Disinfestations of the Oriental Tobacco Budworm in Green Hot Pepper by Ultra High Carbon Dioxide: Implications for Postharvest Fruit Quality

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To develop environmentally amenable insect disinfestations, effects of a carbon dioxide (CO_2) controlled atmosphere (CA) on the control of the oriental tobacco budworm *Helicoverpa assulta* were investigated in green hot peppers. Green hot peppers (cv. Nokgwang) were exposed to CO_2 at 80% and 100% in 0.08-mm polyethylene film bags for 24 and 48 h at 20°C. Mortality percentages of oriental tobacco budworm larvae were determined after gas exposure. The CO_2-CA at both concentrations for 24 h greatly reduced survival of the larvae, showing approximately 65% mortality when compared with control fruit. Prolonged exposure at both concentrations up to 48 h completely disinfested the larvae. To evaluate plausible deleterious effects of the ultra high CO_2-CA on green hot peppers, the fruit were stored at 10°C, and postharvest quality was analyzed in terms of firmness, electrolyte leakage, respiration rate, and content of vitamin C and capsaicin. There were no significant differences in postharvest fruit quality up to 20 days of storage, compared with control fruit. Meanwhile, respiration rates of exposed pepper fruit were approximately half the control's rate after 20 days of storage. These results suggested that ultra high CO_2-CA could disinfest *H. assulta* without significant differences in postharvest quality of green hot peppers, compared with control fruit. Exposure of 80% CO_2 for 24 h would be recommended as a reliable control means that is harmless to humans and can alleviate concern regarding pesticide residues.

Keywords : *Capsicum annuum*, carbon dioxide, *Helicoverpa assulta*, postharvest quality

The oriental tobacco budworm *Helicoverpa assulta* G. (Lepidoptera: Noctuidae) is one of the most destructive insects to threaten production of green hot peppers (*Capsicum annuum* L.). *H. assulta* is mostly found in Asia, Africa, and Australia and control of the pest relies mainly on chemical practices of periodic pesticide sprays on pepper fruit (Choi et al., 1998). Meanwhile, it is hard to determine infested fruit and control the pest because the larvae grow in the fruit, degrading fresh fruit quality undetected by visual assessments. Owing to increased consumer awareness of environmental safety and nutritional value of fresh crops, non-chemical insect extermination methods have been reported as alternatives to methyl bromide fumigation for horticultural crops. Controlled atmospheres (CA) have been known to be an effective alternative or complement to other disinfestation practices for preventing and controlling arthropod infestations (Fields and White, 2002). In particular, a low oxygen (O_2) and high CO_2-enriched atmosphere has been widely used as an alternative disinfestation system to fumigants for postharvest disinfestations. Denterner et al. (1992) reported that the light brown apple moth and long-tailed mealy bug could be controlled by 0.5% O_2 and 5.3% CO_2 insecticidal controlled atmosphere (ICA). Leong and Ho (1995) studied the control efficacy of *Liposcelis bostrychophila* Badonnel and *L. entomophila* (End.) (Psocoptera: Liposcelidiae) using 45% and 60% CO_2, respectively. Mitcham et al. (1997) examined effects of 0.25% O_2 and 40% CO_2 of ICA on the quality of 'Fuyu' persimmons. Downes et al. (2003) demonstrated methods of determining effects of low oxygen and high CO_2 on green peach aphids (*Myzus pericase* L.) and codling moths (*Cydia pomonella* L.). Van Epenhuijsen (2002) also investigated effects of various CO_2 concentrations from 0% to 95% with 5% O_2 on the control efficacy of green peach aphids. Although many studies on non-chemical disinfestations of horticultural crop insects were reported, little information is available on control of the oriental tobacco budworm in green hot peppers by ultra high CO_2-treated conditions or the effects of gas exposure on the storage characteristics of green hot peppers. Meanwhile, preliminary experiments have shown that the vacuum package of green hot peppers rendered the fruit easily broken (data not shown). Objectives of this study were to (i) investigate effects of ultra high CO_2-treated conditions on insect disinfestations in green hot peppers and (ii) determine the effect of ultra high CO_2 on external and internal postharvest quality of green hot pepper fruit.

MATERIALS AND METHODS

Larvae Disinfestations

Plant materials. Green hot peppers (cv. Nokgwang) were collected from greenhouses at Gongju, Korea. Fruit with punctures (ca. 2 mm in diameter) that were probably due to insect penetration on the fruit surface were preferentially selected for mortality assessment at the green mature stage. Gas treatments were initiated the following day.

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Gas treatments

Green hot peppers were placed in two types of containers (50 fruit per container): (i) polyethylene (PE) film bags (0.08 mm in thickness) and (ii) acrylic boxes (ca. 2.0 L in volume). Fruit in PE film bags and acrylic boxes was subjected to four regimes of gas treatments: (i) atmospheric air (control), (ii) 80% CO_2 with air, (iii) 100% CO_2, and (iv) 100% N_2, and followed by complete sealing. Gas-exposed fruit were kept in air at room temperature (18 to 20°C) for 24 h and 48 h, respectively. Treated fruit was then opened along the longitudinal axis, and larvae in the fruit were transferred to a Petri dish containing a few pieces of fresh green hot peppers to check whether larvae could resume growth under ambient conditions after one day. Larvae that had not shown movement by visual assessment were considered to be dead. Mortality of the larvae was determined by counting the number of dead larvae out of the total larvae from the fruit. The experiment was conducted as a completely randomized design with three replications per treatment.

Analysis of Postharvest Quality

To evaluate plausible deleterious effects of an ultra high CO_2-CA on green hot peppers, Fruit of uniform size at full maturity were harvested for analysis of postharvest quality in Gongju, Korea. Fruit in PE film bags (0.08 mm in thickness) were subjected to three of the same types of gas treatments as infected green hot peppers, since the PE film package method was more practicable than the acrylic box package. The treatments were (i) atmospheric air (control), (ii) 80% CO_2 with air, and (iii) 100% CO_2 at room temperature for two days. Fruit were then placed in a storage chamber at 10°C (low temperature) for up to 20 days, and at room temperature for up to 12 days. In a few cases, severely deteriorated Fruit were exempt from periodic monitoring. The experiment was conducted as a completely randomized design with three replications per treatment and 15 fruit per replicate.

Fruit firmness

Firmness, an external fruit characteristic, was determined using a texture analyzer (TA-XT2; Stable Micro System, Haslemere, UK). Each fruit was punctured to 10 mm in depth using a probe (5 mm in diameter) at a rate of 5.0 $mm{\cdot}s^{-1}$.

Fruit deterioration

As deterioration indexes, symptoms of seed browning and calyx softening were monitored to evaluate quality deterioration. Fruit was visually assayed according to its appearance. Deterioration symptoms included rot showing, watery flesh softening, seed browning, and calyx softening on the fruit. The deterioration index was recorded by counting fruit showing at least one symptom from 15 fruit in each replicate.

Electrolyte leakage

Fruit were washed in distilled water and kept dry. Twenty disks (10 mm in diameter) of fruit were immersed in 30 mL of 0.4 M mannitol solution. The first electrolyte leakage was measured using an electrical conductivity meter (H8633; Hanna Instrument, Seoul, Korea). After freezing at -20°C for 12 h and thawing at room temperature, the second electrolyte leakage was measured to calculate the final electrolyte leakage as previously reported (Bae et al., 2003).

L-Ascorbic acid (vitamin C)

Ten grams of fresh fruit were homogenized with 25 mL of 6% metaphosphoric acid and centrifuged at 15,000 rpm at 4°C for 20 min. The supernatant was filtered through Whatman filter paper (No. 2) and a 0.45-μm Millipore filter, and then purified using a Sep-Pak C18 cartridge (Waters, Milford, MA). Chemicals were analyzed using a liquid chromatograph (P2000; Spectra-Physics, San Jose, CA) and a YMC-Pack Polyamine II column (250 mm×4.6 mm, YMC, Kyoto, Japan). The mobile phase was acetonitrile:$NH_4H_2PO_4$ (70:30, v/v), flow rate was 1.0 $mL{\cdot}min^{-1}$, and vitamin C was detected at 254 nm (Kim et al., 2006).

Capsaicinoids

Five grams of fresh fruit were homogenized with 20 mL of acetone and stirred at room temperature for 5 h. The mixture was filtered and the filtrate was added to 4 folds of methanol. Capsaicinoids including capsaicin and dihydrocapsaicin were quantified with liquid chromatography as described above. The mobile phase was methanol:1% acetic acid in water (70:30, v/v), and flow rate was 1.5 mL min^{-1}. Capsaicinoids were detected at 280 nm (Lee et al., 2006).

Respiration rate

Gas-treated green hot peppers were placed in a sealed chamber (ca. 1.0 L in volume) at room temperature. Air samples were collected after 1 h, and CO_2 production was analyzed using a gas chromatograph (Varian 3400, Palo Alto, CA) equipped with a thermal conductivity detector and a column (2.4 mm×2 m) packed with 60 to 80 mesh active carbon (Alltech, Deerfield, IL). Flow rate of the carrier gas (He) was 30 $mL{\cdot}min^{-1}$. Injector temperature was set at 110 C, column temperature at 70°C, and detector temperature at 150°C.

RESULTS AND DISCUSSION

Larvae Disinfestations

This study demonstrated the effectiveness of ultra high CO_2 treatment for disinfestations of *H. assulta* in green hot peppers. No larvae in control fruit were dead in the 0.08 mm PE film bags after 24 h, whereas mean mortality reached approximately 35% after 48 h (Fig. 1A). Both 80% and 100% CO_2 treatments caused a significant increase in mortality (ca. 70%) after 24 h, leading to complete disinfestations after 48 h. N_2 gas treatment was also effective at arresting larvae survival, but did not show complete disinfestations even after a 48 h exposure. In addition, significant increases in mortality were evident in CO_2-treated fruit in the acrylic boxes (Fig. 1B). Prolonged CO_2 treatments at the two concentrations up to 48 h completely disinfested the larvae. However, N_2 gas treatment showed lower insecticidal activity than all types of CO_2 treatments. Under laboratory conditions in this study, larvae of *H. assulta* could not

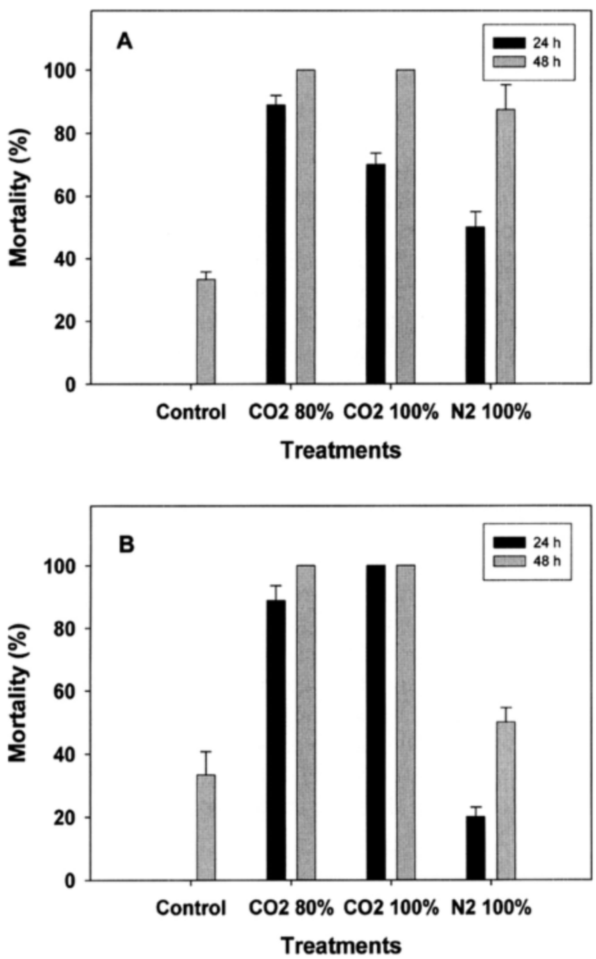

Figure 1. Mortality of *H. assulta* by gas treatments. **A.** Polyethylene film (0.08 mm in thickness) bags. **B.** Acrylic boxes. Bars in the columns indicate standard deviations.

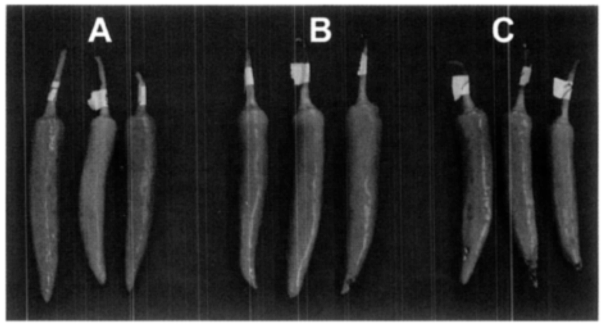

Figure 2. Green hot pepper fruit after 15 days of storage at 10°C under different CO_2 treatments. **A.** Control. **B.** 80% CO_2. **C.** 100% CO_2.

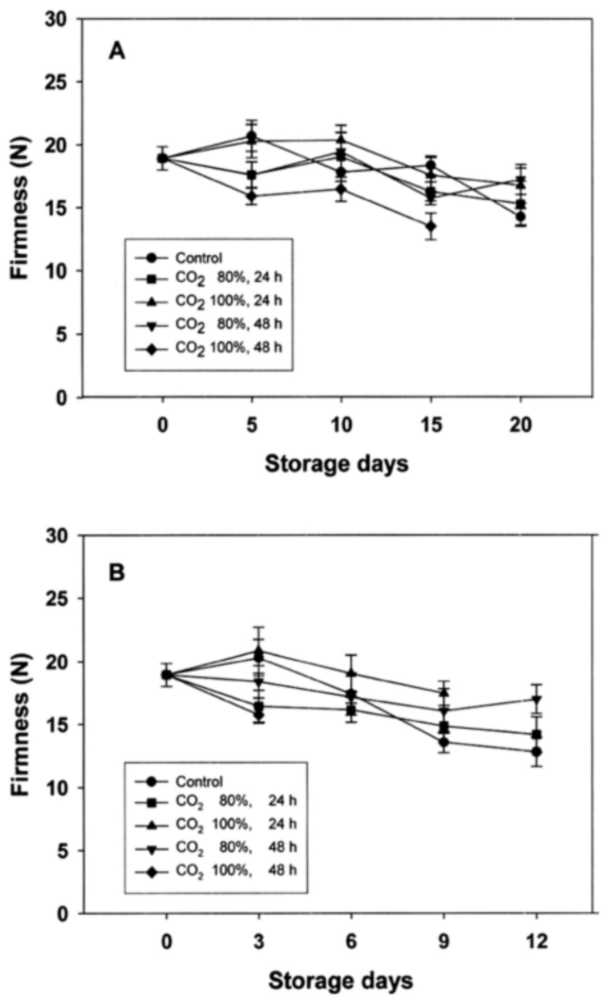

Figure 3. Temporal progress of fruit firmness of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

survive the high concentration CO_2 atmosphere. Mechanisms underlying budworm mortality are known to be associated with water loss in insects. Carbon dioxide acts directly on the insect's spiracle muscle to cause immediate opening (Alonso et al., 2005). Sustained opening of the spiracle can induce uncontrolled water loss, leading to dehydration and mortality (Lehmann et al., 2000).

Analysis of Postharvest Quality

In this study, temporal changes of comprehensive postharvest quality of green hot pepper fruit were monitored during storage under different concentrations of CO_2-treated conditions. Despite apparent insecticidal activity of elevated CO_2 conditions on the larvae of *H. assulta*, these CO_2 treatments did not produce significant detrimental effects on external postharvest quality of the green hot pepper fruit (Fig. 2). Control fruit appeared to be normal after 15 days of storage at 10C. Instances were noted where some tip blight or discoloration occurred on pepper fruit under 80% or 100% CO_2 treatments. Firmness, an external characteristic of CO_2-exposed fruit, decreased as the storage period progressed at 10°C (Fig. 3A). Starting at approximately 18 N, firmness decreased to approximately 15 N after 20 days of storage. Due to degeneration of the fruit from the 100% CO_2 treatment, firmness could not be determined in the later stages of storage. Greater firmness decreases were observed in fruit stored at room temperature (Fig. 3B). Storage temperature appeared to have significant effects on firmness of the control fruit, showing a relatively steeper decrease at room temperature than at 10°C. The critical temperature for chilling injury was known to range

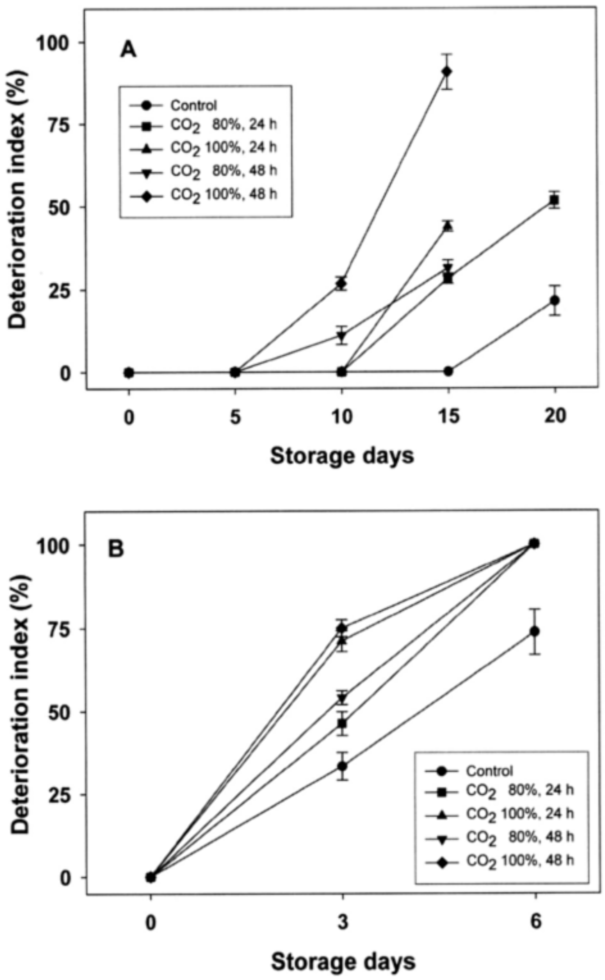

Figure 4. Temporal progress of fruit deterioration of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

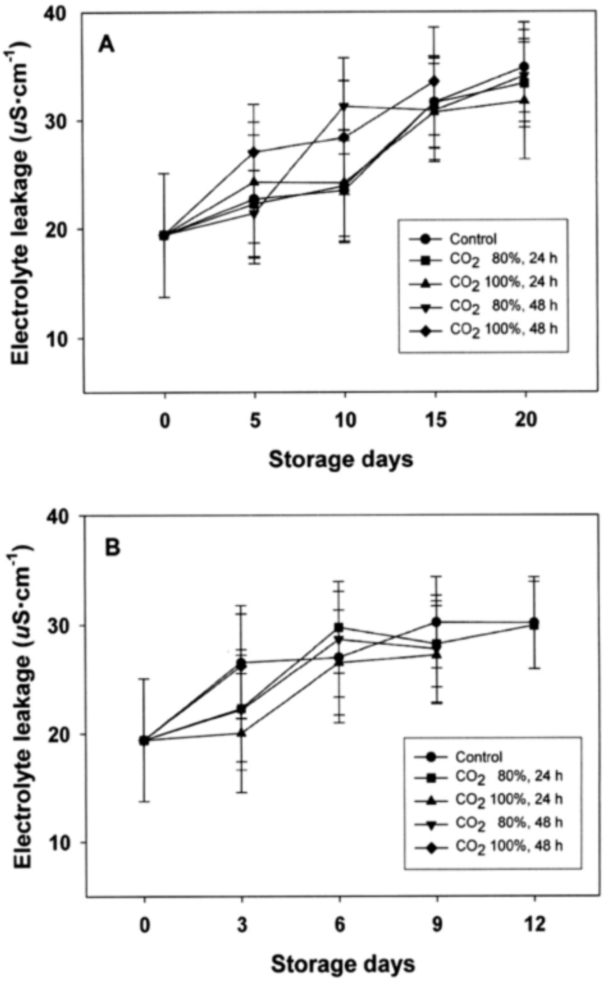

Figure 5. Temporal progress of electrolyte leakage of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

from 0 to 10°C in green hot peppers (Kader, 1992), so the 10°C in this study can be considered a very low temperature for storage of green hot peppers. Meanwhile, chilling injury could be minimized by CA storage with 3% CO_2 (Yang and Lee, 1997) or modified atmosphere packing (Ding et al., 2002). Thus, it was assumed that there were no significant differences between firmness of the control or CO_2-exposed fruit. The mechanical properties of fruit are a function of cell wall strength, cell-to-cell adhesion, cell packing, and the internal pressure or turgor of cells (Harker et al., 2000 and references therein). In strawberry fruit, the mechanism for CO_2-induced firmness enhancement was due to changes in the pH of the apoplast. Unraveling of the exact mechanisms of decreased fruit firmness in green hot pepper by ultra high CO_2-CA awaits further studies.

Another external characteristic of CO_2-exposed fruit monitored in this study was fruit deterioration. Seed browning and calyx softening appeared during the shelf period as storage disorders. CO_2-exposed fruit seemed to have more severe deterioration than control fruit stored at 10°C (Fig. 4A). Up to 10 days of storage, no remarkable differences in deterioration were found among 100% CO_2-treated fruit and control fruit stored at 10°C. Fruit deterioration was more pronounced at room temperature (Fig. 4B), thus it was hard to determine fruit deterioration during later stages of storage. In 'Fuji' apple fruit, internal browning and softening occurred due to high CO_2 content (Park et al., 1997). The general pattern of electrolyte leakage from pepper fruit stored at 10°C was an increase in electrolyte leakage from each type of fruit after storage (Fig. 5A). No striking differences were detected among 100% CO_2-treated fruit and control fruit. A similar pattern of electrolyte leakage increase was also observed in fruit stored at room temperature (Fig. 5B). Electrolyte leakage reflects a decrease in membrane integrity (Thompson, 1988). Since lower storage temperatures slow down the metabolic activity of green hot peppers and preserve membrane integrity, storage at 10°C could contribute to better retention of membrane integrity and less electrolyte leakage than at room temperature.

Temporal analysis of vitamin C content showed a gradual decrease in the control fruit stored at 10°C (Fig. 6A) and

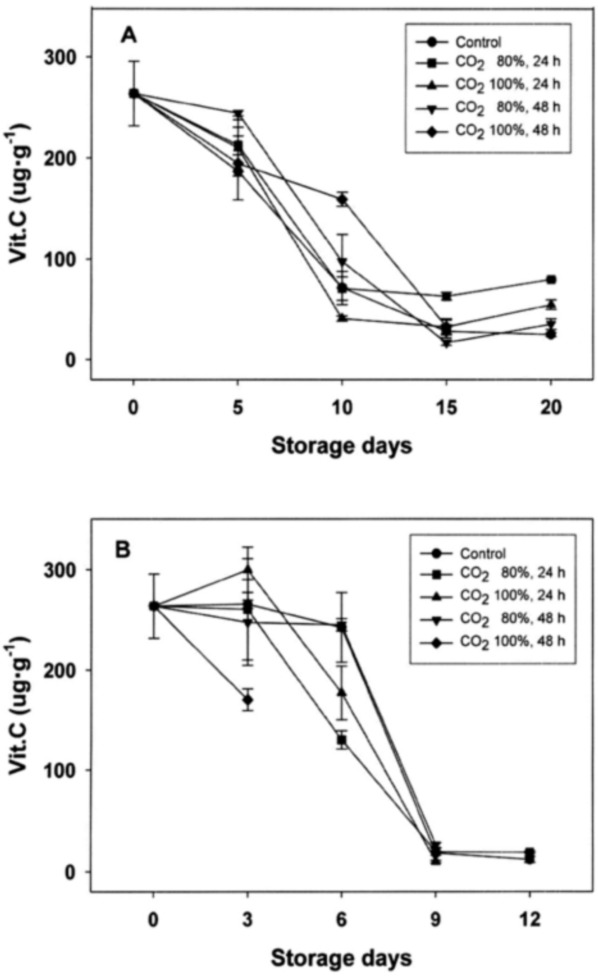

Figure 6. Temporal progress of vitamin C of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

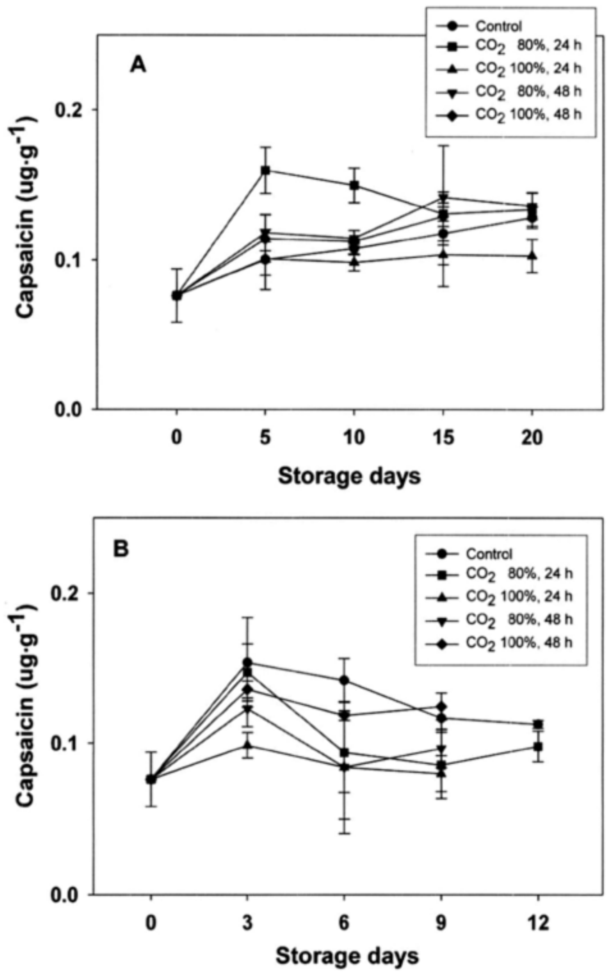

Figure 7. Temporal progress of capsaicin of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

room temperature (Fig. 6B). CO_2-exposed fruit also showed a similar pattern of decrease, although several variations occurred during storage. Capsaicin content was a little higher in CO_2-exposed green hot peppers than in control peppers at 10 C (Fig. 7A), but different results were found at room temperature storage, showing that the capsaicin content was a little lower in CO_2-exposed green hot peppers than in control (Fig. 7B).

Meanwhile, respiration rates of the CO_2-exposed fruit were approximately half the rates of the control after 20 days of storage at 10°C (Fig. 8A) and room temperature (Fig. 8B). Respiration rates of green bell peppers increased after harvest at room temperature storage; however, the value decreased under certain storage conditions (Mikal and Saltveit, 1977). It is widely accepted that CA storage inhibits respiration of horticultural fruit, resulting in prolonged postharvest quality (Kader, 1986).

In conclusion, the overall results indicated that ultra high CO_2-treated conditions could disinfest the oriental tobacco budworm without significant differences in postharvest fruit quality of green hot peppers, compared with control fruit. Ultra high CO_2-treated regimes could be further tested for evaluation of their possible implementation as shipment or in-transit disinfestation treatments for green hot pepper quarantines. It is reasonable to conclude that ultra high CO_2-CA is effective for disinfestations of *H. assulta* while maintaining reasonable deterioration of postharvest quality of green hot peppers. Considered that both 80% and 100% CO_2 treatments caused a significant increase in mortality after 24 h, fruit exposure to 80% CO_2 for 24 h would be recommended as a reliable means of control that is harmless to humans, and can alleviate concern regarding pesticide residues. Such a strategy of employing 80% CO_2 treatment will lower the possibility for deterioration of postharvest fruit quality, concomitantly getting around the difficulty of maintaining 100% CO_2 concentration in commonly used PE film bags. The control efficacy of CO_2 exposure on diverse pepper species and on other horticultural crops needs to be validated at a large scale to confirm its broad-spectrum characteristics and establish optimal dose in further studies.

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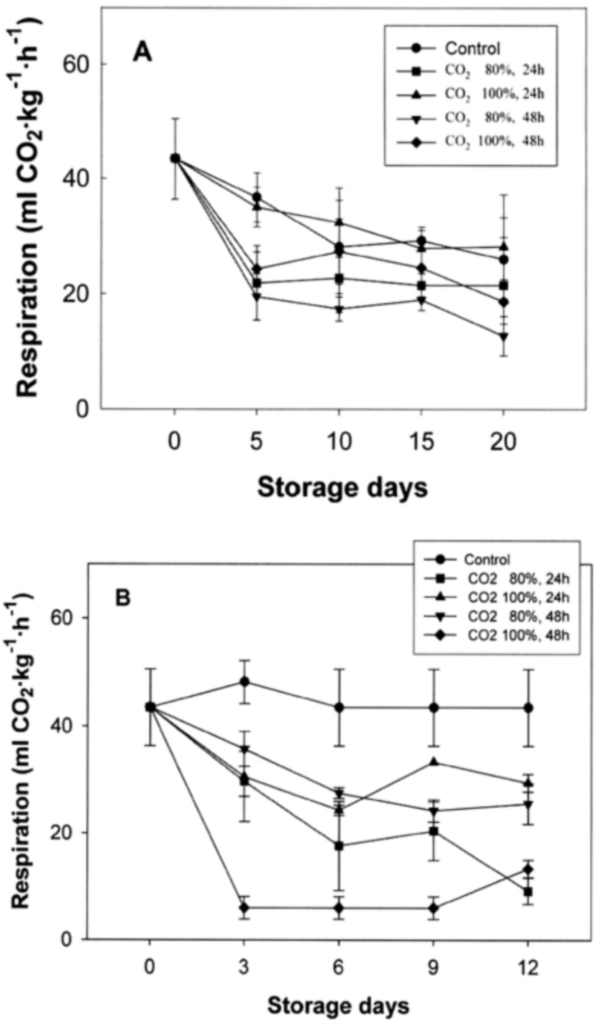

Figure 8. Temporal progress of respiration rates of green hot peppers according to CO_2 treatments and temperatures during storage. **A.** Low temperature (10°C). **B.** Room temperature.

LITERATURE CITED

Alonso M, Del Rio MA, Jacas, JA (2005) Carbon dioxide diminishes cold tolerance of third instar larvae of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in 'Fortune' mandarins: implications for citrus quarantine treatments. Postharv Biol Tech 36: 103-111

Bae RN, Choi JH, Mok IJ, Jung DS (2003) Effects of perforated PE film packaging and storage temperature on quality of red pepper (*Capsicum annuum*). J Kor Soc Hor Sci 44: 49-51

Choi MY, Tatsuki S, Boo KS (1998) Regulation of sex pheromone biosynthesis in the oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: Noctuidae). J Insect Physiol 44: 653-658

Denterner PR, Peetz SM, Britles DB (1992) Modified atmospheres for the postharvest disinfectation of New Zealand persimmons. NZ J Crop Hortic Sci 20: 203-208

Ding CK, Chachin K, Ueda Y, Imahori Y, Wang CY (2002) Modified atmosphere packaging maintains postharvest quality of loquat fruit. Postharv Biol Tech 24: 341-348

Downes CJ, Carpenter A, Hansen LD, Lill RE (2003) Microcalorimetric and mass spectrometric methods for determining the effects of controlled atmospheres on insect metabolism. Thermoch Acta 397: 19-29

Fields PG, White NDG (2002) Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu Rev Entomol 47: 259-331

Harker FR, Elgar HJ, Watkins CB, Jackson PJ, Hallett IC (2000) Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharv Biol Tech 19: 139-146

Kader AA (1986) Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Tech 40: 99-104

Kader AA (1992) Postharvest biology and technology: An overview, *In* A Kader, ed, Postharvest Technology of Horticultural Crops, University of California, Oakland, California, pp 15-20

Kim S, Kim KS, Park JB (2006) Changes of various chemical components by the difference of the degree of ripening and harvesting factors in two single-harvested peppers (*Capsicum annuum* L.). Kor J Food Sci Tech 38: 615-620

Lee SY, Sim SY, Seo MW, Lee SW, Lim JW, Lee YB (2006) Changes in fruit characteristics after brining of pickling pepper (*Capsicum annuum* L.) cultivars. Kor J Hort Sci Tech 24: 168-176

Lehmann FO, Dickinson MH, Staunton J (2000) The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophilia spp.*) J Exp Biol 203: 1613-1624

Leong ECW, Ho SH (1995) Effects of carbon dioxide on the control efficacy of *Liposcelis bostrychoplia* Bad. and *Liposcelis entomophila* (End.) (Poscoptera: Liposcelididae). J Stored Prod Res 3: 185-190

Mikal E, Saltveit Jr (1977) Carbon dioxide, ethylene, and color development in ripening mature green bell peppers. J Amer Soc Hort Sci 102: 523-525

Mitcham EJ, Attia MM, Biasi W (1997) Tolerance of 'Fuyu' persimmons to low oxygen and high carbon dioxide atmospheres for insect disinfestation. Postharv Biol Tech 10: 155-160

Park YM, Kweon HJ, Kim HY, Ryu OH (1997) Preharvest factors affecting the incidence of physiological disorders during CA storage of 'Fuji' apples. J Kor Soc Hort Sci 38: 725-729

Thompson JE (1988) The molecular basis for membrane deterioration during senescence, *In* L Nooden, A Leopold, eds, Senescence and Aging in Plants, Academic Press, San Diego, pp 51-83

Van Epenhuijsen CW, Carpenter A, Butler R (2002) Controlled atmospheres for the post-harvest control of *Muzus persicae* (Sulzer) (Homoptera: Aphididae): effects of carbon dioxide concentration. J Stored Prod Res 38: 281-291

Yang YJ, Lee KA (1997) Physiological characteristics of chilling injury and CA effect on its reduction during cold storage of pepper fruit. J Kor Soc Hort Sci 38: 478-482