the longan fruit was observed when exposed to ozone fumigation for 10 h (93.50% in the pericarp and 81.54% in the aril). Moreover, as demonstrated in the kinetic model in Figure 4, the reaction rate was found to constantly decrease with increase in the exposure time, indicating that the sulfur degradation rate increases with increase in ozone exposure time. Other studies have shown that ozone treatment could be effective in reducing pesticide residues in fruits and vegetables. Similarly, Savi *et al.* (2015) reported that ozone gas can reduce the residue levels of deltamethrin and fenitrothion in stored wheat grain. Inan *et al.* (2007) also found that the aflatoxin B_1 content in red pepper was reduced after exposure to 66 mg/L ozone for 60 min and that there was no effect on the color quality. According to

Haberhauer *et al.* (1999), the use of high concentration ozone can destroy the residue deposit of pesticides due to ozone possessing the efficiency to expedite the degradation of the pesticide. Residues of fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin were found to have reduced by 68.5%, 75.4%, 83.7% and 100.0% respectively, after fumigation with ozone 10,000 μL.L^{-1} for 1 h on table grapes (Glabler *et al.,* 2010). In addition, Hwang *et al.* (2001) also reported that ozone-containing water removed 56–97% of mancozeb residue concentrations of 1 μg.mL^{-1} and 10 μg.ml^{-1} from apple. As well as the study of patulin degradation by ozone. Ozone could degrade patulin toxicity in pear juice and apple juice (Cataldo, 2008)

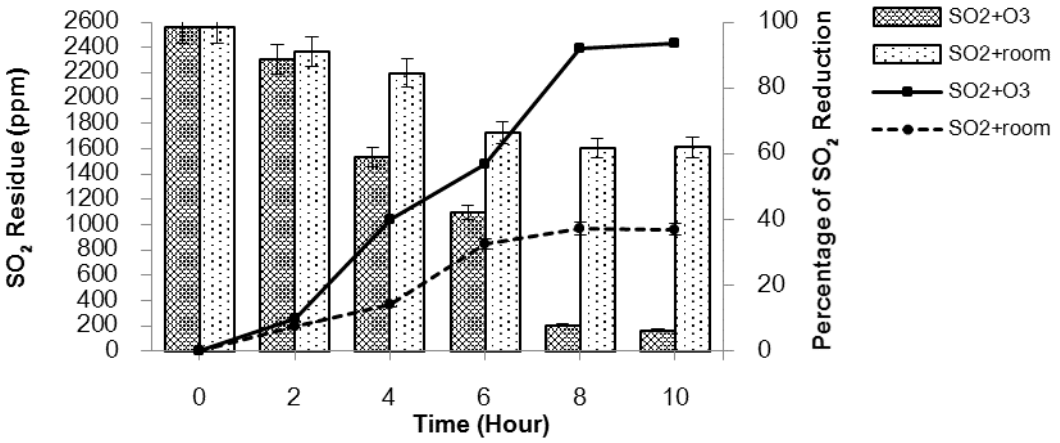

Figure 2 The sulfur residue in the pericarp of the longan fruit after exposure to ozone fumigation for 0, 2, 4, 6, 8, and 10 h

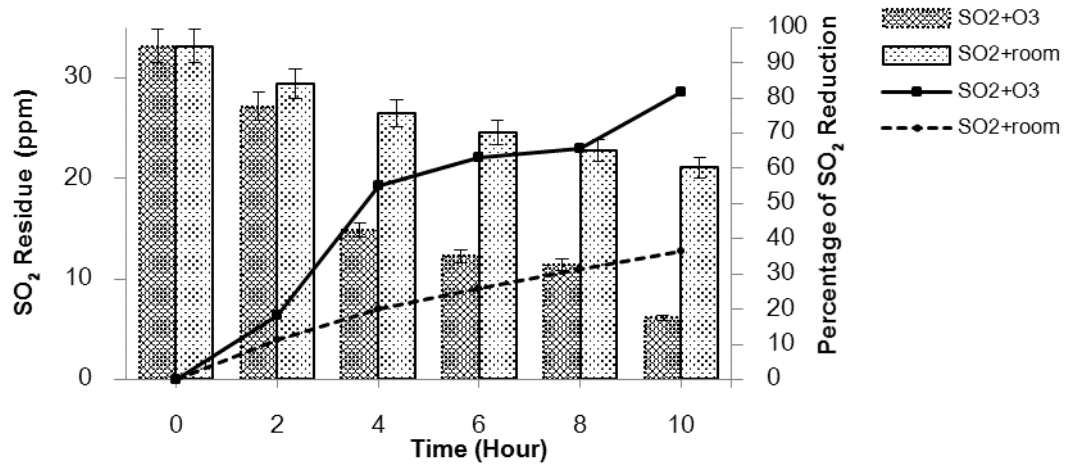

Figure 3 The sulfur residue in the aril of the longan fruit after exposure to ozone fumigation for 0 h, 2 h, 4 h, 6 h, 8 h, and 10 h

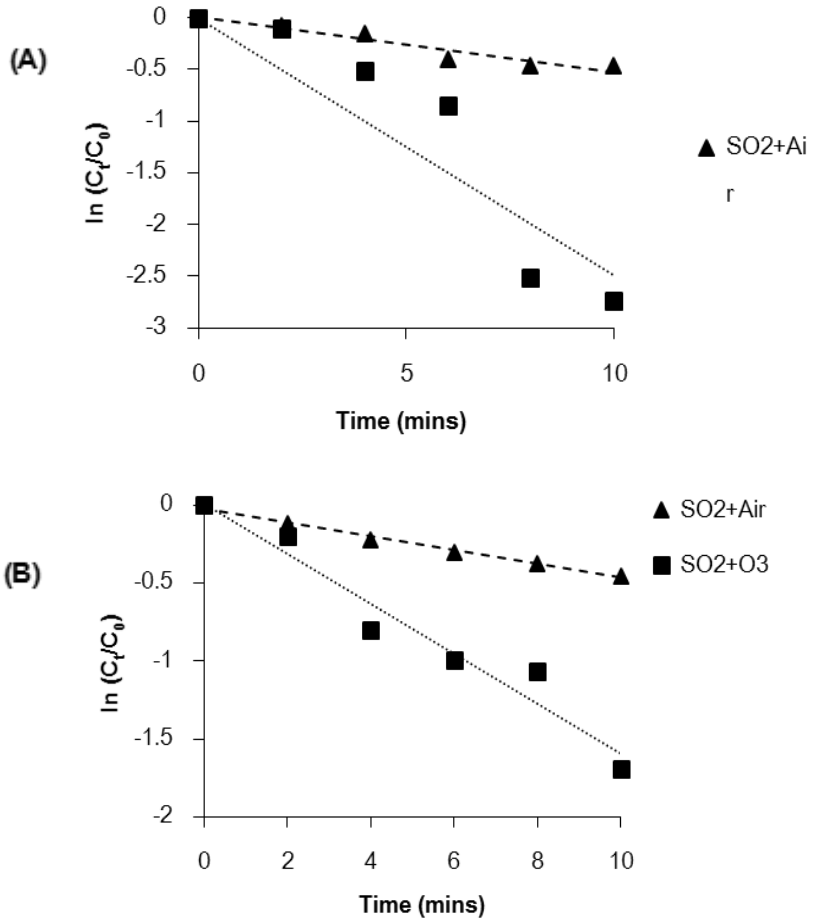

Figure 4 The first order kinetic model fitted to sulfur degradation by ozone on the pericarp (A) and the aril (B) of the longan fruit

3.2 Effect of ozone fumigation on pericarp browning and PPO activity of sulfur dioxide treated longan fruit

Pericarp browning is indicated by browning index. As shown in Figure 5, all fruit treatments in which SO_2 was fumigated significantly reduced the browning index when compared to the control group throughout the storage time (Figure 5). As far as the PPO activity of the longan pericarp during storage at 25°C for 5 days is concerned, the activity of the PPO enzyme was observed to have significantly decreased in all the treated fruit, and it was less than 1 unit.mg protein^{-1} as compared to the control group (Figure 6). Low PPO activity correlates with low browning appearance. This study showed that ozone has no effect as regards inhibition of browning, but that browning is affected by SO_2 fumigation. Commercially, SO_2 fumigation has been used to inhibit pericarp browning of the longan fruit. It has been reported that SO_2 can control enzymatic browning in several ways. First, it may inhibit PPO by modification of the protein. Second, it may prevent fumigation of the brown

pigments, and, lastly, sulfites are reducing agents that reduce colored orthoquinones back to being colorless. This also correlates with the pericarp browning index. All the treatments produced low scores for the browning index throughout the storage period. This is because the longans were SO_2-fumigated, which controls color bleaching and inhibits browning of the longan fruit pericarp in reactions that are both related and unrelated to enzymes (Siriparnich, 2006). Nevertheless, ozonation is also an effective treatment for bleaching and can oxidize pigments rapidly (Rakpatum, 2000).

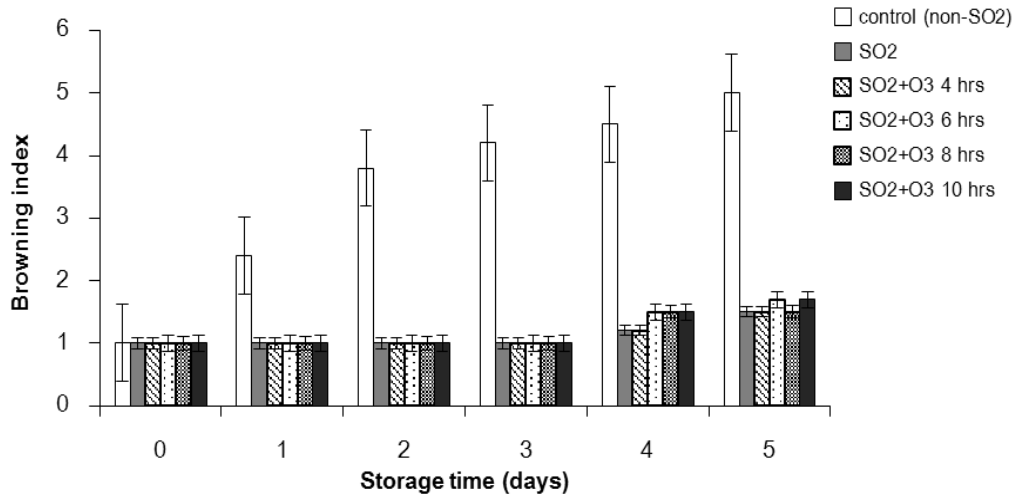

Figure 5 The pericarp browning index of the longan fruit after various treatments during storage at 25°C for 5 days

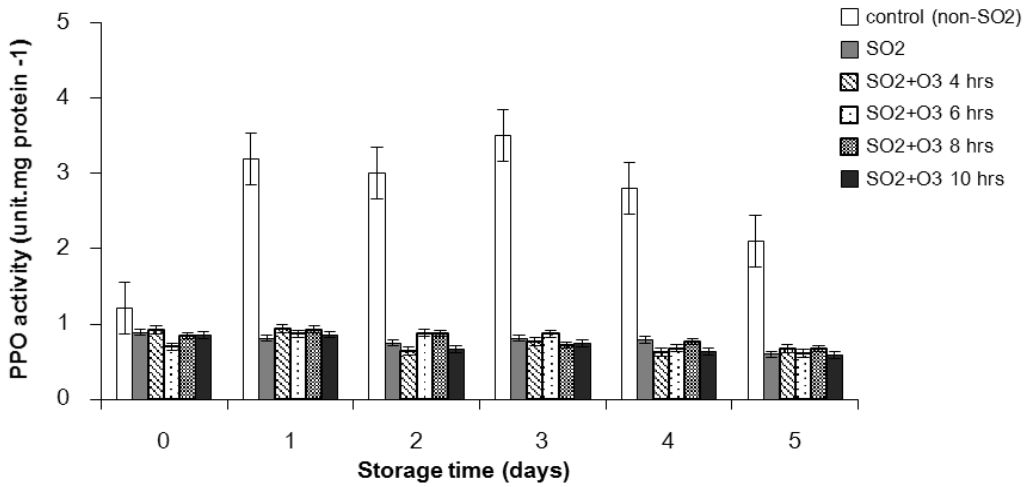

Figure 6 The PPO activity of the longan fruit after various treatments during storage at 25°C for 5 days

3.3 Effect of ozone fumigation on disease incidence and microorganism population of sulfur dioxide treated longan fruit

Disease incidence was significantly inhibited after 4 h, 6 h, 8 h, and 10 h of O_3 exposure and throughout this investigation (Figure 7). None of the treated fruits showed evidence of microorganism population immediately after fumigation and after 5 days of storage, in comparison with the control group (Figure 8A). Exposing fruits to ozone for 4 h, 6 h, 8 h and 10 h significantly inhibited yeast and mold population, while the control and the SO_2-fumigated group exhibited yeast and mold populations of 3.04 log cfu.mL^{-1} and 0.48 log cfu.mL^{-1}, respectively, after 5 days of storage at 25°C (Figure 8B), which is related to disease incidence. This result shows that ozone can control postharvest disease incidence by reducing the microorganism contamination of the longan fruit, and that the effect is extremely good with SO_2 due to the SO_2 gas reacting with water within the pericarp to become H_2SO_3 and having pH 3–5, which inhibits microbial growth effectively. Similarly, Glabler *et al.* (2010) reported that fumigation with high doses of ozone gas controls postharvest decay. Guzel-seydim *et al.* (2003) also reported that ozone can degrade unsaturated lipids of the microbial cell membrane. This would effect membrane leakage and cell breakdown. Victorin (1992) reported that there are two pathways of microbial growth inhibition by ozone. First, ozone oxidizes the sulfhydryl group, amino acid, peptide and peptein. Second, ozone oxidizes the polyunsaturated fatty acid to acid peroxide. Both pathways collapse the microbial cell.

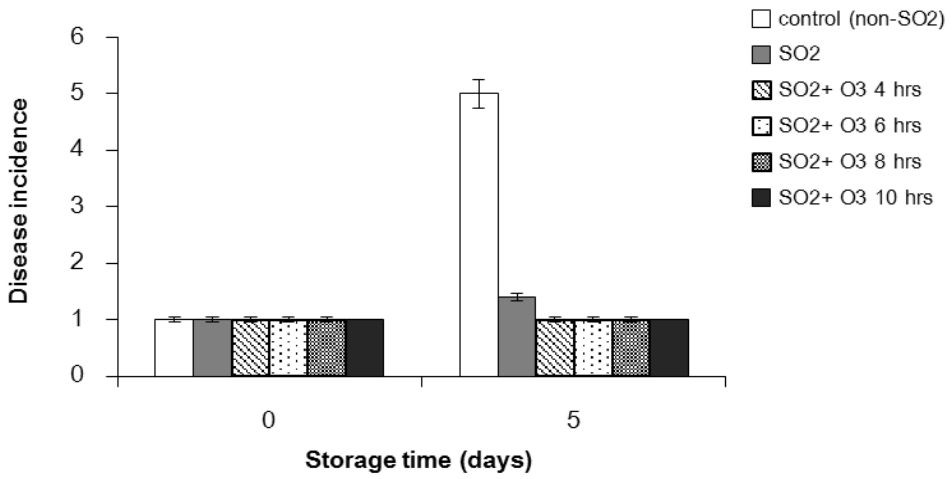

Figure 7 Effect of ozone on disease incidence of the longan fruit immediately after fumigation and after 5 days of storage at 25°C

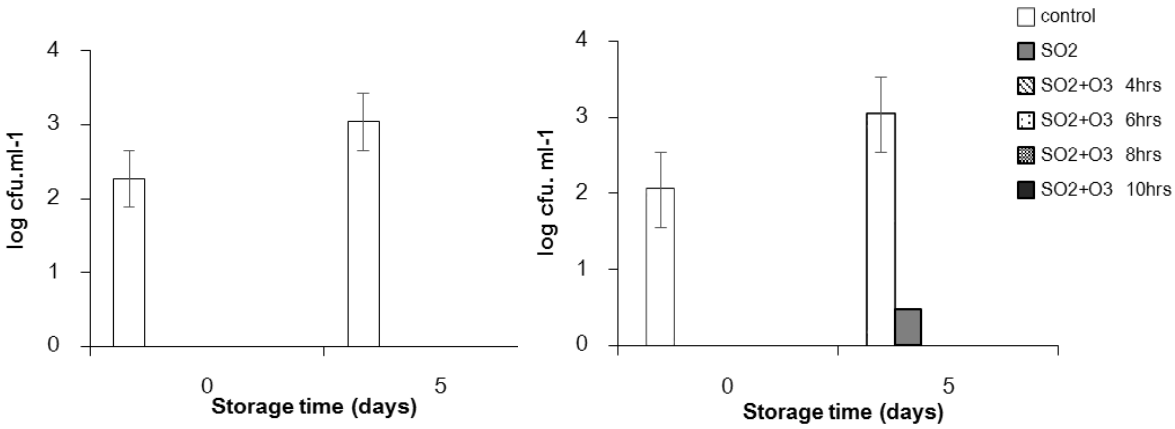

Figure 8 Effect of ozone on microorganism populations, total microorganism (A), and yeast and mold (B) of the longan fruit immediately after fumigation and after 5 days of storage at 25°C

3.4 Effect of ozone fumigation on postharvest quality of sulfur dioxide treated longan fruit during storage at low temperature

As shown in Table 1, the L* (lightness) and the b* (yellowness) values of all the treated fruits demonstrate that there was a significant delay in the decrease of these values when compared to the control group, indicating that SO_2 could maintain the lightness and the yellowness of the longan pericarp and that ozone also delayed the color change of the pericarp of the longan fruit because of its bleaching property. There was no significant difference between the groups with regard to the total soluble solid (TSS). All of the fumigated treatments had higher scores in sensory evaluation (taste, odor, texture, and overall acceptance) as well than the control group, and there was no disease incidence or browning index after storage at 5°C for 35 days. This result shows that exposing to ozone for 10 h can control disease incidence and also delay pericarp browning and changes in the postharvest qualities of SO_2-treated longan fruit. Similarly, Glowacz and Rees (2016) reported that exposure to ozone at 0.45 μmol.mol^{-1} and 0.9 μmol.mol^{-1} reduced disease incidence and that the skin color of red chili pepper was observed to have been bleached after exposure to ozone at 2 μmol.mol^{-1}, while ozone 0.9 μmol.mol^{-1} also reduced the loss of quality during storage of both red and green chili peppers. Boonkorn *et al.* (2012) also reported that the qualities of the tangerine fruit were not affected by ozone exposure and that no phytotoxicity occurred in the fruit when exposed to high doses of ozone throughout the experiment. In addition, Gross (1987) also found that ozone-containing water can delay pericarp color change during storage in the tangerine fruit.

Table 1 Effect of ozone fumigation on quality changes in longan fruit after storing at 5°C for 35 days

Treatment	Pericarp color		Disease incidence	Browning index	TSS (%Brix)	Sensory evaluation (score)			
	L* (lightness)	b* (yellowness)				Taste	Odor	Texture	Overall acceptance
Control (Non-SO_2)	21.29b	13.21b	3.2b	4.7b	19.80a	1.20b	1.20b	1.00b	1.20b
SO_2	26.26a	17.71a	1a	1a	19.88a	2.60a	3.00a	2.90a	2.60a
SO_2+O_3 10 h	26.69a	17.98a	1a	1a	20.06a	3.00a	3.00a	3.00a	3.00a

4. Conclusion

Ozone fumigation treatment can reduce sulfur residue in longan fruit by 93.50% in the pericarp and 81.54% in the aril after exposure for 10 h. Exposure to ozone also inhibits disease incidence and microorganism populations during storage. Moreover, ozonation can delay pericarp browning and changes in the postharvest qualities of sulfur dioxide treated longan fruit throughout the storage period.

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