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Full Length Research Paper

Effect of chlorine dioxide on the control of postharvest diseases and quality of litchi fruit

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Litchi (*Litchi chinensis* Sonn.) is an important subtropical fruit crop with high market value. However, rapid pericarp browning and decay of litchi fruits caused by infection of microorganisms during storage can result in up to 50% of postharvest loss prior to consumption. New methods are urgently needed for effectively overcoming these problems. In this study, we tested the efficacy of chlorine dioxide for the control of postharvest diseases of litchi fruit. Inactivation kinetics of litchi anthracnose by ClO_2 solution concentrations ranging from 5, 10, 20, 40, 60, 80 and 120 mg/l was also studied. The fruits of the cultivar 'Huaizhi' were first treated with 80 and 120 mg/l ClO_2 and then stored at 20°C for 7 days. The effect of the ClO_2 treatments on the postharvest physiology was investigated. The results showed that 5 mg/l ClO_2 solution could significantly inhibit litchi anthracnose spore germination. In addition, treatments with 80 and 120 mg/l of ClO_2 significantly reduced postharvest decay and peel browning of the fruit, inhibited polyphenol oxidase (PPO) and peroxidase (POD) activity, retained total soluble solids (TSS) and titratable acidity (TA) content, and increased phenylalnine ammonialyase (PAL) activity and malondialdehyde (MDA) content. However, ClO_2 solution did not significantly influence CO_2 and C_2H_4 production of the fruits, compared with those in the untreated control. Overall, 120 mg/l ClO_2 solution treatment was effective in inhibiting postharvest diseases and improving the quality of litchi fruits.

Key words: Litchi, chlorine dioxide, *Colletotrichum* spp, polyphenol oxidase, peroxidase.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is an important subtropical fruit crop with high market value. However, rapid pericarp browning and decay of litchi fruits during storage are the main problems that result in a great loss of its market value (Li et al., 2006). Postharvest loss of litchi is estimated to be 20 to 30% of the harvested fruit, even as high as 50% prior to consumption, mainly due to the decay caused by infection of microorganisms such as *Peronophythora* lithci*, Penicillium* spp*., Colletotrichum* spp. (Jiang et al., 2002; Jiang et al., 2001). Currently, sulphur dioxide (SO_2) fumigation is widely used to control the decay caused by some of these fungi such as *Penicillium* spp and C*olletotrichum* spp. Infection of litchi fruits by these fungi can become a major problem in litchi export industry, because SO_2 fumigation or SO_2 fumigation followed by acid dip treatment with HCl to regain appealing red colour, which is practiced in some packhouses, brings undesirable SO_2 and residues, alters the favorable fruit taste and causes hazards for consumers and packhouse workers (Lichter et al., 2004; Sivakumar et al., 2008). In recent years, there are growing concerns regarding SO_2 residue present in the fruits, especially from import countries such as those in Europe, U.S. and Japan. The European community currently permits a maximum limit of sulfur residue

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Abbreviations: ClO_2, Chlorine dioxide; **PPO,** polyphenol oxidase; **POD,** peroxidase; **PAL,** phenylalnine ammonialyase; **MDA,** malondialdehyde; **SO_2,** sulphur dioxide; **TA,** titratable acidity; **BI,** browning index; **DI,** disease incidence.

concentration at as low as only 10 mg/l in the edible portion of the fruits (Paull et al., 1998; Sivakumar et al., 2010). Therefore, a replacement for SO_2 with the safer, environmentally friendly and economical fungicide treatments would be preferred.

Chlorine dioxide (ClO_2) is one of the disinfectants, which has been used to control microbiological growth in different industries. The U.S. Environmental Protection Agency (EPA) has approved the use of ClO_2 as a disinfectant for potable water treatment with a 1.0 mg/l limit to chlorite ion in the treated water (US FDA, 2000). Aqueous ClO_2 has been approved by FDA as an anti-microbial agent for washing fruits and vegetables at a residual concentration of 3.0 mg/l (US FDA, 1995). Aqueous ClO_2 offers several advantages for food sanitization, especially for processing vegetables and fruits. ClO_2 solutions have shown to be efficient in inhibiting pathogens inoculated to different fruits and vegetables, such as apple, cantaloupe, strawberry, tomato and blueberries (Gomez-Lopez et al., 2009; Lee et al., 2006; Wu and Kim, 2007), although, no complete elimination has been obtained.

This study was conducted to investigate the effect of ClO_2 solution on controlling *Colletotrichum* spp spore germination, pericarp browning and the shelf life of litchi fruits. The activities of browning related enzymes including PPO, POD and PAL, as well as MDA content were also evaluated in litchi fruits during storage with 7 days of post-storage at 20°C.

MATERIALS AND METHODS

Strains and spore germination

Litchi anthracnose (*Colletotrichum* spp) was provided by Plant Pathology Department, College of Horticulture, South China Agricultural University Guangzhou, China. The pathogens were cultured for 6 days on potato sucrose agar (PSA: 1 L of distilled water containing 200 ml of extract of boiled potatoes, 20 g of sucrose and 20 g of agar) plates at 26°C. The spores were prepared by washing plates with water agar. Conidial suspensions were then adjusted to 3.0 to 6.0 × 10^6 spores/L using hemacytometer counts of conidia. A 0.1 ml aliquot of conidial suspension was added to 10 ml (0, 5, 10, 20, 40 and 60 mg/l) $C1O_2$ solutions and incubated for 24 h at 26 to 28°C. The spore germination rate was checked by microscopic examination at 40× magnification and colonies were counted. Axioskop 2 plus electron microscope (Carl Zeiss Jena, Germany) was used for the observation of *Colletotrichum* spp spore micro-morphology (Calvo et al., 2007).

Plant materials and ClO_2 treatment

Litchi (*L. chinensis* Sonn.) fruits of the cultivar 'Huaizhi' at 80 to 90% maturity were obtained from a commercial orchard in Guangzhou. Fruits with uniform size and colour were selected and dipped into different ClO_2 solutions (0, 80, 120 mg/l) for 3 min and then air-dried. Each 1000 g of the treated fruits was packed into a plastic punnet and wrapped with a 0.03 mm thick polyethylene bag and then stored in a room at 20±1°C with 80 to 90% relative humidity (RH). During the storage, enzyme activity and overall visual quality were measured at one-day interval. Fruits treated with tap water were used as the control for simulation of the commercial conditions.

A stabilized ClO_2 powder product (Beijing Startech Science and Technology Co. Ltd, China) was used in this research. After activated by tap water, ClO_2 solution (10,000 mg/l) was further diluted with tap water to specific concentrations. ClO_2 concentration in sealed test bottles was measured over time at 360 nm using a UV-2450 spectrophotometer (Shimadzu, Japan) (Ruffell et al., 2000).

Fruit quality evaluation

Postharvest decay was assessed on a 1 to 5 scale, describing the severity of postharvest fungal decay (1 = no decay; 2 = 25% decayed; 3 = 50% decayed; 4 = 75% decayed and 5 = entire fruit decayed) (Sivakumar and Korsten, 2006). The severity of browning was assessed visually as: 1 = no browning; 2 = 1–2 brown spots, acceptable marketability; 3 = some spots with browning, limited marketability; 4 = 50% of the fruit surface with browning; 5 = 75% entire fruit surface with browning, no marketability. The browning index (BI) was calculated as ∑ (browning scale × percentage of corresponding fruit within each scale) (Zhang et al., 2001). A set of twenty-four (24) fruits were randomly selected from each treatment, the pulp were pressed manually through layers of gauze and the total soluble solids (TSS) was determined with a digital refractometer (Atago, Japan). Percentage of titratable acidity (TA) was determined by titration with 0.01 M NaOH and calculated as citric acid equivalents from 10 g of pulp obtained from 10 fruit.

Three groups of samples with 20 fruits per group were enclosed in a 1.9 L container and incubated for 2 h at 20°C. A 1.0 ml of gas sample was withdrawn from the headspace and the concentrations of C_2H_4 and CO_2 were measured using GC2014/G-3900 gas chromatograph (Shimadzu/Hitachi Corp. Ltd, Japan) equipped with a porapak-Q column and flame ionization/thermal conductivity detectors (FID/TCD). Helium was used as the carrier gas with a head pressure of 180 kPa. The oven temperature was maintained at 80/50°C, both the injector and detector temperatures were kept at 150°C. The results were expressed as μl C_2H_2 /kg·h and mg CO_2/kg·h, respectively.

PPO, POD and PAL activities

One gram of pericarp tissue from 10 fruits was homogenized in a 5 ml of 0.05 M phosphate buffer (pH 7.0) and 0.5 g of polyvinylpyrrolidone (PVP insoluble) at 4°C. After centrifugation for 20 min at 19,000×*g* at 4°C, PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate, according to the method of Dong et al. (2004). One unit of enzyme activity was defined as the amount that caused a change of 0.001 in the absorbance per minute. POD activity, using guaiacol as a substrate, was assayed by the method of Zhang et al. (2005) in a reaction mixture (3 ml) containing 25 μl of enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 1% H_2O_2 and 0.1 ml of 4% guaiacol. The increase in the absorbance at 470 nm due to the guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

PAL was extracted according to the methods of Sun et al., (2009). All steps were performed at 4°C. Litchi pericarp tissue (1 g) was homogenized in a 5 ml of 0.05 M sodium borate buffer (pH 8.8) containing 5 mM mercaptoethanol and 0.1 g of PVP. The enzyme

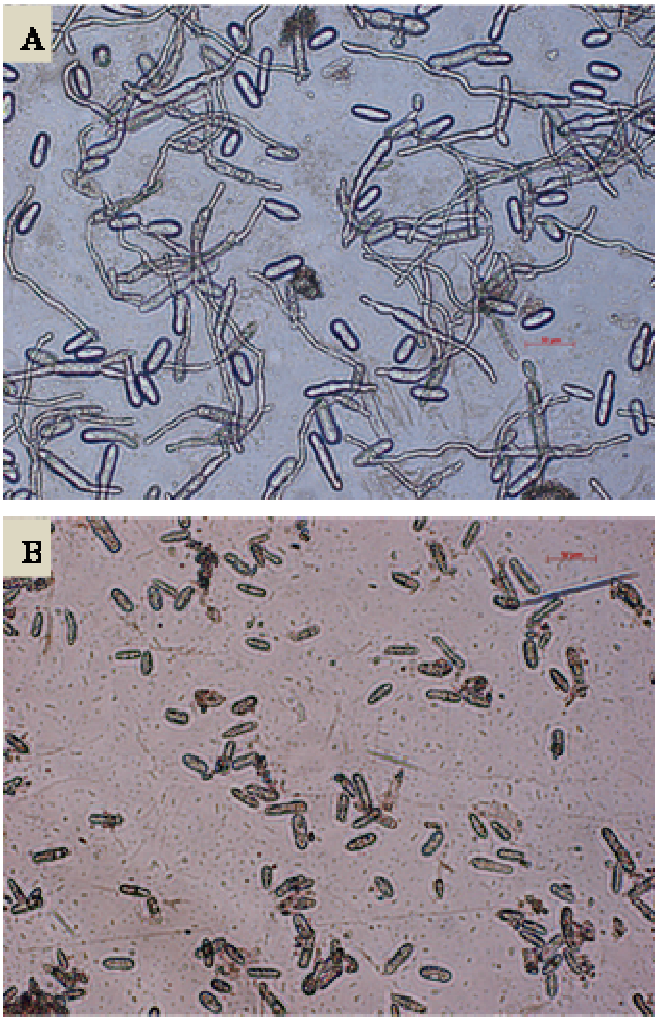


Figure 1. Effect of ClO_2 on the *Colletotrichum* spp spore growth after 24 h incubation. The growth of hyphae dipped in 20 mg/l solution was significantly inhibited (A), when compared with the control (B). 20 mg/l ClO_2 treatment. Red bar: 50 µm.

extract was centrifuged at 12,000×*g* for 15 min and then the supernatant was collected to determine PAL activity. The reaction mixture consisted of 1.9 ml of 0.05 M sodium borate buffer (pH 8.8), 1 ml of 20 mM L-phenylalanine and 0.1 ml of enzyme solution, with a final volume of 3 ml. Enzyme samples were incubated for 1 h at 37°C. In the control sample, the enzymatic extract was replaced by 1 ml of 0.05 M sodium borate buffer (pH 8.8). The reaction was stopped by the addition of 0.2 ml of 6 M trichloroacetic acid. One unit of enzymatic activity was defined as the amount of the enzyme that caused a change of 0.01 in the absorbance at 290 nm per hour.

Malondialdehyde (MDA) determination

MDA content was measured following the method of Sofo et al., (2004) and expressed as µmol per gram dry weight. Litchi pericarp tissue (1 g) was added to a 5.0 ml of 0.05 M phosphate buffer (pH 7.8) and 0.4 g of PVP (insoluble) at 4°C and centrifuged at 10,000×*g* for 15 min. A 1 ml of supernatant was added to 3.0 ml of 0.5% (w/v) thiobarbituric acid in 10% (w/v) trichloroacetic acid. The mixture was heated at 100°C for 30 min and then quickly cooled on ice. After centrifugation at 10,000×*g* for 10 min, A532, A600 and A440 of the supernatant were recorded. The value for non-specific absorption at 600 nm was subtracted and a standard curve of sucrose (from 2.5 to 10 µmol/ml) was used to rectify the results from the interference of soluble sugars in samples, reading A532 and A440. MDA content was calculated using its absorption coefficient of 157 /mmol·cm and expressed as µmol MDA /mg (DW)

Statistical analysis

The entire experiment was done three times with three replicates for each time. Data were plotted using Sigma Plot10.0 software and one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$, $P < 0.01$) using SPSS Version 16.0. Data were presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

Micro-morphology and germination rate of *Colletotrichum* spp spores

The spore micro-morphology of *Colletotrichum* spp is shown in Figure 1. Microscopic examination of the fungus cultured under sustained 20 mg/l ClO_2 confirmed the lack of sporulation in *Colletotrichum* spp. ClO_2 solution completely inhibited *Colletotrichum* spp spore growth. The hyphae in the control cultures were highly branched and lacked spores. After 24 h incubation, the average hyphal length reached a plateau (300±50 µm) in the control (Figure 1a), whereas in the ClO_2 treated group, the average hyphal length was 50±10 µm (Figure 1b).

The spore germination rate of *Colletotrichum* spp is shown in Table 2. The inactivation effect of ClO_2 on *Colletotrichum* spp spore increased as ClO_2 concentrations were increased. Spores were inactivated when the concentration was as low as 5 mg/l. At a ClO_2 concentration of 20 mg/l, spore germination rate was 0.00%, compared with 95% in the control. These results clearly indicated that, ClO_2 treatment significantly ($P < 0.01$) decreased the germination rate of *Colletotrichum* spp spore of litchi fruits. Cell membrane has been identified as the primary target of ClO_2 on microbial cells. Beuchat et al. (2004) found that, treatment of *Bacillus cereus* and *Bacillus thuringiensis* spores in a medium in which cells had grown and produced spores with an equal volume of alkaline (pH 12.1) ClO_2 (400 mg/l) for 30 min reduced spore populations by 4.6 and 5.2 log cfu/ml. Lee et al. (2004) reported that, *Alicyclobacillus acidoterrestris* spore number was reduced by > 4.8 log with 120 mg/l free chlorine dioxide treatment for only 1 min. Pao et al. (2007) demonstrated a significantly sanitizing ability of 20 mg/l ClO_2 against Salmonella enterica and Erwinia carotovora inoculated onto the surface of

Table 1. Effect of ClO_2 on TSS and TA of litchi fruit during storage at 20°C.

Parameter		Day of storage			
		1.0	3.0	5.0	7.0
TSS	Control	18.86± 0.11[a]	17.90±0.10[c]	17.36±0.05d	17.03±0.20d
	80	18.86± 0.11[a]	18.40±0.13[b]	18.26±0.05[b]	18.23±0.06[b]
	120	18.86± 0.11[a]	18.50±0.11[b]	17.90±0.12[c]	17.63±0.06[d]
TA	Control	0.84±0.05[a]	0.71±0.04[c]	0.53±0.04[f]	0.40±0.02[h]
	80	0.84±0.05[a]	0.77±0.05[b]	0.57±0.10[e]	0.49±0.02[g]
	120	0.84±0.05[a]	0.80±0.03[b]	0.64±0.03[d]	0.58±0.04[e]

Means within a column between control and treatment followed by the same letter are not significantly different at the 5% level. Each data point represents the mean ± S.E. (n = 3).

tomato fruits. These results support that ClO_2 solutions can significantly reduce fungal population in litchi fruits.

Changes in pericarp browning and disease index (DI) of ClO_2-treated litchi fruits

Postharvest decay is one of the major problems that reduce the commercial value of litchi fruits. A wide range of fungi, such as *Aspergillus, Penicillium* and *Rhizopus*, can cause decay of litchi fruits, occurring during and after harvest through skin injury, whereas *Colletotrichum* and *Botryodiplodia* infects fruits either in the field or through the cut stem end during harvest or handling (Jiang et al., 2002; Scott et al., 1982). In this study, the postharvest diseases were observed to be significantly reduced by ClO_2 solution at 80 and 120 mg/l (Figure 2b). Additionally, the browning index of the fruits treated with ClO_2 solution at 80 and 120 mg/l were significantly lower than that of the control after 5 days of harvest (Figure 2a). Comparing the inhibitory efficiency of various ClO_2 concentrations on browning, we found that 120 mg/l of ClO_2 was the most effective concentration for litchi, cv. 'Huaizhi'. It is worth noting that, following the increasing of the disease index, the browning index of the fruits increased gradually in all the treatments (Figure 2a). This observation is similar to those made by Li et al. (2005) who reported that the incidence of anthracnose of the fruits was closely related to the browning of the pericarp, the infection of anthracnose fungus accelerated the pericarp browning and lesion larging. These observations indicate that the increased browning index is possibly associated with the disease index development. In this study, we observed that that the pericarp browning was markedly delayed by ClO_2 treatments. This inhibitory effect may contribute to lower disease index of litchi fruits during storage at 20°C.

Respiration rate and ethylene production

Litchi fruit is non-climacteric and does not continue to ripen after harvest (Holcroft and Mitcham, 1996). Respiration rate increased from 2 days after treatment and peaked on day 7 (Figure 3a). In contrast to the control, the respiration rate of the fruit treated with 80 mg/l ClO_2 was decreased. However, no significant difference was found in the respiration rate between untreated and treated samples on day 7 ($P < 0.05$), which were 0.0253±0.024, 0.0223±0.0031 and 0.0254±0.0005 g/h·g, respectively. Another study reported a significant reduction in the respiration rate at 10°C after fumigating green bell pepper with 20 and 50 mg/l ClO_2 gas for 40 days (Du et al., 2007). Furthermore, Gomez-Lopez found an increase in the respiration rate of shredded white cabbage by 22% with ClO_2 gas, but did not affect the respiration rate of grated carrots and shredded iceberg lettuce (Gomez-Lopez et al., 2007, 2008). These contradictory results could be due to the differences of ClO_2 concentration, exposure time or possibly in association with the disease development after harvest.

The pattern of ethylene production was similar to that of respiration rate, where it increased from 3 to 6 days during storage at 20°C, peaked at day 6 and then decreeased (Figure 3b). ClO_2 treatments increased the ethylene production but no significant difference was found between untreated and treated samples ($P < 0.05$). This result might be associated with skin browning and rotting of litchi fruit.

Total soluble solids and titratable acidity

Total soluble solids (TSS) and titratable acidity (TA) are important factors in the assessment of the flavor and nutrition of litchi fruit. Litchi fruit does not continue to ripen and accumulate sugars after harvest, so the TSS increases during fruit ripening, while the TA remarkably decreases (Sivakumar et al., 2010). As shown in Table 1, the concentrations of TA and TSS decreased over time during the shelf life evaluation. TSS in ClO_2-treated fruit generally decreased during storage time from 18.80 (day

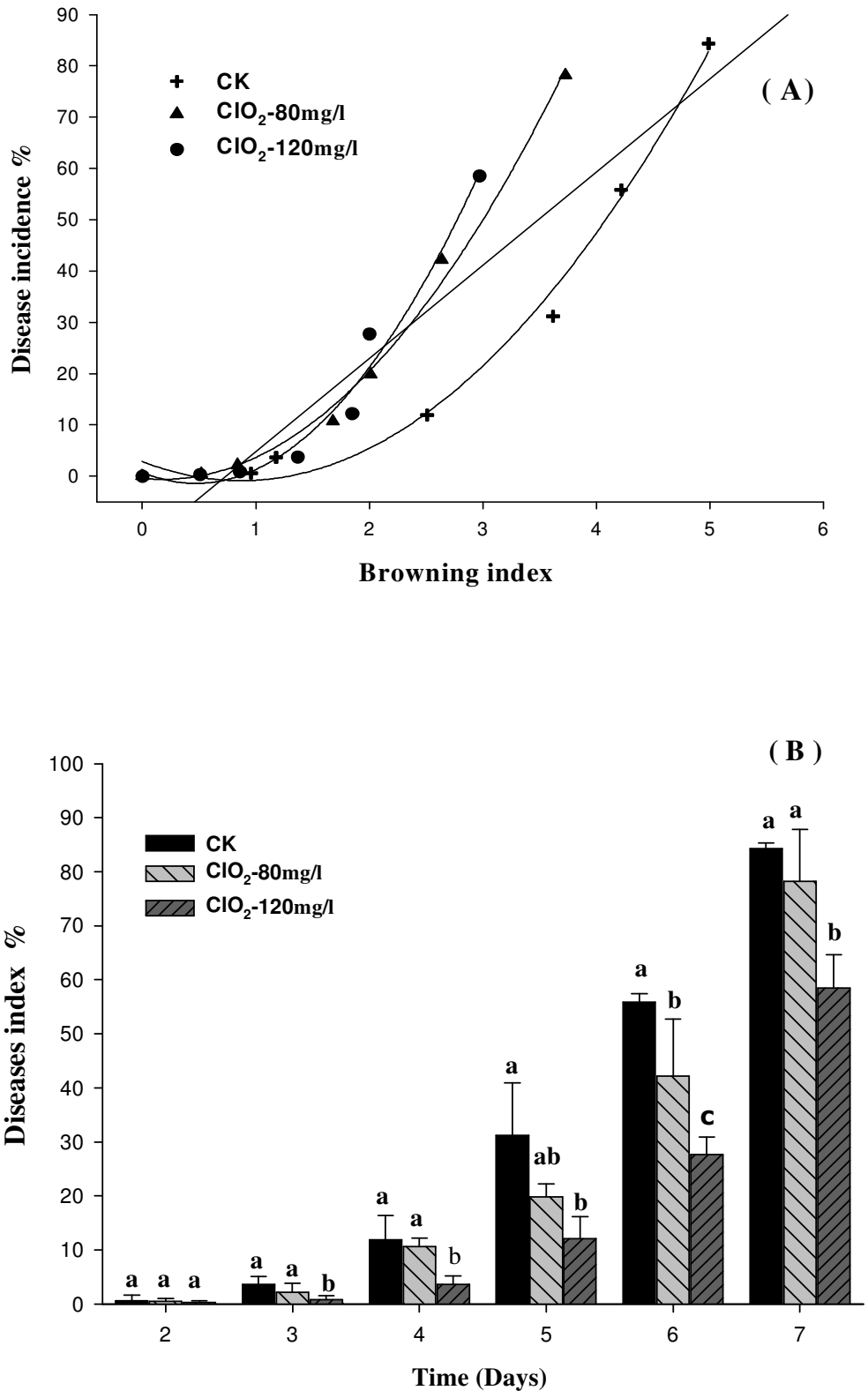


Figure 2. Effect of ClO_2 on browning index with disease incidence (A) and disease index (B) of litchi fruit during storage at 20°C. Each value is presented as the mean ± SE (n = 3). The same letters are not significantly different at $P < 0.05$ level within the same group. BI, Browning index; DI, disease incidence.

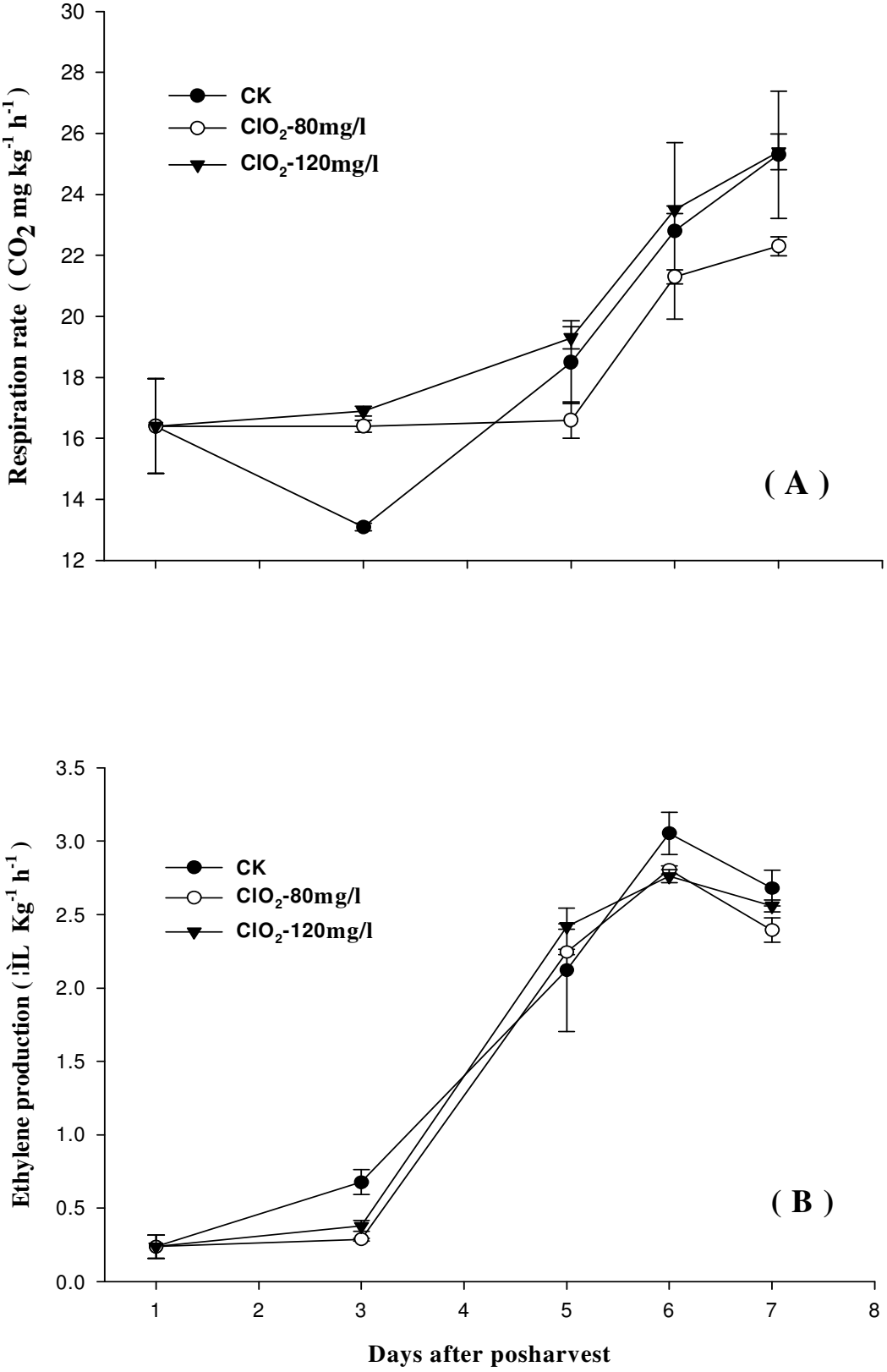


Figure 3. Effect of ClO_2 on ethylene production (A) and respiration rates (B) of litchi fruits during storage at 20℃. Each value is presented as the mean ± SE (n = 3).

Table 2. Effect of ClO_2 on spore germination rate of *Colletotrichum* spp spore growth after 24 h incubation.

ClO_2 concentration (%)	0 mg/l	5 mg/l	10 mg/l	20 mg/l	40 mg/l	60 mg/l
Spore germination rate	95.0±0.5^{A}	10.3±0.2^{B}	3.5±0.8^{C}	0 ^{D}	0 ^{D}	0 ^{D}

*Results are shown as mean ± standard deviation mean. Different letters within the same line indicate statistical significance at $P < 0.01$.

1) to 17.64 (day 7). TSS and TA contents in fruit treated with 80 and 120 mg/l ClO_2 were significantly higher than the control after 7 days. Conversely, Du et al. (2007) reported that, ClO_2 treatment did not influence TA and TSS contents of green bell peppers. In this study, the higher levels of TSS and TA in the pulp with ClO_2 treatment may be due to the reduced postharvest diseases of litchi fruit, but this needs further investigation.

Effect of ClO_2 on PPO, POD and PAL activity

As shown in Figure 4a, PPO activity in litchi fruit increased at the beginning of storage and peaked at 6 days after harvest and then markedly declined. The fruit treated with ClO_2 presented relatively lower POD activity during storage in comparison to the control. Both 80 and 120 mg/l ClO_2 treatments showed the ability to significantly inhibit the PPO activity. Pericarp browning due to decay or desiccation is mediated by PPO activity (Jiang and Fu, 1998). The browning index of litchi fruit pericarp was associated with increased PPO activity. A significantly higher incidence and severity of browning and PPO activity were observed in the control fruit, but moderate browning in the treated fruit. Similarly, an increase in POD activity was apparent during the course of the storage. POD activity in the control fruit slowly increased and then rapidly increased after 5 days of storage. ClO_2 treatment resulted in a slight increase of POD activity throughout 7 days of storage compared with the control fruit (Figure 4b). Peel browning of harvested litchi fruit has largely been attributed to the rapid degradation of red anthocyanin pigments. This process is associated with enzymatic oxidation of phenolics by polyphenol oxidase (PPO) and/or peroxidase (POD) and formation of polymeric browning pigments (o-Quinones) (Jiang et al., 2004; Sivakumar et al., 2010). Since ClO_2 is widely used as a pulp bleaching agent in the paper industry, Du et al. (2007) hypothesized that, it might be useful to preserve color of vegetables. These authors demonstrated that the PPO activities were inhibited with ClO_2 treatment during the storage of the pepper and ClO_2 might relate to the oxidation of amino acids and/or disulfide bonds that are involved in the active site in the PPO. It has been reported that ClO_2 could oxidize phenols (Napolitano et al., 2005). In this study, we showed that the PPO activity was reduced by ClO_2 during storage. This effect may be partially attributed to the inhibition of postharvest diseases and/or react with phenols. It is also possible that, ClO_2 has a direct impact on the PPO and POD content. Further studies are needed to figure out the exact reason for this trend

Figure 4c shows that, PAL activity in the fruits with all ClO_2 treatments increased during storage (Figure 4c). There were significant differences in PAL activity between the control and the groups treated with 80 and 120 mg/l ClO_2 ($P < 0.05$). PAL is the first enzyme in phenylpropanoid pathway and plays a key role in the synthesis of phenolic compounds in plants (Pina and Errea, 2008). These compounds can be further converted to other phenolic compounds via coumarate, such as flavonols, anthocyanins, chlorogenic acid and caffeic acid derivatives which are thought to serve as browning substrates in some plant tissues (Tomas-Barberan et al., 1997). A large number of reports have shown that the increase of PAL activity was related to anthocyanin accumulation (Faragher and Chalmers, 1977; Jiang and Joyce, 2003; Wang et al., 2000). In this study, the activation of PAL activity in the litchi fruits reduced the decay index of litchi fruit treated with ClO_2, might also be beneficial in delaying pericarp browning of the fruits.

MDA content

The malondialdehyde (MDA) content of both treated and control fruits increased during storage (Figure 5). Compared with the control, the MDA content of ClO_2-treated fruits remained significantly lower throughout the storage ($P < 0.05$). A later increase in MDA content after harvest is possibly associated with ClO_2-inhibited post-harvest disease of litchi fruits. MDA is the main product of lipid peroxidation in plant cells (Li and Yu, 2001); there-fore, the lower MDA content might indicate that higher membrane integrity was maintained with ClO_2 treatment.

In conclusion, this study investigated the shelf life of the litchi fruits after 7 days of storage at 20°C, in terms of skin browning and disease development. We observed that application of ClO_2 delayed skin browning, inhibited postharvest diseases and maintained higher concentrations of total soluble solids and titratable acidity. Also, this treatment also significantly reduced spore germination of *Colletotrichum* spp. We observed lower PPO, POD and higher PAL activity during storage after ClO_2 treatment. The mechanism of the inhibition of PPO and POD activity by ClO_2 requires further investigation. Our results

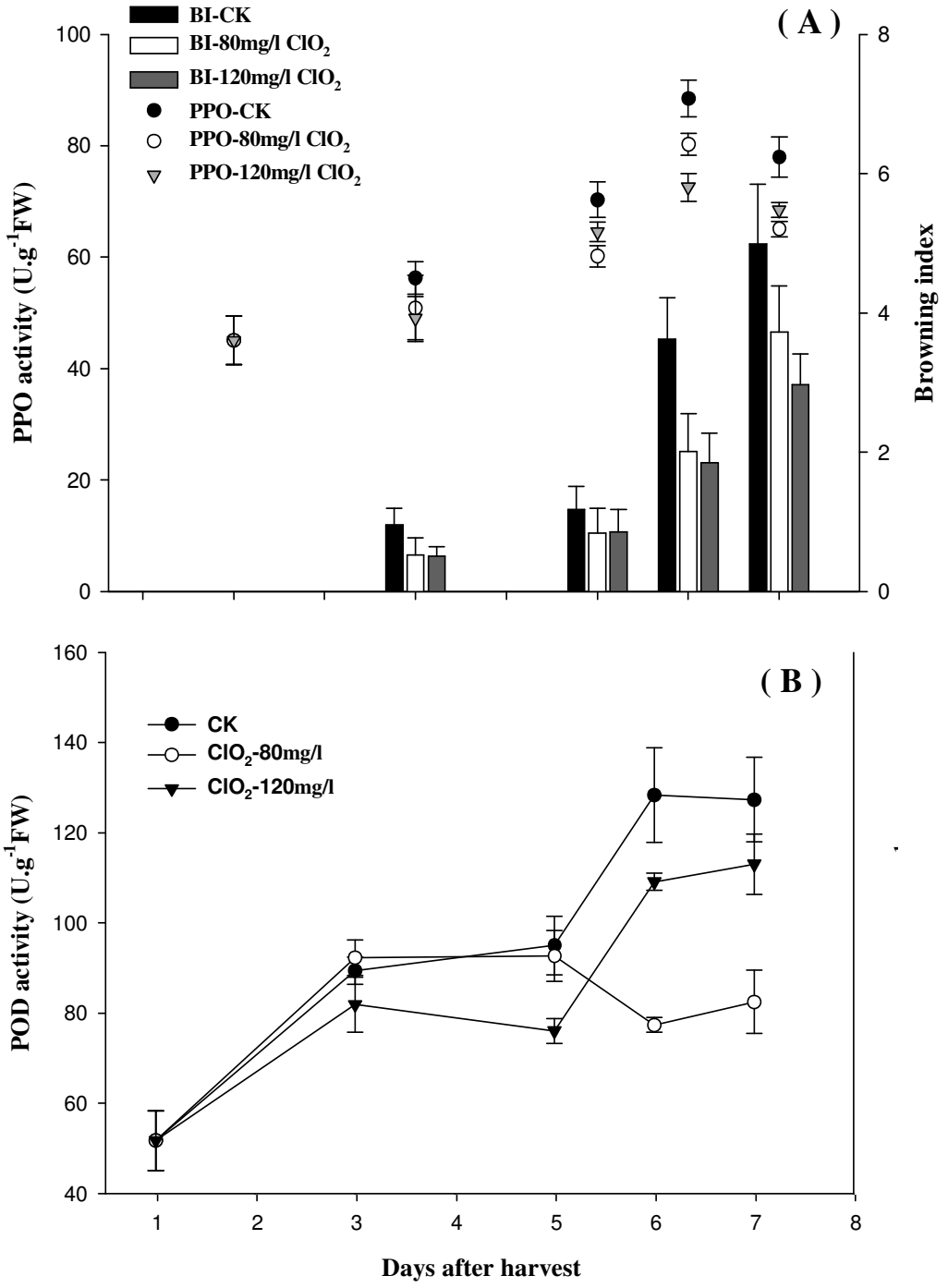
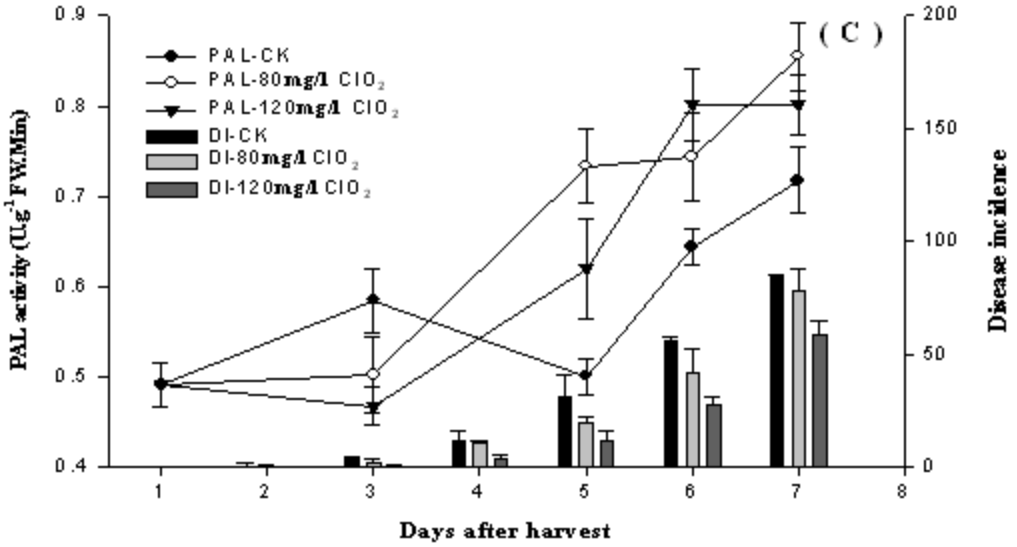


Figure 4. Effect of ClO_2 on PPO activity and browning index (A), POD activity; (B) PAL activity and disease incidence; (C) in the pericarp of litchi fruit during storage at 20°C. Each value is presented as the mean ± SE (n = 3). PAL, Phenylalnine ammonialyase; DI, disease incidence.

indicated that, ClO_2 treatment might be suitable to retain better quality of litchi fruits. Therefore, as a non-toxic oxidizing agent during storage and transportation for marketing chains, ClO_2 treatment could be used at least as a partial alternative to SO_2 fumigation, for health conscious consumers in domestic and overseas markets. Further research on the commercial packhouses at different geographical locations prior to application of



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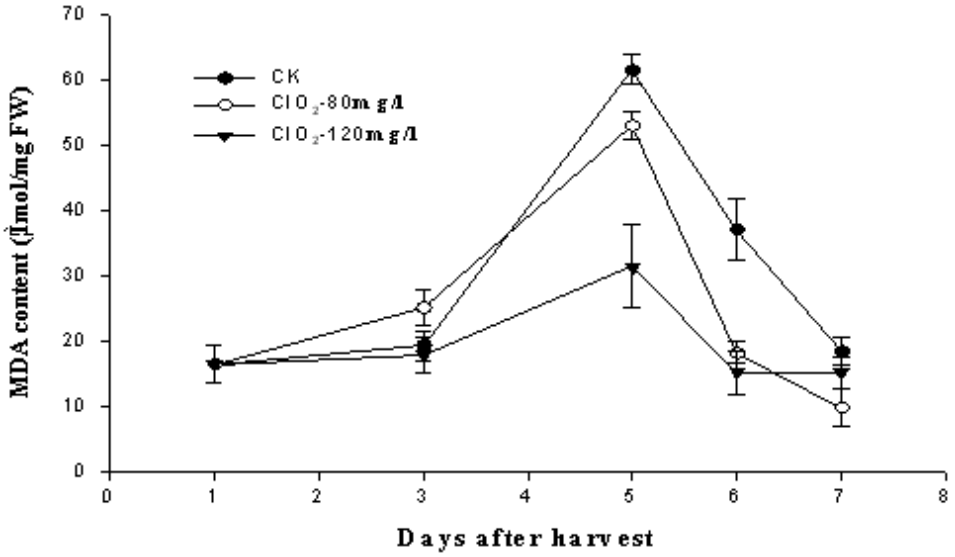

Figure 5. Effect of ClO_2 on MDA content in the pericarp of litchi fruit during storage at 20°C Each value is presented as the mean ± SE (n=3).

ClO_2 application needs to be conducted at commercialization.

ACKNOWLEDGEMENTS

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REFERENCES

Beuchat LR, Pettigrew CA, Tremblay ME, Roselle BJ, Scouten AJ (2004). Lethality of chlorine, chlorine dioxide, and a commercial fruit and vegetable sanitizer to vegetative cells and spores of Bacillus cereus and spores of *Bacillus thuringiensis*. J. Food Prot. 67: 1702-1708.

Calvo J, Calvente V de Orellano, ME Benuzzi, D Sanz de Tosetti, MI (2007). Biological control of postharvest spoilage caused by Penicillium expansum and Botrytis cinerea in apple by using the bacterium Rahnella aquatilis. Int. J. Food Microbiol. 113: 251-257.

Du JH, Mao R, FU MM, LI Wei (2007). Effects of Chlorine Dioxide Gas on Postharvest Physiology and Storage Quality of Green Bell Pepper (*Capsicum frutescens* L. var. Longrum). Agric. Sci. China. 6: 214-219.

Dong H, ChengL, Tan J, Zheng K, Jiang Y (2004). Effects of chitosan coating on quality and shelf life of peeled litchi fruit. J. Food Eng. 64: 355-358.

Faragher J, Chalmers D (1977). Regulation of Anthocyanin Synthesis in Apple Skin. III. Involvement of Phenylalanine Ammonia-lyase. Funct. Plant Biol. 4: 133-141.

Gomez-LopezVM, Devlieghere F, Ragaert P, Debevere J (2007). Shelf-life extension of minimally processed carrots by gaseous chlorine dioxide. Int. J. Food Microbiol. 116: 221-227.

Gomez-Lopez VM, Ragaert P, Jeyachchandran V, Debevere J, Devlieghere F (2008). Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. J. Food Microbiol. 121: 74-83.

Gomez-Lopez VM, Rajkovic A, Ragaert P, Smigic N, Devlieghere F (2009). Chlorine dioxide for minimally processed produce preservation: a review. Trends Food Sci. Technol. 20: 17-26.

Holcroft DM, Mitcham EJ (1996). Postharvest physiology and handling of litchi (*Litchi chinensis* Sonn.). Postharvest Biol. Technol. 9: 265-281.

Jiang YM, Duan XW, Joyce D, Zhang ZQ (2004). Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem. 88: 443-446.

Jiang YM, Fu JR (1998). Inhibition of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. Food Chem. 62: 49-52.

Jiang YM, Joyce DC (2003). ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Regul. 39: 171-174.

Jiang YM, Yao LH, Lichter A, Li JR (2002). Postharvest biology and technology of litchi fruit. Food, Agric. Environ. 1: 76-81.

Jiang YM, Zhu XR, Li YB (2001). Postharvest control of litchi fruit rot by Bacillus subtilis. LWT-Food Sci. Technol. 34: 430-436.

Lee SY, Gray PM, Dougherty RH, Kang DH (2004). The use of chlorine dioxide to control Alicyclobacillus acidoterrestris spores in aqueous suspension and on apples. J. Food Microbiol. 92: 121-127.

Lee SY, Dancer GI, Chang SS, Rhee MS, Kang DH (2006). Efficacy of chlorine dioxide gas against Alicyclobacillus acidoterrestris spores on apple surfaces. J. Food Microbiol. 108: 364-368.

Li HY, Yu T (2001). Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. J. Sci. Food Agric. 81: 269-274.

Li L, Peng CZ, Li MF, Zheng XQ (2006). Proteomic analysis of seedless litchi at embryo developmental stage. J. Trop. Subtropical Bot. 14: 312-317.

Li X, Liu AY, Chen W (2005). Studies on Development and Control of Anthracnose of Lychee Fruit before and after Harvest. Acta Hortic. 665:409-413.

Lichter A, Dvir O, Ackerman M, Feygenberg O, Pesis E (2004). Acidified peel of litchi fruits selects for postharvest Penicillium decay. Phytoparasitica, 32: 226-236.

Napolitano MJ, Green BJ, Nicoson JS, Margerum DW (2005). Chlorine Dioxide Oxidations of Tyrosine, N-Acetyltyrosine, and Dopa. Chem. Res. Toxicol. 18: 501-508.

Pao S, Kelsey DF, Khalid MF, Ettinger MR (2007). Using aqueous chlorine dioxide to prevent contamination of tomatoes with Salmonella enterica and Erwinia carotovora during fruit washing. J. Food Prot. 70: 629-634.

Paull RE, Reyes MEQ, Reyes M (1998). Sulfite residues on litchi fruit treated with sulfur dioxide. Postharvest Biol. Tech. 14: 229-233.

Pina A, Errea P (2008). Differential induction of phenylalanine ammonia-lyase gene expression in response to in vitro callus unions of *Prunus* spp. J. Plant Physiol. 165: 705-714.

Ruffell KM, Rennecker JL, Mariñas BJ (2000). Inactivation of *Cryptosporidium parvum* oocysts with chlorine dioxide. Water Res. 34: 868-876.

Scott KJ, Brown BI, Chaplin GR, Wilcox ME, Bain JM (1982). The control of rotting and browning of litchi fruit by hot benomyl and plastic film. Sci. Hortic. 16: 253-262.

Sivakumar D, Arrebola E, Korsten L (2008). Postharvest decay control and quality retention in litchi (cv. McLean's Red) by combined application of modified atmosphere packaging and antimicrobial agents. Crop Prot. 27: 1208-1214.

Sivakumar D, Korsten L (2006). Influence of modified atmosphere packaging and postharvest treatments on quality retention of litchi cv. Mauritius. Postharvest Biol. Tech. 41: 135-142.

Sivakumar D, Terry LA, Korsten L (2010). An Overview on Litchi Fruit Quality and Alternative Postharvest Treatments to Replace Sulfur Dioxide Fumigation. Food Rev. Int. 26: 162-188.

Sofo A, Dichio B, Xiloyannis C, Masia A (2004). Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Sci. 166: 293-302.

Sun J, Xiang X, Yu CY, Shi JY, Peng HX, Yang B, Yang SY, Yang E, Jiang YM (2009). Variations in contents of browning substrates and activities of some related enzymes during litchi fruit development. Sci. Hortic. 120: 555-559.

Tomas-Barberan FA, Gil MI, Castaner M, Artes F, Saltveit ME (1997). Effect of Selected Browning Inhibitors on Phenolic Metabolism in Stem Tissue of Harvested Lettuce. J. Agric. Food Chem. 45: 583-589.

US FDA (2000). United States Food and Drug Administration. Maximum containment level for disinfection by products. In 2000.40 CFR. Part 141: 64.

US FDA (1995). United States Food and Drug Administration. Secondary direct food additives permitted in food for human consumption. In 1995.60 (42) CFR. Part 173.

Wang H, Arakawa O, Motomura Y (2000). Influence of maturity and bagging on the relationship between anthocyanin accumulation and phenylalanine ammonia-lyase (PAL) activity in 'Jonathan' apples. Postharvest Biol. Tech. 19: 123-128.

Wu VCH, Kim B (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. Food Microbiol. 24: 794-800.

Zhang ZQ, Pang XQ, Duan XW, Ji ZL, Jiang YM (2005). Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. Food Chem. 90: 47-52.

Zhang ZQ, Pang XQ, Ji ZL, Jiang YM (2001). Role of anthocyanin degradation in litchi pericarp browning. Food Chem. 75: 217-221.