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Microbiological and Quality Responses of Strawberry Fruit to High CO_2 Controlled Atmosphere and Modified Atmosphere Storage

Yuji Nakata
Faculty of Biology-Oriented Science and Technology, Kindai University, 930 Nishimitani, Kinokawa, Wakayama, 649-6493 Japan

Hidemi Izumi
Faculty of Biology-Oriented Science and Technology, Kindai University, 930 Nishimitani, Kinokawa, Wakayama, 649-6493 Japan

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Abstract. **'Minomusume' strawberries were stored in high CO_2 atmospheres (20%, 30%, and 40%) by means of a controlled atmosphere (CA) and active modified atmosphere packaging (MAP) for 10 days at 5 °C. The CA of 20% to 40% CO_2 was effective in delaying an increase in fungal count and preventing the external formation of mold mycelia, but a CA of >30% CO_2 induced black discoloration on the surface of strawberry due to CO_2 injury. When strawberry fruit were stored in a MAP flushed with either air or high CO_2, all packages approached an equilibrium of ≈20% CO_2 and 2% O_2 by the end of storage. Fungal counts of strawberry fruit stored in a MAP remained constant throughout the storage period and the diversity of fungal flora was partially similar regardless of the difference in the MAP method. Visual quality (mold incidence and severity of black discoloration) and physicochemical quality (weight loss, firmness, pH, and total ascorbic acid content) were unaffected by CO_2 atmospheres as the flushing gas during active MAP storage, except that the fruit in a MAP flushed with 20% and 30% CO_2 were firmer than those with air and 40% CO_2. After transfer to ambient conditions for 6 days at 10 °C, however, external formation of mold mycelia identified as *Botrytis cinerea* and surface black discoloration were induced in strawberry fruit in MAP flushed with 30% and 40% CO_2.**

Strawberry has a short shelf life because of susceptibility to fungal infections, which is associated with soft or leaky fruit, and this results in its rapid deterioration in appearance and texture (Kader, 1991). The fungal pathogen *Botrytis cinerea*, which causes grey mold, can infect senescent leaves and flower parts at the field stage, and then it can remain latent during storage (Petrasch et al., 2019). It is therefore essential to apply appropriate preharvest and postharvest practices such as use of fungicides, disinfecting agents, biocontrol agents, and physical treatments to control fungal spoilage (Romanazzi et al., 2016).

High CO_2 atmospheres have been shown in vitro (García-Gimeno et al., 2002; Hoogerwerf et al., 2002) and in strawberry fruit (Chambroy et al., 1993; Nielsen and Leufvén, 2008) to be an effective postharvest treatment to reduce or delay the development of *B. cinerea*. An in vitro study by García-Gimeno et al. (2002) reported that no growth of *B. cinerea* was observed above 30% CO_2 when inoculated on agar in a petri dish and stored for 10 d at 18 °C. Chambroy et al. (1993) found that with strawberry fruit, CO_2 of 10% to 20% markedly reduced the development of *B. cinerea* inoculated on fruit and decayed fruit stored for 7 d at 10 °C. Because high CO_2 atmospheres have also been shown to reduce the development of other diseases such as Rhizopus rot caused by *Rhizopus stolonifer* and Mucor rot by *Mucor* species in addition to grey mold by *B. cinerea* (Mitcham, 2016), the high CO_2 treatment could be one of the best methods for disease control of strawberry fruit.

However, the high CO_2 atmospheres for controlling postharvest decay are close to the highest concentrations tolerated by strawberry fruit, thereby making them susceptible to CO_2 injury (Watkins, 2000). Ke et al. (1991) reported that CO_2 levels of >20% caused injury with a change in skin and/or flesh color from red to dark–blue-red in strawberry fruit after 10 d of storage at 0 or 5 °C. In addition to injury, elevated CO_2 atmospheres adversely affected fruit color due to a remarkable decrease in anthocyanin content (Gil et al., 1997; Holcroft and Kader, 1999a, 1999b). Their studies revealed that changes in pH induced by CO_2 levels of 20% to 40% affected the anthocyanin stability and color expression. On the contrary, Picón et al. (1993) reported that anthocyanin content of strawberry fruit did not change during storage in a MA packaging (MAP) equilibrated to 7% O_2 and 14% CO_2 for 11 d at 2 °C. Nunes and Morais (2002) also observed that strawberry fruit in a CA of 5% O_2 and 15% CO_2 maintained their initial anthocyanin content and remained lighter colored than when stored in air for 2 weeks at 10 °C. Responses of strawberry fruit to high CO_2 atmospheres seemed to be CO_2 level- and cultivar-dependent. The influence of cultivar on physiological and physicochemical responses of strawberry fruit to high CO_2 exposure was investigated in several strawberry cultivars grown in the United States (Watkins et al., 1999) and Sweden (Nielsen and Leufvén, 2008), whereas work on microbiological response to high CO_2 concentrations was limited to few cultivars. The microbial quality response of a target cultivar of strawberry to elevated CO_2 needs to be investigated to determine the optimum ranges of CO_2 acceptable in CA and MAP.

In this study, 'Minomusume' strawberries grown in Japan were used to determine the microbiological and qualitative responses of the fruit to high CO_2 atmospheres. In Japan, CA storage of strawberry fruit is not used commercially, and MAP is the backbone technology for strawberry fruit. Therefore, CA study was initially conducted to simulate MAP with a similar gas composition for assessing the quality of strawberry fruit. Subsequently, active MAP flushed with high CO_2 concentrations that were selected on the basis of the results from the CA study was applied to evaluate the microbiological and overall quality of strawberry fruit stored under high CO_2 atmospheres. In addition, we also removed the fruit from MAP to ambient conditions by unsealing after 10 d of storage at 5 °C and then storing for 6 d at 10 °C to determine whether elevated CO_2 had a residual effect on microbiological quality.

Materials and Methods

Plant materials. 'Minomusume' strawberry fruits (*Fragaria ×ananassa* Duch.) were obtained from a distribution center in Gifu Prefecture, Japan in Apr. 2018 for CA study and in Apr. 2019 for MAP study. Forty-five plastic trays, with 14 to 15 fruits placed in each 1-L tray, were transported to the laboratory at Kindai University at 10 °C, and then 39 trays containing fruit were selected after removal of underripe, overripe, and damaged fruit. The weight of a strawberry fruit was 19.5 ± 1.0 g.

High CO_2 CA storage. Six trays each weighing 278 ± 1.0 g (14 to 15 fruits) were placed into a 7-L glass jar containing 5 mL of distilled water in a plastic beaker to maintain a high relative humidity. Three independent replicates were stored under a continuous flow of air or high CO_2 atmospheres (20%, 30%, and 40%) with the balance being air at a flow rate of 20 mL·min^{-1} for 10 d at 5 °C.

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Current address for Y.N.: Agricultural R and D center, Zen-noh, 18-1-4 Higashiyawata, Hiratsuka, Kanagawa, 254-0016 Japan.
H.I. is the corresponding author. E-mail: izumi@waka.kindai.ac.jp.

Active MA packaging of high CO_2. For active MAP, the added gas concentration was based on the results of CA storage. Each tray containing 14 to 15 fruits was placed in oriented polypropylene (OPP) film (30 µm thick, 25 × 26 cm, O_2 permeability of 1170 mL·m^{-2}·d^{-1}·1013 hPa^{-1}) and then flushed and evacuated three times with air or 20%, 30%, and 40% CO_2 before sealing. Three replicated packages of each treatment were stored for 10 d at 5 °C. Thereafter, part of the packages were unsealed and then stored under the ambient conditions for 6 d at 10 °C to simulate the storage condition following market distribution to consumers.

Gas analysis. The CO_2 and O_2 concentrations in packages were measured daily during the MAP storage period with a gas chromatograph equipped with a thermal conductivity detector (Model GC-8AIT; Shimazu Corp., Kyoto, Japan). The columns used for CO_2 and O_2 analyses were Porapak Q (60–80 mesh/A, 3.2 mm × 1.5 m) at 90 °C and Molecular Sieve (60–80 mesh/A, 3.2 mm × 1.5 m) at 60 °C, respectively.

Microbial counts. Strawberry fruit during either CA storage or active MAP followed by unsealed packaging storage were taken periodically, and microbial analysis of each sample was performed three times. Each sample was assessed for counts of fungi from the surface of strawberries as previously described (Izumi and Watada, 1994). Two fruits with the calyx (≈40 g) were transferred aseptically to 200 mL of sterile saline solution and stirred with a sterile magnetic stirrer for 1 min. Then serial dilutions of this solution were poured onto triplicate plates of potato dextrose agar (PDA; Nissui Pharmaceutical Co., Tokyo, Japan) with 100 mg·L^{-1} of chloramphenicol and incubated at 25 °C for 72 h for enumeration of fungi. The microbiological plate count was expressed as log colony-forming units (cfu)/g sample.

Microbial identification. The fungi on strawberry fruit stored in an active MAP and subsequently unsealed packaging were identified. One hundred and thirty-five fungal isolates were selected from colonies with different appearances on PDA plates prepared for the fungal enumeration from strawberry samples. The MicroSeq microbial identification system (Applied Biosystem Inc., Foster City, CA) was used for identification of fungi, and the sequencing data were analyzed with Analysis Software (Microseq D2 LSU rDNA sequence databases Version 2.0 for fungi) as previously described by Poubol and Izumi (2005). A cut off of the highest matching score with the sequence in the database was chosen for species identity. Upon transfer from active MAP to ambient conditions, mold mycelia observed on strawberry fruit were also identified using the MicroSeq system.

Visual quality. External mycelial development was evaluated as the mold incidence occurred during CA or MAP followed by unsealed packaging storage. Mold incidence score was determined based on the percentage of the total number of fruits with mold mycelia in each tray and expressed as a rating scale of 0 = no mold, 1 = slight mold (<10%), 2 = moderate mold (10% to 30%), and 3 = severe mold (>30%). Fruits in a tray in each CA treatment or active MAP followed by unsealed package treatment were evaluated

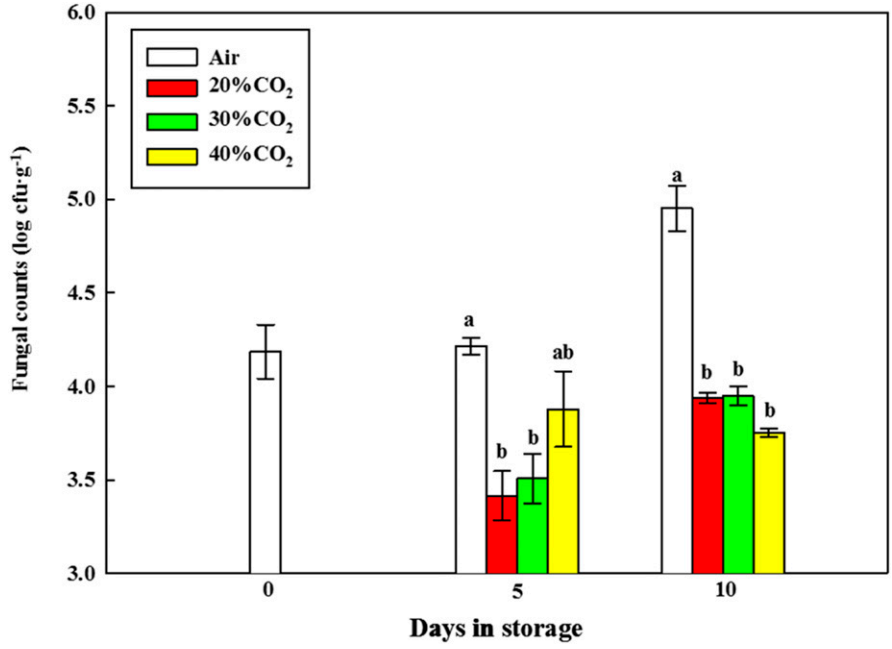

Fig. 1. Fungal counts on strawberry fruit stored in either air or high CO_2 CAs (20%, 30%, and 40%) for 10 d at 5 °C. The counts on the initial day were not analyzed separately for each treatment. Bars with different letters within the same day are significantly different ($P \leq 0.05$). Vertical lines represent ± SE.

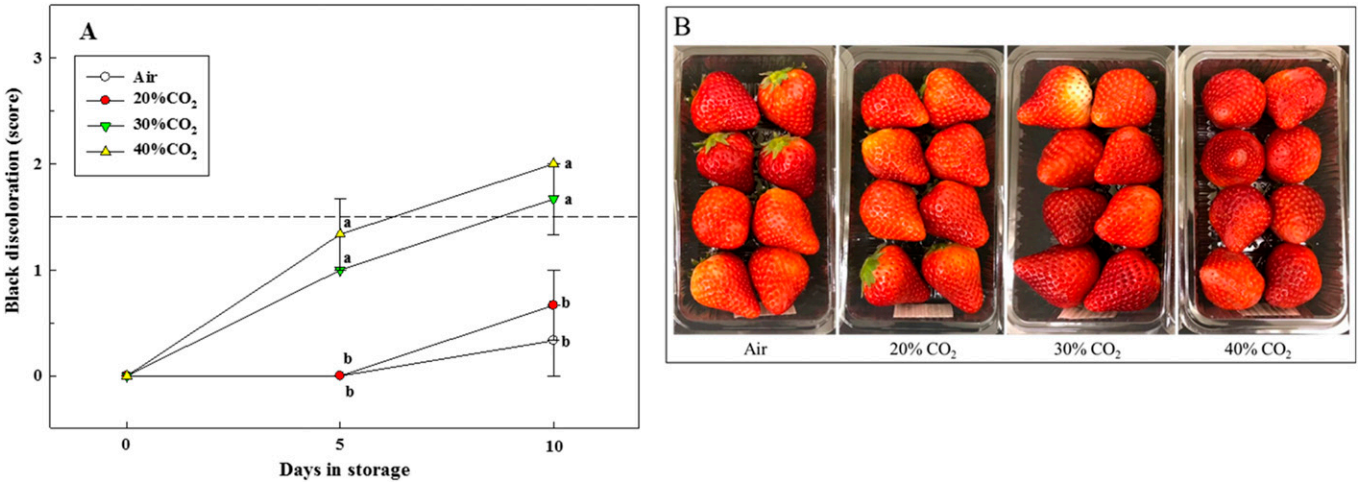

Fig. 2. Black discoloration of strawberry fruit stored in either air or high CO_2 CAs (20%, 30%, and 40%) for 10 d at 5 °C (**A**), and photographs of the strawberry fruit after 10 d of storage at 5 °C (**B**). Black discoloration score: 0 = none, 1 = slight, 2 = moderate, and 3 = severe. Broken line (score = 1.5) indicates the limitation of marketability. Symbols with different letters within the same day in each graph are significantly different ($P \leq 0.05$). Vertical lines represent ± SE.

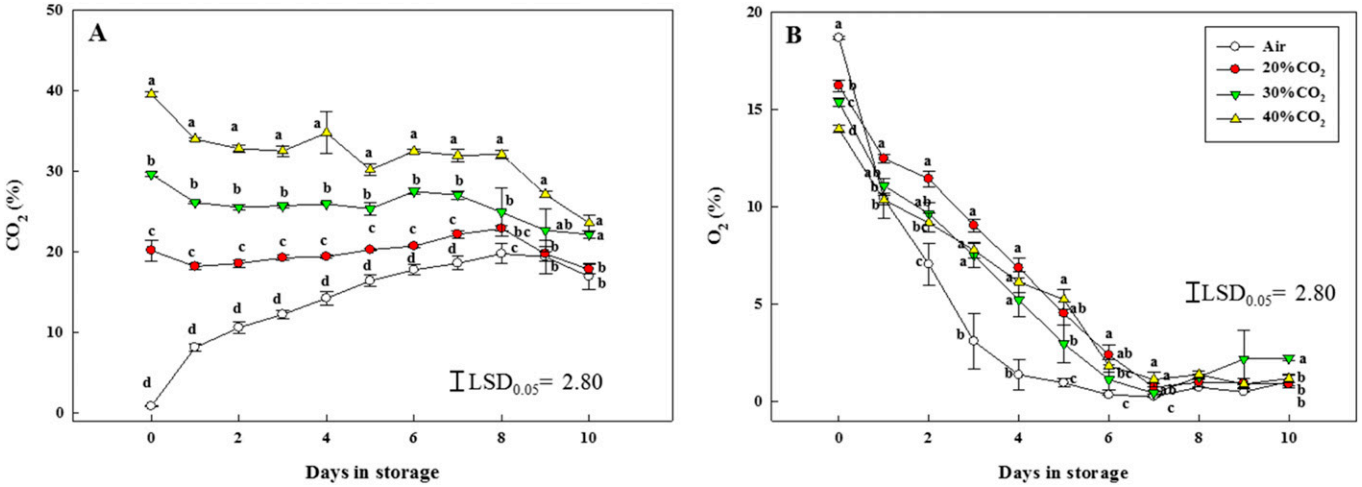

Fig. 3. Concentrations of CO_2 (**A**) and O_2 (**B**) in a MA packaging flushed with either air or high CO_2 (20%, 30%, and 40%) containing strawberry fruit during storage for 10 d at 5 °C. Symbols with different letters within the same day in each graph are significantly different ($P \leq 0.05$). Vertical lines represent ± SE.

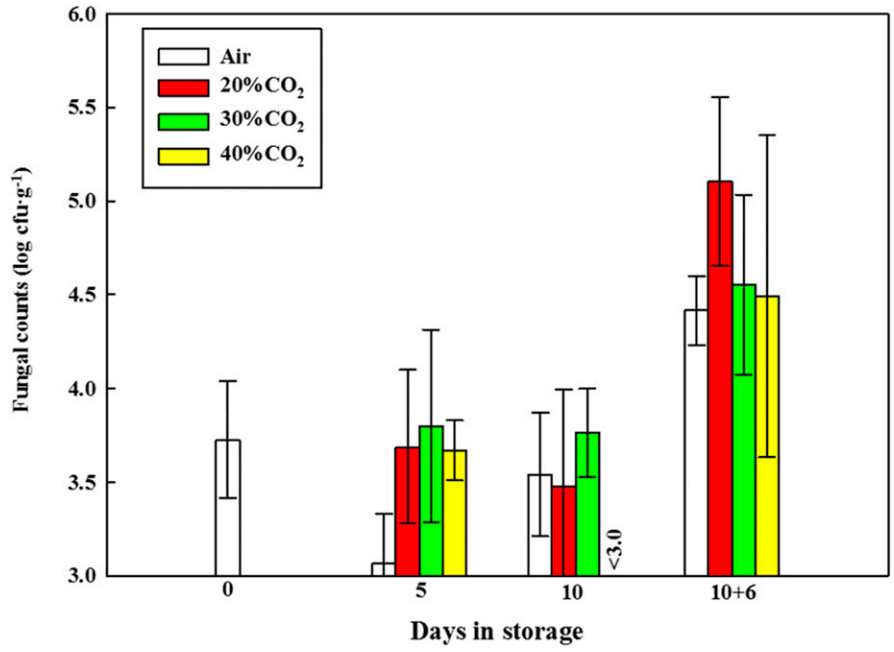

Fig. 4. Fungal counts on strawberry fruit stored in a MA packaging flushed with either air or high CO_2 (20%, 30%, and 40%) for 10 d at 5 °C followed by unsealed packaging for 6 d at 10 °C. The counts on the initial day were not analyzed separately for each treatment. Vertical lines represent ± SE. Less than 3.0 log cfu/g means below the level of detection.

for severity of black discoloration. Samples were scored on a scale of 0 = none, 1 = slight, 2 = moderate, and 3 = severe. Three replications of one tray each were used in each treatment for the evaluation. Strawberry fruit were considered at the lower limit of unmarketability when the score of mold incidence and black discoloration reached 1.5.

Physicochemical quality. Strawberry samples stored in an active MAP were taken periodically for evaluation of physicochemical quality including weight loss, firmness, surface pH, and total ascorbic acid content as previously described by Poubol and Izumi (2005). The evaluation of physicochemical quality was carried out in three replications for each package. Percentage of weight loss was calculated from the weight of each tray containing fruits before and after storage. Firmness of three fruits per replication was measured using a fruit firmness tester (KM-1; Fujiwara Co. Ltd., Tokyo, Japan) equipped with a 5-mm conical plunger by the force to penetrate into the equatorial zone of the fruit and expressed in N. Surface pH of three fruits was determined with a compact pH meter (Model B-113; Horiba Ltd., Kyoto, Japan). Total ascorbic acid content of the three fruits was extracted with a 4% metaphosphoric acid solution containing 3% dithiothreitol as a reducing agent, and determined by high-performance liquid chromatograph (Model LC-10A; Shimazu) equipped with a PLRP-S 100A column (4.6 mm × 25 cm, 5 µm; Polymer Laboratories, Varian, Inc., Amherst, MA) and an electrochemical detector (Model ECD 300; Eicom Corp., Kyoto, Japan). The mobile phase was 0.02 M KH_2PO_4 (pH 2.2) and the flow rate was 1 mL·min^{-1}.

Statistical analysis. Statistically significant differences ($P \leq 0.05$) were determined for the microbial counts, gas concentration, and visual and physicochemical evaluation data based on an analysis of variance using the SAS system, Release 9.4 (SAS Institute Inc., Cray, NC). The mean values were compared using the Tukey's honestly significant difference method.

Results and Discussion

High CO_2 CA storage. Strawberry fruit showed a fungal count of 4.2 log cfu/g on the initial day (Fig. 1). The counts increased to 4.9 log cfu/g after 10 d of storage in air, whereas the high CO_2 (20%, 30%, and 40%) CA inhibited the fungal count increase throughout 10 d of storage. The fungistatic effect was similar in the high CO_2 atmospheres. The desirable effect of CA/MA with 10% to 40% CO_2 levels on fungal growth has been observed on strawberries (Nielsen and Leufvén, 2008; Wszelaki and Mitcham, 2000), table grapes (Teles et al., 2014), blueberries (Cantín et al., 2012), raspberries (Haffner et al., 2002), cranberries (Gunes et al., 2002), and sweet cherries (De Vries-Paterson et al., 1991). The fungistatic action of high CO_2 involves its dissolution into the aqueous phase of the produce and the fungi. Dissolved CO_2 causes a decrease of the intercellular pH, which inhibits enzymatically catalyzed reactions and enzyme synthesis, interacts with cell membranes, and changes the physicochemical properties of proteins (Farber, 1991; Molin, 2000).

External fungal development was also visually inhibited by high CO_2 atmospheres (data not shown). In contrast, surface color of strawberry fruit held in 30% and 40% CO_2 atmospheres changed from red to black at the unmarketable level (score 1.5) by Day 10,

Table 1. Fungi isolated from strawberry fruit before storage and stored in a MA packaging flushed with either air or high CO_2 (20%, 30%, and 40%) for 10 d at 5 °C.

Before storage			After 10 d of storage			
Fungi	Genus	Species	Treatment	Fungi	Genus	Species
Molds	*Cladosporium*	*cladosporioides*	Air	Molds	*Cladosporium*	*cladosporioides*
Yeasts	*Pseudozyma*	*antarctica*		Yeasts	*Candida*	*geochares*
	Sporidiobolus	*johnsonii*			*Pseudozyma*	*antarctica*
					Rhodotorula	*mucilaginosa*
					Sporidiobolus	*johnsonii*
			20% CO_2	Molds	*Cladosporium*	*cladosporioides*
				Yeasts	*Cryptococcus*	*laurentii*
					Pseudozyma	*antarctica*
					Sporidiobolus	*johnsonii*
			30% CO_2	Molds	*Cladosporium*	*cladosporioides*
				Yeasts	*Cryptococcus*	*laurentii*
					Pseudozyma	*antarctica*
					Rhodotorula	*graminis*
			40% CO_2	Molds	*Cladosporium*	*cladosporioides*
					Penicillium	*olsonii*
				Yeasts	*Aureobasidium*	*pullulans*
					Pseudozyma	*antarctica*
					Sporidiobolus	*johnsonii*

Table 2. Fungi isolated from strawberry fruit and visible mycelia that formed on fruit stored in an unsealed packaging for 6 d at 10 °C following a MA packaging flushed with either air or high CO_2 (20%, 30%, and 40%) for 10 d at 5 °C.

Strawberry fruit				Mycelia formed on strawberry fruit			
Treatment	Fungi	Genus	Species	Treatment	Fungi	Genus	Species
Air	Molds	*Cladosporium*	*cladosporioides*	Air	Molds	*Botrytis*	*cinerea*
		Penicillium	*camembertii*			*Cladosporium*	*cladosporioides*
	Yeasts	*Cryptococcus*	*laurentii*	20% CO_2	Molds	*Botrytis*	*cinerea*
		Pseudozyma	*antarctica*			*Cladosporium*	*cladosporioides*
		Sporidiobolus	*johnsonii*	30% CO_2	Molds	*Botrytis*	*cinerea*
20% CO_2	Molds	*Cladosporium*	*cladosporioides*			*Cladosporium*	*cladosporioides*
		Sydowia	*polyspora*	40% CO_2	Molds	*Botrytis*	*cinerea*
	Yeasts	*Cryptococcus*	*laurentii*			*Cladosporium*	*cladosporioides*
30% CO_2	Molds	*Cladosporium*	*cladosporioides*			*Epicoccum*	*nigrum*
	Yeasts	*Cryptococcus*	*laurentii*				
		Pichia	*barkeri*				
		Pseudozyma	*antarctica*				
		Sporidiobolus	*johnsonii*				
40% CO_2	Molds	*Cladosporium*	*cladosporioides*				
	Yeasts	*Cryptococcus*	*laurentii*				
		Pseudozyma	*antarctica*				
		Sporidiobolus	*johnsonii*				

while the score of black discoloration in fruit held in air and 20% CO_2 atmosphere was below the unmarketable level throughout 10 d of storage (Fig. 2). Ke et al. (1991) reported that CO_2 injury with a change in skin and/or flesh color from red to dark-blue was observed in strawberry fruit stored under 50% or 80% CO_2 atmospheres for 10 d at 0 or 5 °C. Gil et al. (1997) showed that storage in 20% and 40% CO_2 atmospheres for 10 d at 5 °C induced a paling or bleaching of internal flesh color of strawberry fruit by decrease in anthocyanin content. Judging from the feature of the observation in this study, the surface discoloration would be because of CO_2 injury rather than degradation in biosynthesis of anthocyanins. During the 10-d storage period under CA, strawberry fruit tolerated CO_2 concentrations as high as 20%.

CO_2 and O_2 concentrations in an active MA packaging. Izumi et al. (2017) illustrated that an active MAP by application of the gas could be helpful in achieving the ideal equilibrium atmosphere readily and quickly as compared with a passive MAP that is achieved by the natural interaction between respiratory O_2 consumption and CO_2 evolution of the packaged produce and the transfer of gasses through the package film. Because our CA study revealed that high CO_2 of 20% to 40% inhibited fungal growth of strawberry fruit and CO_2 of >30% induced CO_2 injury indicated as black discoloration, an active MAP having an initial 20%, 30%, or 40% CO_2 was performed to evaluate the effects of high CO_2 and tolerances to high CO_2 for commercial usage.

When strawberry fruit were stored in a MAP flushed with high CO_2 for 10 d at 5 °C, the CO_2 concentration remained at the initial level of 20% in an active MAP of 20% CO_2, and it decreased gradually from 30% and 40% to 23% in an active MAP of 30% and 40%, respectively, by the end of storage (Fig. 3). The CO_2 concentration in the packages flushed with air approached a 20% equilibrium after 8 d of storage. Thus, all packages approached an equilibrium of 20% to 23% CO_2 irrespective of the initial CO_2 level, probably as a result of low respiration rates of the sample and high gas permeability of the film used in this study. The O_2 concentration decreased from 14% to 18% to 2% in the packages during the storage period regardless of the treatment, with the decrease occurring more rapidly in packages with air than with high CO_2.

Microbiological quality in an active MA packaging. Fungal counts of strawberry fruit stored in a MAP flushed with air or high CO_2 were 3.7 log cfu/g on the initial day and remained constant during 10 d of storage at 5 °C, except that flushing with 40% CO_2 reduced the counts to levels below the limit of detection (3.0 log cfu/g) on Day 10 (Fig. 4). After transfer of fruit from active MAP to ambient conditions for 6 d at 10 °C, fungal counts of all samples increased to 4.5 to 5.0 log cfu/g. No residual effects on inhibiting the fungal growth were obtained with CO_2 treated fruit.

Fungi isolated from strawberry fruit on the initial day of MAP storage consisted of one mold genus *Cladosporium* and two yeast genera *Pseudozyma* and *Spordiobolus* (Table 1), which are phytopathogenic and/or soilborne organisms found in fruits and their environments (Izumi et al., 2008a).

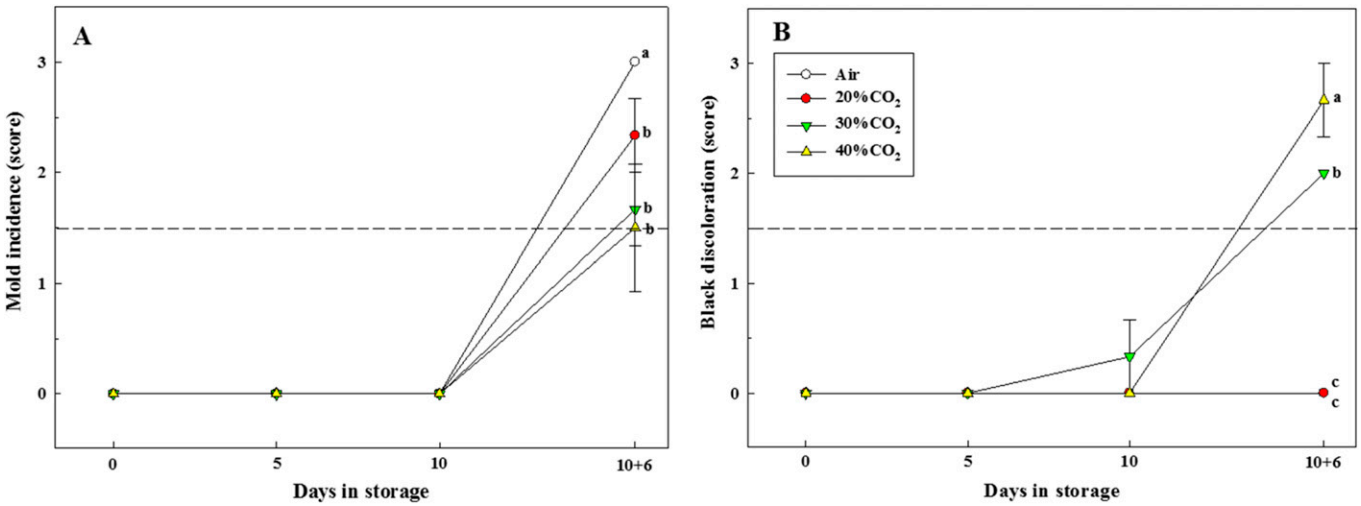

Fig. 5. Mold incidence (**A**) and severity of black discoloration (**B**) of strawberry fruit stored in a MA packaging flushed with either air or high CO_2 (20%, 30%, and 40%) for 10 d at 5 °C followed by unsealed packaging for 6 d at 10 °C. Mold incidence score based on the % of the total number of fruits with mold mycelia: 0 = none, 1 = slight (<10%), 2 = moderate (10% to 30%), and 3 = severe (>30%). Black discoloration score: 0 = none, 1 = slight, 2 = moderate, and 3 = severe. Broken line (score = 1.5) indicates the limitation of marketability. Symbols with different letters in each graph are significantly different ($P \leq 0.05$). Vertical lines represent ± SE.

These fungi were commonly detected on strawberry fruit stored in packages with either air or high CO_2 for 10 d. *Cladsporium* species are the causal agent of postharvest disease that generally show negligible levels, but the incidence could increase among strawberry cultivars that show resistance to gray mold (Feliziani and Romanazzi, 2016). Other frequently isolated fungi from stored fruit included the mold genus *Penicillium* and the yeast genera *Rhodotorula* and *Cryptococcus*. CO_2 atmospheres in MAP did not affect the diversity and variety of fungal flora of strawberries.

After transfer to ambient conditions for 6 d at 10 °C, the fungi living in a plant-soil environment were also found on strawberry fruit (Table 2). Interestingly, *B. cinerea* was identified from mycelia that formed on strawberry fruit. It is implicated that the main strawberry pathogen is *B. cinerea*, followed by *Rhizopus* spp., *Mucor* spp., *Colletotrichum* spp., and *Penicillium* spp. (Feliziani and Romanazzi, 2016). Although the phytopathogens excluding *Botrytis* and *Penicillium* were not found on strawberry fruit in this study, the mold flora of a given strawberry is not necessarily the same as that of other strawberries because potential sources of microbial contamination for fruit are from soil, agricultural water, pesticide solution, and packing-shed equipment in different environments (Izumi et al., 2008b, 2008c). Because infection of *B. cinerea* can result from nesting, which is direct penetration of mycelia growing on fruit, and the infection can proceed rapidly (Petrasch et al., 2019), it is important to prevent *B. cinerea* from developing and spreading from rotted fruit to nearby healthy fruit during storage.

Visual quality in an active MA packaging. Mold mycelia were not observed visually on strawberry fruit with all treatments during MAP storage for 10 d at 5 °C, but the mycelia on all fruits developed to a visible size and reached unmarketable level upon transfer to ambient conditions for 6 d at 10 °C (Fig. 5). The growth of mycelia, identified as *B. cinerea* as described above, was greater in samples flushed with air than those with high CO_2. Black discoloration was expedited to an unmarketable level in strawberry fruit in packages with 30% and 40% CO_2 only after transfer from MAP to ambient conditions, while fruit in packages with air and 20% CO_2 showed no black discoloration throughout the storage period. This fact was like the result of CA storage.

Physicochemical quality in an active MA packaging. Weight loss of strawberry fruit held in an active MAP approached 0.5% to 0.7% regardless of the treatment during storage for 10 d at 5 °C (data not shown). The total loss on Day 10 was obviously under the limit for marketability of 3% to 6% in a soft fruit (Hardenburg et al., 1986). The firmness of strawberry fruit decreased to about 10% and 20% of the initial day by the end of storage in packages with 20% and 30% CO_2, and packages with air and 40% CO_2, respectively (data not shown). Several studies have shown that strawberry fruit treated with high CO_2 levels of 15% to 30% were firmer than those treated with air during storage at 0 °C (Ke et al., 1991), 1 °C (Choi et al., 2016), 2 °C (Li and Kader, 1989), 3 °C (Shin et al., 2008), 4 °C (Chandra et al., 2015; Nunes and Morais, 2002), 5 °C (Ke et al., 1991), and 18 °C (García et al., 1998). In this study, because fruits rotted by fungal development were not observed during active MAP storage, the changes of fruit firmness may involve physiological and physical responses rather than microbiological responses. It is recognized that fruit softening is attributed to the middle lamellar pectin degradation of cell walls due to activity of cell wall hydrolase such as endopolygalacturonase, pectin methylesterase, rhamnogalacturonase, and xyloglucan endotransferase (Smith et al., 2003). Recently, transcriptomic analyses revealed that expression levels of genes encoding cell wall-degrading enzymes decreased in response to the treatment with 30% CO_2 (Bang et al., 2019). The surface pH and total ascorbic acid content of all samples remained constant throughout 10 d of storage and were 4.5 to 5.0 and 75 to 80 mg/100 g, respectively (data not shown). These results were like those of Li and Kader (1989) on strawberry fruit stored in 10% to 20% CO_2 CA for 7 d at 2 °C.

In conclusion, a 20% CO_2 atmosphere by means of either a CA or active MAP could be an effective postharvest treatment to improve microbiological quality without any deterioration on 'Minomusume' strawberries during storage for 10 d at 5 °C. The strawberry fruit in a MAP flushed with 20% CO_2 did not induce visible mold development and black discoloration after transfer to unsealed packaging for 6 d at 10 °C, although the residual effects of addition of 20% CO_2 on microbiological quality were not observed. This technology will be useful for a realistic MAP for a domestic market and a versatile MA pallet for a foreign export market. Postharvest management after transfer from high CO_2 atmospheres to ambient conditions is necessary so as not to cause any mold mycelia to become visible because its appearance would give rise to consumer rejection.

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