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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**High CO₂ Prolongs Postharvest Life of
Strawberry Fruit by Reducing Decay
and Cell Wall Degradation**

고농도 이산화탄소 처리에 의한 수확 후
딸기 부패 및 세포벽 분해 억제

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High CO₂ Prolongs Postharvest Life of Strawberry Fruit by Reducing Decay and Cell Wall Degradation

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ABSTRACT

Extending postharvest life of fruit, particularly strawberry, is a challenge due to rapid loss of firmness and freshness during ripening. To overcome this problem, short-term high CO₂ treatment has been widely used to maintain firmness and reduce decay. Previous studies of high CO₂ treatment have mostly been related to the issue of proper treatment concentration and time. However, the mechanism of the high CO₂ treatment to maintain fruit firmness is still unclear. Therefore, the objective is to understand postharvest physiological mechanism by high CO₂ in strawberry fruit after harvest. In this study, harvested strawberry fruit was stored at 10°C after 3 h of treatment with 30% CO₂ or air. The effect on the cell wall and lipid metabolism was examined through pectin analysis and cell wall degradation-related gene expression. The high CO₂ treatment maintained higher pectin content

and firmness and lower decay compared to the control. Cell wall degradation-related gene expression was lower with high CO₂ treatment. High CO₂ induced oligogalacturonides and thereby provided the defence mechanism against *Botrytis cinerea* in strawberry fruit. High CO₂ lowered decay incidence after 7 days after inoculation of *Botrytis cinerea*. The high CO₂ treatment is expected to maintain the quality by reducing decay and cell wall degradation.

Keywords: *Botrytis cinerea*, Cell wall degradation, High CO₂, Oligogalacturonides, Pectin.

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LIST OF ABBREVIATIONS

CO ₂	Carbon dioxide
CTAB	Cetyl trimethylammonium bromide
DP	Degree of polymerization
EG	Endo- β -1, 4-glucanase
EXP	Expansin
GalA	Galacturonic acid
LOX	Lipoxygenase
OGA	Oligogalacturonides
PE	Pectin methylesterase
PG	Polygalacturonase
PL	Pectate lyase
qRT-PCR	Quantitative real-time polymerase chain reaction
XTH	Xyloglucan endotransglucosylase/hydrolase

INTRODUCTION

Carbon dioxide (CO₂) plays an important role in agriculture. During cultivation, CO₂ is used in CO₂ enrichment or plant factory system for better yield and quality. Because of acting as a substrate of photosynthesis, CO₂ is an indispensable element in agriculture. During cultivation as well as postharvest process, CO₂ is used in controlled atmosphere storage or short-term high CO₂ treatment to maintain the freshness and shelf-life of fruits. However, the mechanism of CO₂ to maintain fruit freshness in postharvest process is still unclear.

Strawberries are representative fruit in short-term high CO₂ treatment. Because strawberry fruits are easily perishable, their fast softening changes the texture and reduces their postharvest life and shelf-life during ripening (Perkins-Veazie, 1995). To solve this problem, high CO₂ treatment is used as postharvest technology. In addition to the strawberry fruit, various fruits have a potential to perform high CO₂ treatment.

Increasing firmness and reducing decay are key effects of high CO₂ treatment. Plant cell walls are systems that are physically and chemically associated with firmness. In ripening progresses, plant cell wall is degraded during softening. Pectin is a major component of plant cell wall. Disassembly of pectin is largely responsible for the softening and textural changes (Brummell and Harpster, 2001). Through the loss of pectic side chains in ripening stage, increased plant cell wall

porosity enhances cell wall degradation enzymes activity (Brummell, 2006).

High CO₂ treatment can be hypothesized to increase firmness by reducing cell wall degradation process. Suppression of fruit decay can be also hypothesized with oligogalacturonides (OGA). OGA are pectin breakdown products. Specific OGA with a degree of polymerization (DP) between 10 and 15 DP activates plant immune responses by reducing decay. So far, there is no direct correlation between OGA and high CO₂ treatment.

In this study, fruit qualities and cell wall degradation gene expressions were examined after treatment of high CO₂ of 30% for 3 h in strawberry. Through the polyuronide assay, cell wall degradation was estimated in strawberry fruit. Specific OGA produced by high CO₂ treatment were also examined. A model for the mechanism of high CO₂ treatment was presented in the study.

LITERATURE REVIEW

High CO₂ treatment

High CO₂ treatment has been widely applied to reduce postharvest losses in horticultural fruits. High CO₂ treatment in the postharvest was effective for reducing respiration rate and fruit decay and for increasing firmness (Bai et al., 2001; Kerbel et al., 1988; Wang et al., 2014). In strawberry fruit, short-term high CO₂ treatment for about 3 h increased fruit firmness with CO₂ concentrations ranging from 10 to 100 kPa. Higher CO₂ concentrations are usually more effective (Harker et al., 2000), but can cause physiological injuries such as fruit discoloration and off-flavors (Watkins et al., 1999).

Short-term high CO₂ treatment induced a change of pectin methylesterase (PE) activity, resulting in the modification of pectins. However, specific tendency of PE activity after high CO₂ treatment has been controversial (Goto et al., 1995; Hwang et al., 2012). Increase of firmness in strawberry fruit involves the modification of pectic polymer including a decrease of water-soluble pectins (Goto et al., 1995; Matsumoto et al., 2010). Specific increase in fruit firmness after high CO₂ treatment was maintained for several days (Matsumoto et al., 2010), by suggesting reducing cell wall degradation in fruit with high CO₂ treatment.

Plant cell wall

The primary walls of plant cells are major components of dietary fiber. (Englyst et al., 2007). Structures, functions, and properties of cell wall show wide and complex biological variation (Knox, 2008). Secondary cell walls are minor components of dietary fiber.

Celluloses, hemicelluloses, and pectins are the main components of the plant primary cell walls that contribute fruit texture (Daas et al., 1999). Celluloses are unbranched, β -1, 4-linked-linked-glucan chains of cellulose aggregate together by hydrogen bonding to form microfibrils each about 3 nm in diameter. Hemicelluloses have some degree of conformational resemblance to cellulose and are capable of hydrogen bonding to cellulose microfibrils, possibly in a similar manner to the aggregation of the cellulose chains themselves (Jarvis, 2009). The principal hemicelluloses of primary cell walls in most plants (Harris and Smith, 2006) are xyloglucans and glucomannans, but arabinoxylans and mixed-linkage β -glucans replace these in grasses, cereals (Cui and Wang, 2009) and a small number of other plants such as palms. Pectins are homopolymer of α -1, 4-linked-D-galacturonic acid (GalA) with various degrees of methyl esterification of the carboxyl groups (Carpita and Gibeaut, 1993). Change of fruit cell wall component is responsible for softening (Brummell and Harpster, 2001).

Cell wall degradation enzymes

Cell wall degradation by hydrolytic enzymes, including polygalacturonase (PG), PE, and pectate lyase (PL) are the reason for fruit softening (Figueroa et al.,

2008).

Expansin (EXP), endo- β -1, 4-glucanase (EG), xyloglucan endo-trans-glucosylase/hydrolase (XTH) are hemicellulase. Hemicellulase is a type of enzyme that degrades the plant cell wall polymer hemicellulose. Hemicellulase are a diverse group of enzymes that hydrolyze hemicelluloses, one of the most abundant groups of polysaccharide in nature (Shallom and shoham, 2003).

Oligogalacturonides

Oligomers of α -1, 4-linked-D-GalA (OGA) are pectin breakdown fragments (Nothnagel et al., 1983). OGA called endogenous elicitors were trigger defense response as damage-associated molecular patterns. However, not all OGA are endogenous elicitors. Specific OGA with a DP between 10 and 15 DP activates plant immune responses, such as accumulation of phytoalexins (Davis et al., 1986), deposition of callose, and production of reactive oxygen species (Galletti et al., 2008) and nitric oxide (Rasul et al., 2012).

MATERIALS AND METHODS

Plant materials

Strawberry (*Fragaria × ananassa* Duch. ‘Seolhyang’) fruits were harvested at the ripe stage (about 80% of red colored) and sorted according to uniform size and external skin color. Fruit with relatively uniform color and size were selected and used in this experiment. After high CO₂ treatment, the firmness of fruit was immediately examined and half of fruit was frozen in liquid nitrogen and stored at –80°C until RNA analysis.

Gas treatment and storage

About 250 fruits were placed in containers at 20°C. Carbon dioxide (30% CO₂ + air) or air (the control) was provided at 10 L/min for 1 min to replace the container atmosphere and was then adjusted to 1 L/min for 3 h. The gas concentrations were determined by using a gas analyzer (YL6500, Younglin, Anyang, Korea) every 30 min. After treatment, each lot was randomly divided into 15 groups (approximately 1 kg per each group) and placed in a plastic tray without cover. They were stored at 10°C for with ambient air of relative humidity of 90 ± 5%, which not controlled. Three groups per each treatment were used for quality evaluation and gene expression assay.

Firmness measurement

Fruit firmness of randomly selected 15 fruits per each replicate was measured using a texture analyzer (CT3 Texture Analyzer, Brookfield, MA, USA) fitted with a 5 mm flat probe. Each fruit was compressed by 5 mm probe at a rate of 1 mm/s, and the maximum force in Newtons (N) developed during the test was recorded.

Respiration rate measurement

About 200 g of fruits from each replicate were placed in 1-L sealed jars with a septum in the lid for sampling headspace gas. Before closing the lids, fruits were allowed to accommodation at room temperature for 1 h then the jars were closed and kept for 3 h, to allow gases to accumulate in the headspace. A 1-mL sample of headspace gas was removed from each jar using a gas tight syringe. CO₂ concentration was measured with gas chromatography system (YL6500, YoungLin) equipped with a thermal conductivity detector and porapak column (1.5 m × 6 mm) for CO₂. Oven, injector, and detector temperatures were set at 70, 110, and 150°C, respectively.

Color measurement

Chromaticity was measured from strawberry peel from 30 randomly selected fruits. The parameters CIELab: L*, a*, and b*, were measured with a CM-2500c

spectrophotometer (Minolta, Osaka, Japan). The parameter L^* indicates brightness or lightness (0 = black, 100 = white), a^* indicates chromaticity on a green (–) to red (+) axis, and b^* indicates chromaticity on a blue (–) to yellow axis (+). Numerical values of a^* and b^* were converted into hue angle ($H^\circ = \tan^{-1} (b^*/a^*)$) and chroma ($C = (a^{*2} + b^{*2})^{1/2}$). The hue is an angle in a color wheel of 360° , with 0° , 90° , 180° and 270° representing the hues red-purple, yellow, bluish-green and blue respectively, while chroma is the intensity or purity of the hue (McGuire, 1992).

RNA extraction

Total RNA was isolated following the method described by Gambino et al. (2008). Cetyl-trimethylammonium-bromide (CTAB) extraction buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2.5% polyvinylpyrrolidone, and 2% of β -mercaptoethanol, at pH 8.0) were pre-heated at 65°C . Three grams in half fruit tissue was ground to a fine powder. The tissue was chilled with liquid nitrogen during the grinding process. The powdered sample was then transferred to 50 mL polypropylene tube containing 15 mL of CTAB extraction buffer. The tube was vortexed and then incubated at 65°C for 10 min. Afterward, 15 mL of chloroform:isoamyl alcohol (24:1, v/v) was added and the tube was vortexed and centrifuged at $11,000 \times g$ for 10 min at 4°C . The supernatant was transferred to new 50 mL tube and LiCl (3 M at final concentration) was added. The mixture was

incubated at 4°C for 30 min and then centrifuged at $21,000 \times g$ for 20 min at 4°C. The pellet was resuspended with 0.5 mL of SSTE buffer (1 M NaCl, 0.5% SDS, 10 mM Tris-HCl, 1 mM EDTA, at pH 8.0) pre-heated at 65°C and transferred to microcentrifuge tube. Chloroform:isoamyl alcohol (24:1, v/v) of 0.5 mL was added and the tube was vortexed and centrifuged at $11,000 \times g$ at 4°C for 10 min. The supernatant was transferred to new microcentrifuge tube and 0.7 mL of cold isopropanol was added and centrifuged at $21,000 \times g$ at 4°C for 15 min. The pellet was mixed with 50 μ L of 70% ethanol and centrifuged at $21,000 \times g$ at 4°C for 5 min. Total RNA was briefly dried and resuspended with 100 μ L of diethylpyrocarbonate treated-water.

cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

For qRT-PCR analysis, the first strand cDNA was synthesized using the amfiRivert Platinum cDNA Synthesis Master Mix (GenDEPOT, Katy, TX, USA) from 5 μ g total RNA. qRT-PCR was performed using candidate primers including eight genes (Table 1) with a template of an 1:10 diluted solution of first strand cDNA product. Candidate primers sequences obtained in GenBank of the National Center for Biotechnology Information.

qRT-PCR was carried out in 10 μ L reaction volume equipped with CFX Connet™ (Bio-Rad, Hercules, CA, USA). The PCR reaction mix contained 2 μ L

Table 1. Candidate primers of housekeeping gene and cell wall degradation-related genes of ‘Seolhyang’ strawberry fruit.

Gene	Acession No.	Sequence	Size (bp)
18s	X15590	F: GTGCTCAAAGCAAGCCTACG R: ATCTGATCGTCTTCGAGCCC	241
PE	AY324809	F: GAAACCTGTGGTTGCCCTCT R: TTTCAAACAACACCAATGCG	256
PG	EF441274	F: GCAACAACGCCGAGTAGAGT R: TGCAGTCGTTGTCTTTCCAAG	247
PL	U63550	F: ATGCTGTTGGGGCATAGTGA R: TTCGCAAAACGGTTGTTAGG	236
LOX	AJ578035	F: CTGATGTGGCCTTCCTCAAA R: TGAGGTGCTGGGATAGAGCA	284
EG	AJ006348	F: AACTTCGCTATCGTTCCTGCTT R: GGGTTTGGATTTCGGA CT CAG	297
EXP	AF159563	F: AGAGGAGGGGGATCGAGATT R: AGAACAAGCAACTGCCCCA	271
XTH	GQ367550	F: CATCCCAATCCGAGTGTTCA R: CTCCTTCTGATCCCACCACC	250

F, forward; R, reverse.

of cDNA, 4 pM each primer, and 5 μ L of 2 \times LabopassTM SYBR Green Q Master (Cosmogenetech, Seoul, Korea). The thermal cycling parameters were 95°C for 30 s; 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 20 s; 95°C for 10 s and a melting curve of 65°C to 95°C, increment of 0.5°C for 10 s. Each reaction was performed in triplicate, and no-template-controls for each primer pair were included in each run. Relative intensity of 1 day control sample was set to 1.

Cell wall extraction

Each tissue was immediately frozen in liquid nitrogen and stored at -80°C until analysis. The frozen tissue was freeze-dried. Three grams of dried fruit powder was dissolved in 100 mL of 80% (v/v) ethanol and homogenized for 1 min. The homogenate was shaken at 250 rpm for 1 h and centrifuge at 11,000 rpm at 10°C for 30 min. Supernatant was discarded and 30 mL water was added to the residue and transferred to centrifuge tube. Mixture was shaken at 250 rpm for 1 h and centrifuge at 11,000 rpm at 10°C for 30 min. Supernatant was transferred to new Falcon tube with filtration through one layer of Miracloth and then 30 mL of water was added to the residue. Mixture was shaken at 250 rpm for 1 h and centrifuge at 11,000 rpm for 30 min at 10°C . Transfer supernatant to same Falcon tube with filtration by Miracloth and mix. Supernatant in the tube was used for size-exclusion chromatography.

The residue washed sequentially with 100 mL of chloroform:methanol (1:1,

v/v), stirred for 20 min, and filtered through a sintered glass filter. The crude cell wall material was extracted twice in 100 mL of acetone, stirred slowly for 10 min, and filtered through a sintered glass filter. The pellet was placed in a vacuum oven and dried at 35 – 40°C in the presence of P₂O₅ for 3 days. The crude cell wall material was extracted in 100 mL of 20 mM HEPES-NaOH (pH 7.0) containing 1 unit/mL of α -amylase and incubated at 37°C for 12 – 18 h. The sample was filtered and sequentially washed with 200 mL of 20 mM HEPES-NaOH (pH 7.0), 200 mL of distilled water, and 200 mL of acetone. Finally, the pellet was dried in a vacuum oven as described above.

Size-exclusion chromatography

Supernatant in falcon tube was immediately frozen in liquid nitrogen and stored at –80°C until analysis. The frozen tissue was freeze-dried. Dissolve dried sample in 10 mL of 0.2 M ammonium acetate (pH 5.0) and mix. The mixture of 1.5 mL was transferred to microfuge tube and centrifuge at 3,000 rpm for 5 min. One mL of supernatant was passed through a Bio-Gel P-4 column (25 cm length \times 2 cm I.D, Bio-Rad) and 1.1 mL fractions were collected. Size-exclusion chromatography was determined according to the method of An et al. (2005).

Polyuronides assay

Sample (0.4 mL) was added to 40 μ L of 4 M potassium sulfamate/sulfamic

acid solution, pH 1.6, and mixed with 2.4 mL of sulfuric acid containing 75 mM Na-tetraborate (Borax). The sample mixture was heated at 100°C for 20 min. After cooling at room temperature, 80 µL of 0.15% (v/v) m-phenyphenol in 0.5% NaOH was added and the solution was allowed stand in the dark for 30 min to develop the pink-red color and the absorbance was read at 525 nm. Polyuronide content was determined according to the method of Chatkaew and Kim (2013).

***Botrytis cinerea* inoculation**

Botrytis cinerea conidia collection and fruit inoculation were performed as previously described by Mengiste et al. (2003). Conidia were suspended in distilled water for fruit inoculation, and 50 µL of spore suspension (2×10^5 spores/mL) was injected into each fruit, which were kept under a 16 h light/8 h dark cycle for 3 days. The fruit were kept under a transparent cover to maintain high humidity.

Data analysis

The experiments were designed to be completely randomized. All experimental analyses were performed in triplicate, and means and standard deviations were calculated. The figures were generated using Sigma Plot 10.0 (SPSS, Chicago, IL, USA).

RESULTS

Firmness and fruit quality measurement

Firmness is a major index that strongly impacts on strawberry fruit quality. The firmness in strawberry after high CO₂ treatment was slightly increased (Fig. 1), whereas the firmness in control fruit decreased during storage. After 10 days treatment, the firmness was maintained at the level of 1 day after treatment.

For 10 days after treatment, the respiration rate was not associated with high CO₂ treatment and steadily increased in both of control and treatment fruit (Fig. 2). Although the control at 14 days was showed a higher respiration rate than high CO₂ treatment, but this different respiration rate between the control and treatment did not affect change of qualities because whole strawberries already decayed within 14 days.

Color indices (lightness, chroma, hue angle) were not significantly different between treatment and control fruits (Table 2). During the storage period, L* value showed a trend of decrease both treatment and control. High CO₂ had no effect on change of fruit peel color.

Cell wall degradation enzyme gene expressions

The expressions of cell wall degradation enzyme genes have been clearly

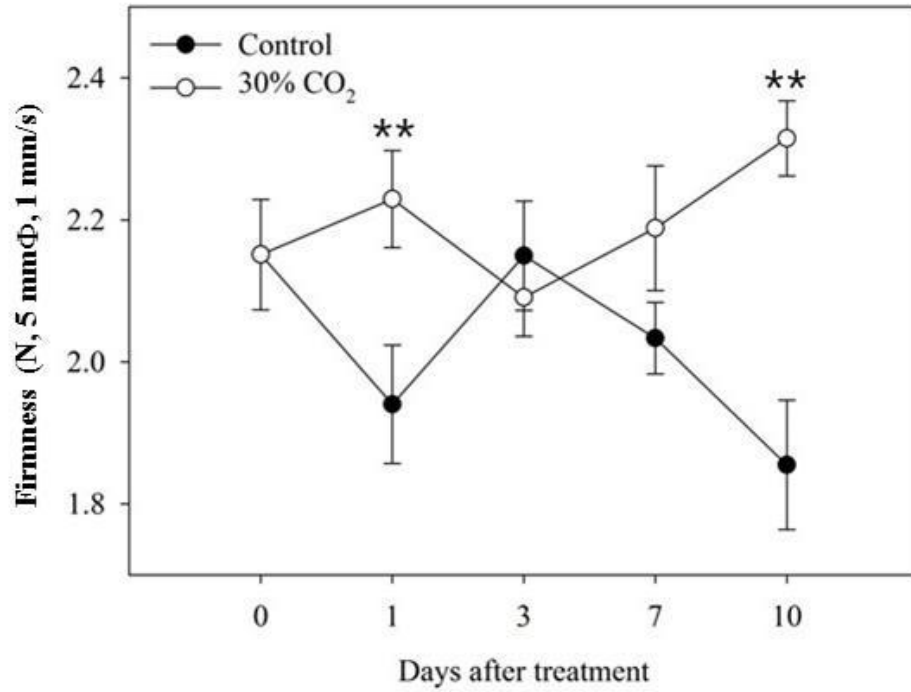


Fig. 1. Changes in fruit firmness of ‘Seolhyang’ strawberry treated with 30% CO₂ for 3 h. Data are the means with SE from 15 replications. **Significant at $P < 0.01$.

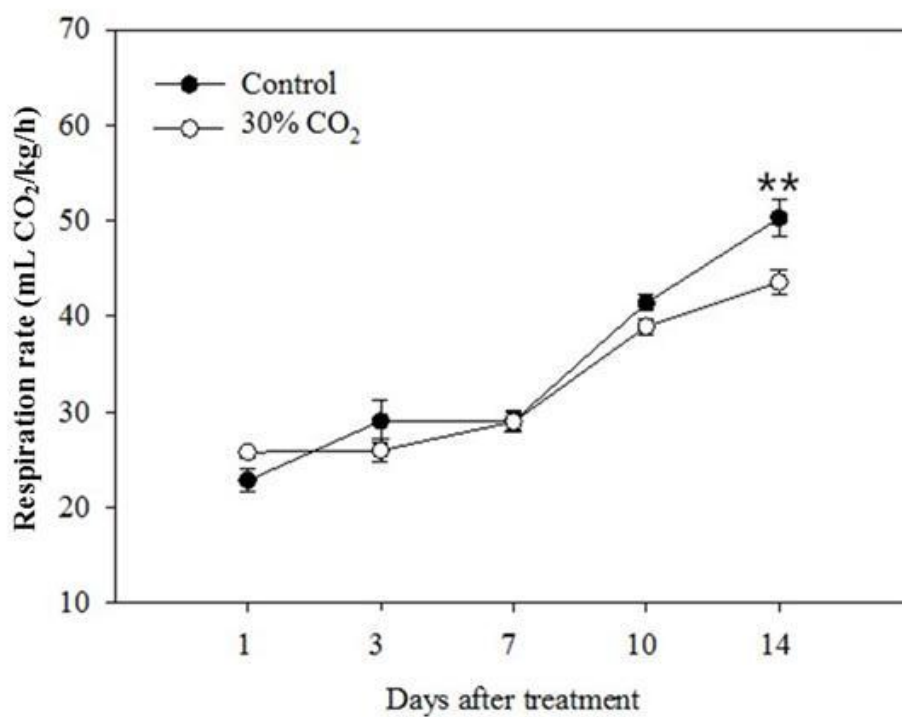


Fig. 2. Changes in respiration rate of 'Seolhyang' strawberry fruit treated with 30% CO₂ for 3 h. Data are the means with SE from 3 replications. **Significant at $P < 0.01$.

Table 2. Effects of 30% CO₂ treatments for 3 h on peel color (L*, a*, b*), chroma, and hue angle of ‘Seolhyang’ strawberry fruit.

Treatment	Storage day	L*	a*	b*	Chroma	Hue angel
Control	0	47.2	24.3	20.7	32.0	40.5
	1	47.5	24.2	20.8	31.9	40.6
	3	42.0	30.6	23.7	38.7	37.8
	7	40.6	30.8	26.3	40.5	40.6
	10	40.2	29.1	21.2	36.0	36.1
	14	40.5	26.9	20.7	33.9	37.6
30% CO ₂	0	47.2	24.3	20.7	32.0	40.5
	1	53.2	19.5	17.6	26.3	42.1
	3	40.0	29.6	22.5	37.2	37.2
	7	40.2	29.3	22.3	36.8	37.2
	10	41.2	28.6	21.5	35.8	36.9
	14	39.7	26.8	19.8	33.3	36.5

related with strawberry fruit firmness (Benítez-Burraco et al., 2003; Figueroa et al., 2008). The effect of high CO₂ treatments on strawberry fruit cell wall degradation enzyme gene expressions has not been clearly understood. In order to observe gene responses to high CO₂ treatment, the level of seven candidate gene expressions were analyzed (Fig. 3). Three enzymes of PE, PG, and PL are pectinase. Lipoxygenase (LOX) is lipid metabolism associated enzyme. Transcript levels of PE, PG, PL, and LOX were detected relatively low in high CO₂ treated fruit during storage. For hemicelluloses modifying genes, EG, EXP, and XTH, higher gene expressions were observed in high CO₂ treatments than in the control fruit for 3 days after treatment (Fig. 3). After then, expressions levels of EG, EXP, and XTH in high CO₂ treated fruit were reduced to the same level or lower than the control.

Polyuronides in cell wall components

The content of polyuronides in the crude cell wall extract (Fig. 4) and water-soluble pectin (Fig. 5) showed diversity of pectin polymers. Significant differences in polyuronides content were observed in the crude cell wall between the CO₂ treatment and control. The content of polyuronides in the crude cell wall extract was 2.13 mg/g dry weight at 1 day in control, and 2.36 mg/g dry weight at 1 day in high CO₂ treatment. The contents of polyuronides in the crude cell wall extract were 2.06 mg/g dry weight at 10 days in control, and 2.22 mg/g dry weight at 10 days in high CO₂ treatment (Fig. 4).

The contents of polyuronides in water-soluble pectin were significantly

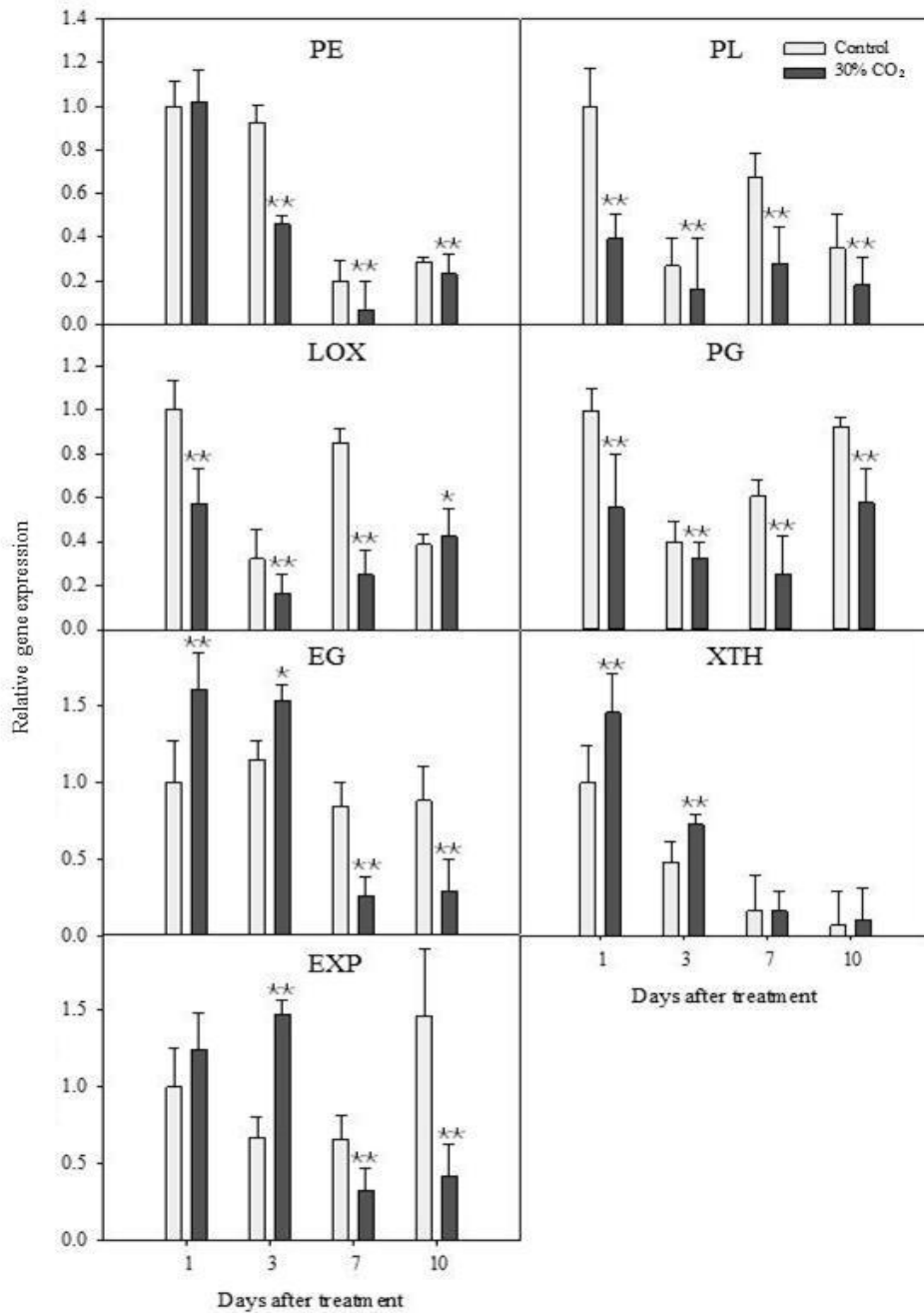


Fig. 3. Cell wall degradation enzyme gene expressions in 'Seolhyang' strawberry fruit treated with 30% CO₂ for 3 h. *, **Significant at $P < 0.05$ or 0.01, respectively.

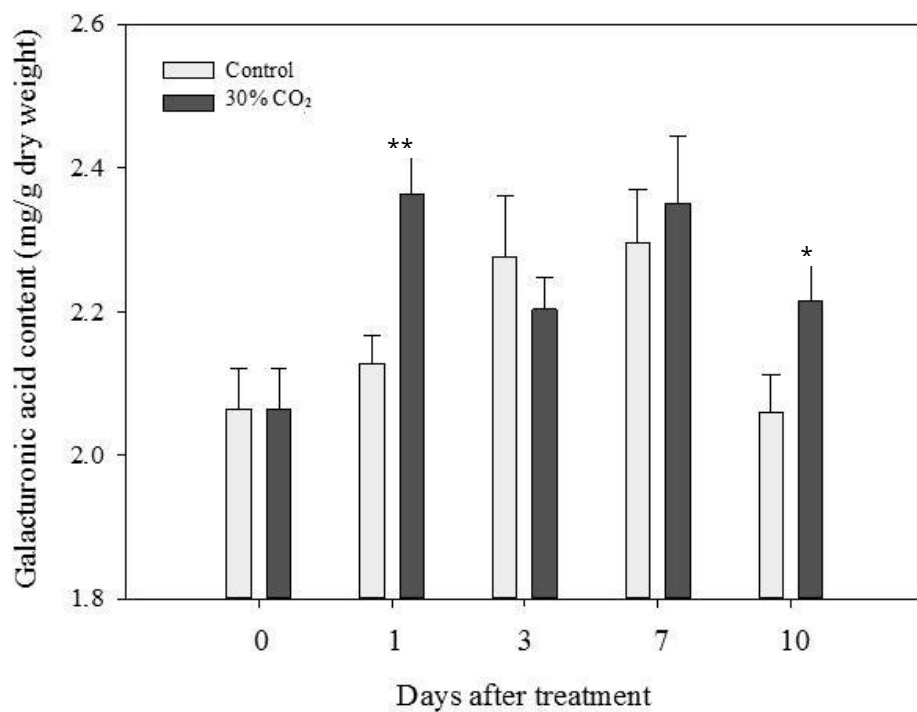


Fig. 4. Polyuronides content of crude cell wall material of 'Seolhyang' strawberry fruit treated with 30% CO₂ for 3 h. **Significant at $P < 0.01$.

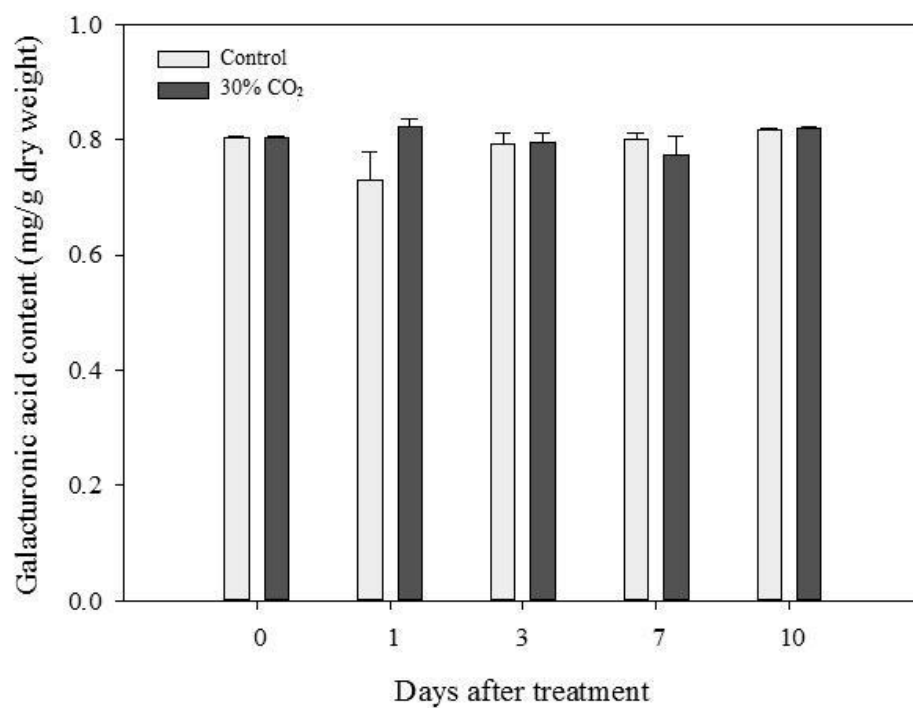


Fig. 5. Polyuronides content of water-soluble pectin of 'Seolhyang' strawberry fruit treated with 30% CO₂ for 3 h.

different between high CO₂ treatment and the control. The contents of polyuronides in water-soluble pectin were 0.73 mg/g dry weight at 1 day in control, and 0.82 mg/g dry weight at 1 day in high CO₂ treatment (Fig. 5).

Size-exclusion chromatography

Size-exclusion was carried out by using a column chromatography with a water-soluble pectin. Total 35 numbers of fraction were collected and the galacturonic acid content was measured in each fraction (Fig. 6). The galacturonic acid content showed almost similar trend between high CO₂ treatment and control. Difference appeared in the processing interval was observed between number 10 and 20 of fractions. Galacturonic acid content was higher in high CO₂ treatment than in control after 3 h and 10 days treatment. The contents of galacturonic acid in the fraction of number 15 were 0.04 mg/mL at 3 h in control, and 0.1 mg/mL at 3 h in high CO₂ treatment (Fig. 6A). The contents of galacturonic acid in the fraction of number 12 were 0.12 mg/mL at 10 days in control, and 0.17 mg/mL at 10 days in high CO₂ treatment (Fig. 6B).

***Botrytis cinerea* inoculation**

Strawberry fruit, inoculated with *B. cinerea*, showed typical gray mold symptoms. There was difference between high CO₂ treatment and the control in lesion diameters, which ranged from 10 to 25 mm after 7 days inoculation. In all

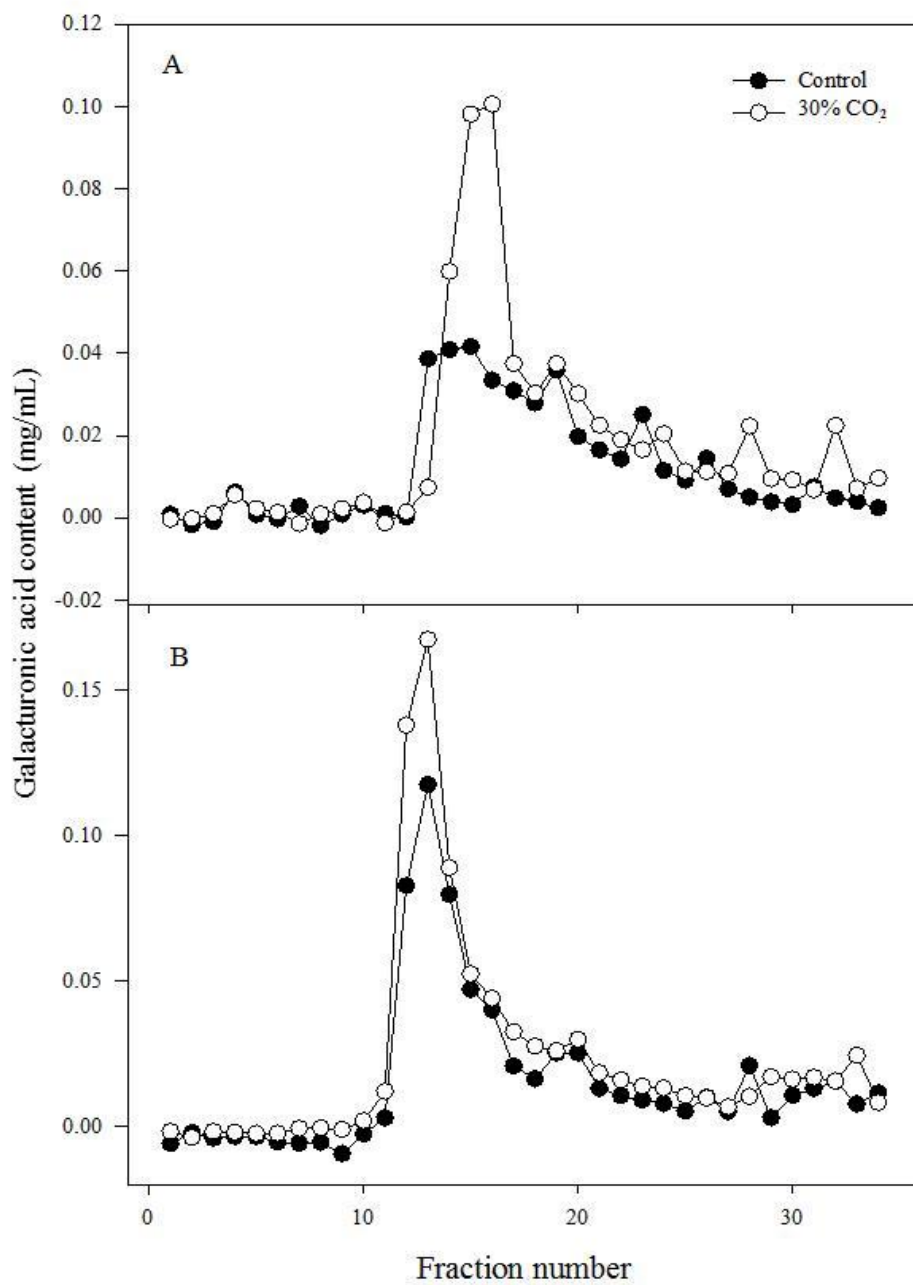


Fig. 6. Size distribution of water-soluble pectin of 'Seolhyang' strawberry fruit treated with 30% CO₂ for 3 h. A, 3 h after treatment; B, 10 days after treatment.

fruits with high CO₂ treatment and the control, gray mold was found, but the incidence was higher in the control with the increased lesion diameters (Fig. 7). Growth of *B. cinerea* on fruits in high CO₂ treatment was about 10% lower than control.

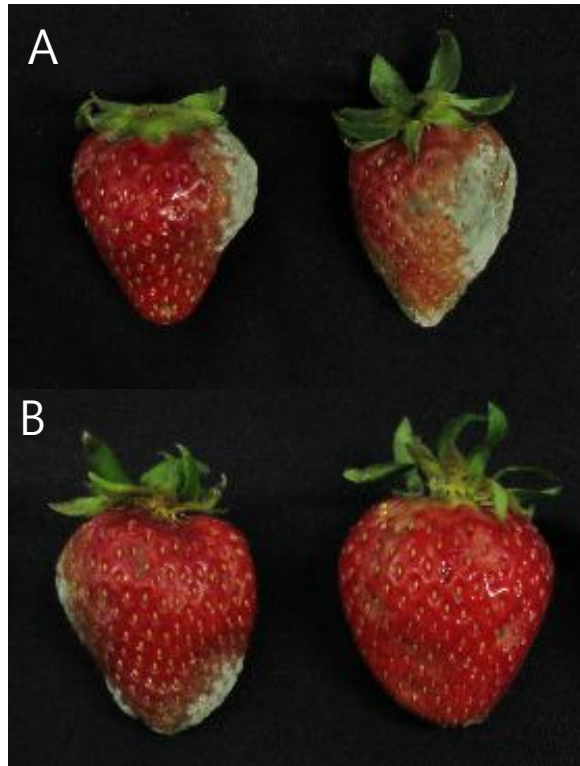


Fig. 7. Growth of *Botrytis cinerea* on strawberry fruits with 7 days after inoculation. A, control; B, 30% CO₂ treatment.

DISCUSSION

Short-term high CO₂ treatment affects on freshness and shelf-life of strawberry fruit during storage. It shows a significant effect on firmness and decay compared then other quality parameters (Figs. 1, 7).

Firmness has been increased by short time treatment and maintained during the storage period (Fig. 1). Pectin is a major component of cell wall that directly affects the firmness of strawberry fruit (Brummell and Harpster, 2001). In previous studies, firmness increase was associated with increased calcium ions binding to cell wall and the suppression of pectin degrading enzyme activity, such as PL (Wang et al., 2014). In this study, reasons of firmness change could be explained by three ways, such as comparing of pectin content in strawberry fruit cell wall (Fig. 4), the expression level of cell wall degrading enzyme genes (Fig. 3), and contents of water-soluble pectin (Fig. 5).

Show the high contents of pectin on 1 day after high CO₂ treatment (Fig. 4). Gene expressions levels of pectin degrading enzymes, such as PE, PG, and PL, were reduced to the same period (Fig. 3). However, gene expression level of hemicellulose degrading enzymes, such as EG, EXP, and XTH, showed a tendency to increase at 1 day after high CO₂ treatment. Therefore, high CO₂ treatment reduces pectin degradation by suppressing of pectin degrading enzymes gene expression. Non-pectin degrading enzymes did not affect content of pectin and

their gene expression levels were not involved in reduction of firmness.

Water-soluble pectin is a general term of small size pectin fragments that indicating water solubility (Aspinall et al., 1968). Because of differences in firmness, water-soluble pectin content was also expected that the difference comes out. However, contents of the water-soluble pectin were not different between high CO₂ treatment and the control (Fig. 5). In order to find the reason of the high water-soluble pectin content in the treatment, additional experiments associated with decay was carried out.

Reducing decay of strawberry fruit by high CO₂ treatment was demonstrated by *B. cinerea* inoculation experiments. High CO₂ treatment could confirm that there is a reliable decay suppressing effect (Fig. 7), through the previous studies that OGA plays an important role in plant defense mechanism (Davis et al., 1986; Galletti et al., 2008; Rasul et al., 2012). Size exclusion experiment showed that there were many specific sizes of pectin in high CO₂ treatment fruit (Fig. 6). Existence of specific size of OGA might support the hypothesis that plant defense mechanisms elicitor is often produced by high CO₂ treatment. Therefore, further studies are needed to clarify the structure of specific OGA and their function.

In conclusion, the results of the present study, high CO₂ treatment could increase firmness of strawberry fruit by reducing pectin decomposition and by suppressing decay. Further studies could clarify that high CO₂ treatment produces specific size of OGA as elicitors of plant defense mechanisms. If that happens, a new postharvest treatment technique could be suggested to prolong freshness of

horticultural crops including strawberry fruit.

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초 록

수확 후 고농도 이산화탄소 처리는 저장 기간이 짧은 딸기의 품질을 유지시키는 효과가 있다고 알려져 있으나 고농도 이산화탄소가 딸기의 세포벽 변화에 관여하는 기작은 확실하지 않다. 따라서 본 연구에서는 고농도 이산화탄소 처리가 ‘설향’ 딸기의 세포벽 및 지질 대사에 미치는 영향을 펙틴 분석과 유전자 발현 실험을 통하여 구명하고자 하였다. 또한 고농도 이산화탄소에 의한 세포벽의 분해와 부패와의 연관성을 구명하고자 잣빛곰팡이병 접종 실험도 함께 진행하였다. 시료는 ‘설향’ 딸기로 80% 착색되었을 때 수확하여 상온에서 30% 이산화탄소로 3시간 처리 후 10°C, 상대습도 90±5% 조건에서 2주 동안 저장하였다. 저장하는 동안 고농도 이산화탄소 처리구가 대조구에 비해 경도와 펙틴의 함량이 높게 유지되었고 부패과 발생률이 낮았다. 세포벽 및 지질 대사 관련 유전자를 RT-PCR로 분석한 결과, 처리구에서 세포벽 분해 효소인 PG, PE, PL 유전자와 지질 대사 관련 효소인 LOX 유전자 발현이 낮게 나타났다. 잣빛곰팡이병균을 접종한 후 7일 뒤에 처리구에서 부패과 발생률이 낮게 나타나 고농도 이산화탄소에 의해 딸기 품질이 유지됨을 확인할 수 있었다. 최종적으로 고농도 이산화탄소는 딸기 세포벽 구성 성분인 펙틴의 분해를 억제하여 딸기

품질을 유지시키는 것으로 보이며, 또한 잿빛곰팡이균에 대한 방어 기작을 향상시키는 것으로 예상된다. 고농도 이산화탄소 처리로 유도되는 딸기 과실 변화를 세포 수준에서 이해함으로써 이산화탄소의 농업적 활용을 기대해 볼 수 있다.

주요어: 고농도 이산화탄소 처리, 세포벽 분해, 펙틴, 잿빛곰팡이병.

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