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（キュウリ果実の低温障害を抑制する環境要因分析に基づく

Modified Atmosphere包装の最適設計）

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The United Graduate School of Agricultural Science,
Gifu University
Science of Biological Production
(Gifu University)

Khandra Fahmy

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INRODUCTION

1.1. Indonesian horticultural and challenges

Fresh fruits and vegetables is a sub-sector of agricultural that gets attentions of the world community, including Indonesia in the last decade. This is not free from public awareness of the benefits for health owing to their nutritional values (Wills et al., 2007). However, they are very perishable and deteriorated easily after harvest. Therefore, the appropriate ways of handling and technologies for maintaining freshness of the products are necessary to minimize loss of the yield and to extend the trading area.

Indonesia is located in a tropical country with vast 1.9 million km^2 and consists of islands with low-lying and mountainous topography, and climate of hot, humid, and moderate (Smith and Dawson, 2004). These advantages provide a high capacity of the growth of various fruits and vegetables. This was shown by the high production of Indonesian fruits and vegetables (Appendix 1 and 2). Fig. 1.1 shows the total production of Indonesian fruits and vegetables in 2010 to 2013. In 2013, total production of Indonesian fruits and vegetables achieves 18.3 and 11.4 million ton, respectively, and these values are expected to increase due to the availability of agricultural lands. Consequently, Indonesia has a potential to develop the market of fresh produces by producing and selling of the fresh fruits and vegetables due to high opportunities at domestic and abroad.

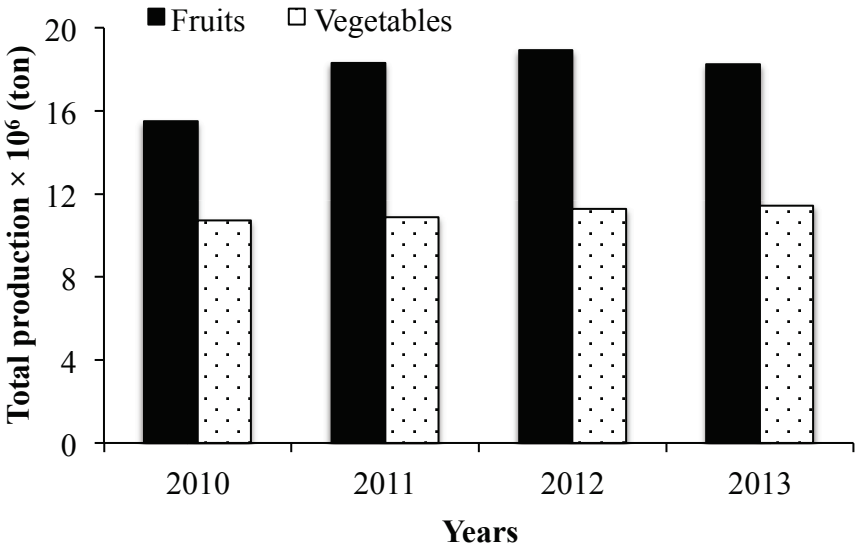

Fig. 1.1. Total production of Indonesian fruits and vegetables in 2010 to 2013 (Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014).

However, these advantages have not been exploited well because of some obstacles of postharvest handling such as the inadequate technology, infrastructure facilities and other complex problems caused by thousands of islands in Indonesia. As a result, there are so many the amounts of losses of fruits and vegetables after harvest, particularly during storage and distribution chain. Paula et al. (1997) and Kader (2005) reported that the amount of postharvest losses has been estimated approximately 30% or more of the total production of fresh produce worldwide. These factors hinder the expansion of Indonesian fruits and vegetables market, not only in domestic but also in overseas. In the facts, the export volume of Indonesian fruits and vegetables is lower than the import volume (Appendix 3~6). Comparing to the total production of Indonesian fruits and vegetables in 2013, the export volume is only 197.9 and 128.3

thousand ton for fruits and vegetables, respectively. These volumes are lower than the import volumes about 535.5 thousand ton for fruits and 994.8 thousand ton for vegetables (Fig. 1.2).

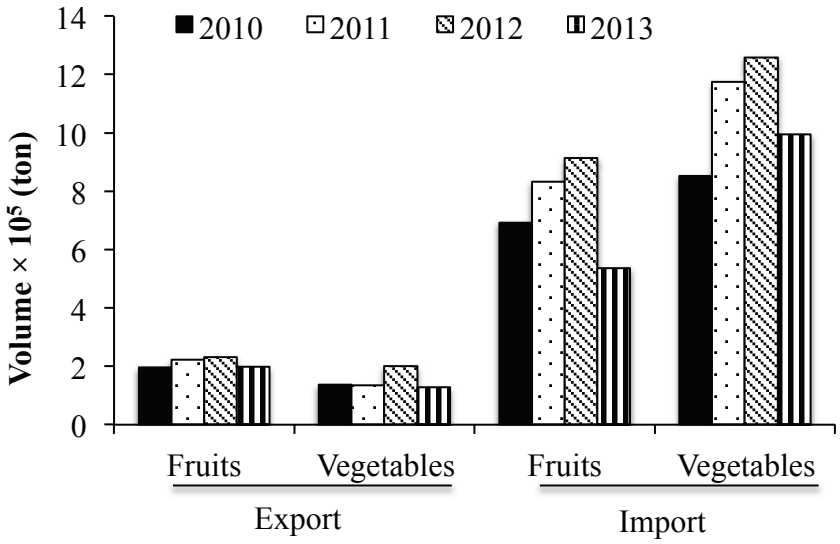

Fig. 1.2. Export and import volume of Indonesian fruits and vegetables in 2010 to 2013 (Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014).

Therefore, the improvement of postharvest handling makes the quality of horticultural products sustain much longer and minimize the amounts of products losses during storage and distribution. Improvement of quality will also induce the increase of the export value of fruits and vegetables from Indonesia to other countries.

1.2. Chilling injury

Controlling of product's temperature is the primary means for quality preservation of fresh horticultural commodities. The lower temperature suppresses the

metabolic rates such as respiration and ethylene production (Biale et al., 1954; Yearsley et al., 1997), resulting in maintaining the product freshness and extends the shelf life. However, almost fruits and vegetables produced in Indonesia are chilling-sensitive products (Appendix 1) and they are sensitive to chilling temperature and injured when stored below critical temperature but still above freezing temperature.

Chilling injury (CI) is a term used to describe the physiological damage that occurs in many plant commodities as a result of exposure to temperatures below 5°–15°C, but above freezing temperatures (Kader, 2002a). It leads to significant destruction of product quality and a concomitant financial loss for producers, processors and consumers.

There are two hypotheses to explain mechanism of CI in chilling-sensitive products caused by storing at low-temperature i.e. primary and secondary response. Primary response is thought to be consequence of change in cell membrane properties at a low-temperature. The bulk membrane lipid phase transforms from liquid crystalline-phase to solid gel-phase (Parkin et al., 1989). The primary response would lead to secondary events which include the accumulation of the reactive oxygen species (ROS) (Karakaş and Yıldız, 2007; Imahori et al., 2008; Yang et al., 2011), increase in malondialdehyde (MDA) (Karakaş and Yıldız, 2007; Imahori et al., 2008; Yang et al., 2011; Mao et al., 2007a; Wongsheree et al., 2009), increase in the activation energy of membrane-associated enzymes such as phospholipase D (PLD) and lypoxigenase (LOX) (Mao et al., 2007a, 2007b), increase in electrolyte leakage (Cabrera and Saltveit, 1990; Palma, 1995; Saltveit, 2002, 2005; Mao et al., 2007a, 2007b; Wongsheree et al., 2009; Dea et al., 2010; Yang et al., 2011; Luengwilai et al., 2012), decrease in photosynthetic rate (Hakim et al., 1999), stimulation of ethylene production (Cabrera

and Saltveit, 1990; Woolf et al., 2003), increase in respiration rate (Eaks and Morris, 1956; Hakim et al., 1999; Dea et al., 2010; Luengwilai et al., 2012;) and then develop into a variety of CI symptoms such as surface and internal discoloration (browning), pitting, water soaked areas, uneven ripening or failure to ripen, off-flavor development and accelerated incidence of surface molds and decay (Kader, 2002a).

1.3. Cucumber fruit

Cucumbers fruits (*Cucumis sativus* L.) are one of the most popular vegetables of the world and usually consumed worldwide as a fresh vegetable. They are frequently transported and stored at low temperature with other kinds of fresh commodities for preserving the quality in most fresh produces. However, cucumbers fruit are chilling sensitive products and susceptible to CI characterized as surface pitting, dark watery and increased susceptibility to decay (Cabrera and Saltveit, 1990).

Several postharvest horticultural treatments have been demonstrated to reduce CI in cucumber fruit. Wang and Qi (1997) reported that packaged cucumber fruit in modified atmosphere packaging (MAP) using perforated and sealed low-density polyethylene bag increased their chilling tolerance. Intermittently warmed of cucumber fruit alleviated development of CI symptoms (Cabrera and Saltveit, 1990) with suppressing the increase of electrolyte leakage and MDA equivalent (Mao et al., 2007a). The application of nitric oxide also effectively reduced CI (Yang et al., 2011). Among postharvest technologies available for limiting CI of cucumber fruit during low-temperature storage, MAP is promising because it has advantages compared with other treatments due to low-cost and easy to implement at the commercial level (Zagory and Kader, 1998).

1.4. Modified atmosphere packaging

Modified atmosphere packaging (MAP) is the promising method to minimize CI, even if chilling-sensitive products were stored at low temperature. Packaging of horticultural crops within a permeable plastic film creates the modified atmosphere condition inside a package such as higher CO_2 and H_2O and lower O_2 comparing with ambient levels, in response to respiration and transpiration of the products. These conditions are beneficial to alleviate CI in chilling-sensitive products due to reduce respiration rate and ethylene production, water loss and other physiological disorder. It has been reported that the MAP are beneficial in alleviating CI in chilling-sensitive products such as eggplants (Fallik et al., 1995), cucumber fruit (Wang and Qi, 1997), avocado (Meir et al., 1997), peach (Fernández-Trujilio et al., 1998), mango (Pesis et al., 2000), melon (Flores et al., 2004), citrus (Porat et al., 2004), carambola (Ali et al., 2004), banana (Nguyen et al., 2004), broccoli (Serrano et al., 2006) and persimmon (Cia et al., 2006). However, limited information on MAP of chilling sensitive products was available, further studies must be conducted to successful MAP for a wide range of chilling-sensitive products.

In MAP, the permeability of the film plays an important role because gas composition inside the package depends on the respiration of the products and the gas exchange between inside and outside of the package occurring through the film used. Therefore, the proper selections and determinations of the film materials, thickness of the film, surface area of the package and weight of products are necessary to create suitable modified atmosphere condition inside the package. If the permeability of the film is lower against product's respiration, the O_2 concentration within the package becomes too low, and then aerobic respiration would soon turn into anaerobic one,

which induces fermentative process due accumulation of ethanol and acetaldehyde (Joles et al., 1994; Petracek et al., 2002). At excess CO_2 concentration also induces the accumulation of acetaldehyde and ethanol (Pesis et al., 2002), leading to reduced aroma biosynthesis and the possibility of off-flavors. High CO_2 level inside the package also increases MDA content, which are products of cell membrane damage (Larrigaudiere et al., 2001). If the permeability of the film is too high, the gas composition inside the package was not regulated as a result the packaging does not give any effect to the product packed. In addition, water vapour exchange in the MAP system affects RH, which plays an important role in physiological responses that influence produce quality. Low RH storage increases water loss, accelerating the deterioration of fresh produce. But the maintenance of high humidity in MAP encourages moisture condensation on commodities and creates favorable conditions for microbial growth.

1.5. Objective of study

With respect to alleviating CI in cucumber fruits using MAP, there is little available information, but cucumber fruit is a popular commodity worldwide and improvement in its storability is desired. Wang and Qi (1997) compared the storability at low temperature among cucumber fruits packed in sealed and perforated low-density polyethylene (LDPE) bags and non-packed fruits and reported that MAP could confer chilling tolerance on cucumber fruits. However, the most influential condition in MAP to reduce CI in cucumber fruit is unclear. The response of chilling-sensitive products to low O_2 and high CO_2 is quite different among commodities (Beaudry, 2000; Watkins, 2000). Moreover, the optimal MAP conditions for alleviating CI in cucumber fruits have not yet been established because the efficacy of MAP depends strongly on O_2 and

CO_2 concentrations inside the packaging. CI is caused by the lipid peroxidation reaction of cell membrane lipids, in which the role of O_2 is critical. On the other side, excessive of CO_2 gives a harmful effect because it stimulates the respiration of cucumber fruit (Kubo et al., 1989)

The main purpose of this study was to design the optimal MAP for alleviating CI in cucumber fruit by analyzing its environmental factors such as low O_2, high CO_2, and relative humidity (RH). Based on analysis of these factors, the most effective conditions for successful MAP designing in alleviating CI in cucumber fruit was determined. Then, MAP was developed using a mathematical model by integrating many variables such as the respiration rate of the product, gas transmission rate through the package, surface area, free volume, and weight of the product.

In chapter 2, the effect of RH on the development of CI symptoms in cucumber fruit during low-temperature storage was determined. Cucumbers fruit were stored at 5°C for 5 days under three RH conditions: 60% (low RH), 80% (medium RH) and 100% or saturated (high RH). During storage gas composition inside the chamber was maintained as same as ambient air. After storage at 5°C, fruits were transferred to ambient air at 24.5°C and stored for 6 days. Quality parameter such as weight loss and firmness as physical indices, skin color as sensory evaluation, and electrolyte leakage and MDA as the CI indices were evaluated (Fahmy and Nakano, 2013).

In chapter 3, the individual and combined effects of low O_2 and high CO_2 on CI suppression in cucumber fruit were investigated. Four gas compositions were tested: low O_2, low O_2 with high CO_2, high CO_2 and ambient air as control. After storage at 5°C, the fruits were transferred to ambient air at 24.5°C and stored for 6 days. Quality parameters including weight loss and firmness as physical indices, skin color as sensory

evaluation and electrolyte leakage and MDA as CI indices were determined (Fahmy and Nakano, 2014a).

In chapter 4, the design technique of MAP for cucumber fruits stored at low temperature using a mathematical model was developed. The critical low O_2 limit of cucumber fruits was determined by monitoring the respiratory quotient (RQ) with decreasing O_2 concentration in the environment. The respiration rate of cucumber under modified atmospheres at various O_2 concentrations was also measured and modeled. The relationship among film permeability, surface area of the package, and weight of packed produce, leading to the equilibration of O_2 concentration in the package at the critical low O_2 was determined by application of the mathematical model to the gas composition change in MAP. Moreover, the effect of CO_2 accumulation inside the package on CI suppression of cucumber fruit was also evaluated by MDA equivalent (Fahmy and Nakano, 2014b).

Finally, in chapter 5 elaborated the conclusions and future perspective of the present study.

CHAPTER 2

INFLUENCE OF RELATIVE HUMIDITY ON DEVELOPMENT OF CHILLING INJURY IN CUCUMBER FRUIT DURING LOW-TEMPERATURE STORAGE

2.1. Introduction

The high demand of agricultural products has been encouraged their trade values in domestic and international market. In side of fresh agricultural products, maintained their quality before achieved by consumers is main objective in order to increase the number of marketability because they are perishable and loss of their quality during distribution process.

Low-temperature storage is main postharvest way to improve storage life of perishable products. It has effect directly in lowering fruit respiration, ethylene production, and fruit metabolism. For fresh agricultural produces, some of them are sensitive to chilling- temperature. Prolonged of storage period may result in chilling injury (CI), whose symptoms are develop when the products are removed from chilling to shelf life condition.

Cucumber fruit is chilling sensitive and susceptible to CI for more than 3 days held at temperatures of less than 10°C indicating in accumulation of the lipid peroxide and malondialdehyde (MDA) equivalent (Karakaş, and Yıldız, 2007). The manifestations of CI are characterized as surface pitting, dark watery patches and increased susceptibility to decay. Modified atmosphere packaging (MAP) has been reported in alleviating CI in cucumber fruits (Wang and Qi, 1997). Increase in humidity,

reduction in O_2 concentration and elevation of CO_2 inside the package are beneficial for preventing the development of CI symptoms (Forney and Lipton, 1990).

In actual distribution chain of fresh agricultural products, control of temperature storage is often conducted in delaying of deterioration, while maintenance the relative humidity (RH) is not always carried out. Water loss is a main cause of postharvest deterioration whose rate depends on the RH. It causes the products loss in quantitative (loss of saleable weight), appearance (wilting and shrivelling), and textural quality (softening, flaccidity and loss of crispness) (Kader, 2002a). Low in RH increases the transpiration damage and leads the products to desiccation; conversely, a higher in RH induces moisture condensation and decay to commodity.

Although recommendation on RH have been made for most commodities, the number of studies in which RH have been independently controlled is limited, and controlling of humidity at low-temperature is difficult to conducted. Thus, the purpose of study was to evaluate the effect of RH on the development of CI symptoms in cucumber fruit at low-temperature storage. Cucumbers fruit were selected in the present study because they are highly perishable product due to water loss. Fruits were stored at 5°C for 5 days under 3 RH conditions: 60% (low RH), 80% (medium RH) and 100% or saturated (high RH). After storage at 5°C, fruits were transferred to 24.5°C under ambient air to check the shelf life for 6 days. Quality parameter such as weight loss and firmness as physical indices, skin color as sensory evaluation, and electrolyte leakage and MDA as the CI indices were evaluated.

2.2. Materials and methods

2.2.1. Plant materials and storage conditions

Cucumber fruits (*Cucumis sativus* L.) were purchased from Kanesue Supermarket in Gifu City, Japan. The fruits were sorted and selected on the basis of uniform size and absence of visual defects. About 2 kg of fruits were placed into an acrylic chamber with a volume of 12.5 L for each RH tested. The chamber was then placed in an incubator (MIR-154-PJ, Panasonic, Japan) set temperature at 5°C for 5 days. Fruits were stored under 3 RH conditions: (1) 60% (low RH), (2) 80% (medium RH) and 100% or saturated (high RH). During storage, the atmosphere composition in the chamber was maintained as same as ambient air. Fig. 2.1 shows schematic diagram of experimental apparatus for controlling of relative humidity (RH). RH in chamber was monitored using a RH controller equipped with a sensor (Japan-Elekit, Japan). Silica gel bed was used to absorb the water vapour in the chamber. The outputs from RH sensor were used as input by solid-state relay (SSR), which controlled an air pump for flushing gas compositions from chamber to the silica gel bed. When the RH in the chamber differed from set value, the air pump turned on to flow the gas composition from the chamber to the silica gel bed for absorbing water vapour and then streamed back into chamber according to set value. RH and temperature changes in the chamber were recorded during storage using a hygrothermograph (TR-52, T&D Corporation, Japan). Gas was circulated inside the chamber using a suction pump with a zirconia O_2 sensor (MC-86, Ijima Electronic, Japan) and a solid state CO_2 probe (GMP221, Vaisala, Finland) to monitor changes in gas composition during storage. The outputs from the O_2 and CO_2 sensors were collected by a data recorder (TR-V550, Keyence, Japan), which controlled an air pump for flowing gas from external atmosphere into the chamber.

When the recorded gas composition in the chamber differed from the ambient air due to respiration by the cucumbers fruit, the air pump turned on and supplies fresh gas from the external atmosphere into the chamber.

After storage at 5°C, fruits were transferred to 24.5°C under ambient air for 6 days. Fruits were packaged in polyethylene film bags to prevent water loss. Some small holes were made so as not to occur the gas modifications inside the package. Skin color, firmness, electrolyte leakage, and MDA equivalent were evaluated before and after storage at 5°C (5d) and followed every 2 days at 24.5°C (7, 9, 11 d), while weight loss was evaluated only before and after storage at 5°C (5d). The flesh was cut into small cubes, frozen in liquid nitrogen quickly and stored in a freezer at −50°C (NF-300SF, Nihon Freezer, Japan) until analysis. The results of quality test were expressed as a percentage (%) i.e. the ratio of the value at time to the value at initial.

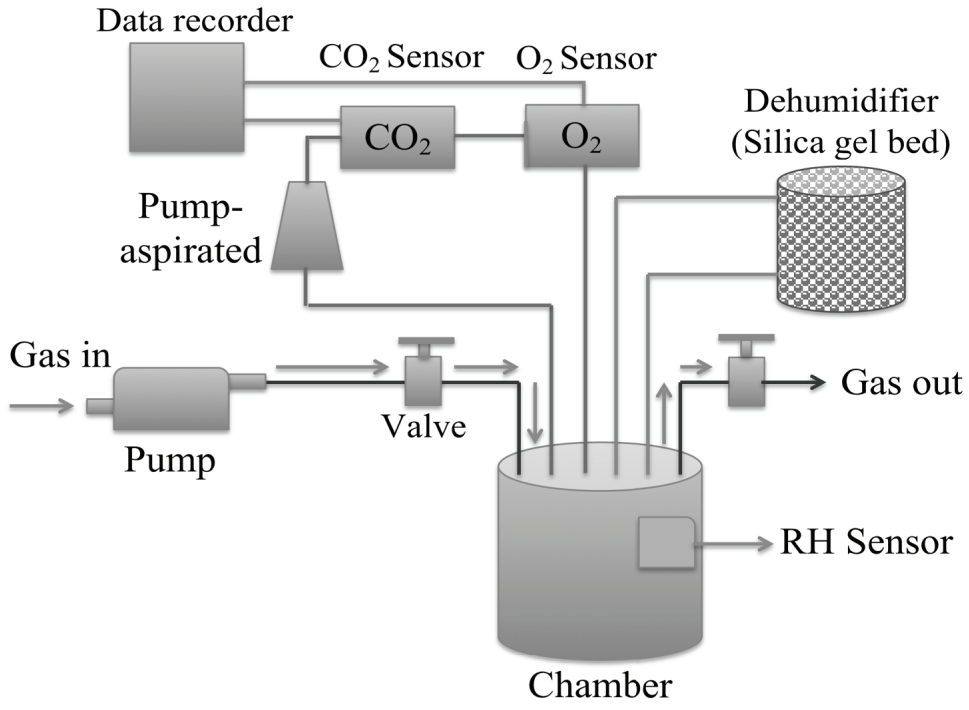

Fig. 2.1. Schematic diagram of experimental apparatus for controlling of relative humidity (RH)

2.2.2. Weight loss

Weight loss (*WL*) was determined in each cucumber plant. Each plant was weighed immediately after arrival at the laboratory (*HW*, harvest weight), and then after removal from refrigerated storage (*SW*, storage weight). Weight loss of each individual plant was calculated as:

$$WL\ (\%) = (1 - \frac{SW}{HW}) \times 100 \qquad (2.1)$$

WL was expressed as percentage of weight loss with respect to fresh mass.

2.2.3. Skin color

Skin color was measured using Minolta chromameter (CR-13, Minolta, Japan) to get parameter L^*, a^* and b^*. The measurements of color were carried out from fives fruits. Four reading were made at equator of the fruit. The results expressed as L^* value correspond to lightness, whereas chorma and hue-angle (h^o) conform to intensity and actual color calculating from $[(a^*)^2 + (b^*)^2]^{1/2}$ and arc-tan b^*/a^*, respectively (McGuire, 1992). Yellowing index (YI) was also determined calculating from $L^*b^*/|a^*|$ (Hirota et al., 2003).

2.2.4. Firmness

Firmness was measured for 5 fruits with a Rheometer (Compac-100 II, Sun Scientific, Japan) equipped with a 30 mm diameter plate plunger and operated at a depth of 1 mm with 30 mm/min of crosshead speed. A stainless steel cork borer was used to

produce 17.5 mm diameter and 20 mm thick sample discs. The results expressed as *F* (N), which represent the force exerted on a sample under compression.

2.2.5. Electrolyte Leakage

Electrolyte leakage was assessed using a method described by Saltveit (2002) with some modifications. Mesocarps of cucumber fruit (11 mm diameter) were excised with a stainless steel cork borer to produce 4 mm thick discs. The discs were soaked into fresh deionized water for 1 min to remove the excess ion on the tissues surface. This treatment was replicated 3 times, after that, the discs were blotted to dry by spreading onto absorbent paper to remove free water present on the surface. Then, 3 selected discs were placed into 50 mL centrifuge tubes with 20 mL of 0.2 M mannitol. The tubes were shaken at 100 cycles/min in a water bath incubator at 25°C (Personal-11, Taitec, Japan). Electric conductivity was measured with a conductivity meter (ES-51, Horiba, Japan) at 0.5 h after addition of the mannitol solution. The tubes were then frozen, thawed and weighed. The contents were incubated for 10 min in a 50 mL flask, allowed to cool to room temperature and transferred back to the plastic tubes. Deionised water was added to the initial weight and total conductivity was measured after an additional 0.5 h of shaking. Individual conductivity readings were converted to percentage of total conductivity.

2.2.6. Molondialdehyde

Malondialdehyde (MDA) was determined according to the method of Hodges et al. (1999) with some modifications. Mesocarp tissue (1 g) of cucumber fruit was homogenised in 10 mL of 80% (v/v) ethanol along with 0.5 g inert sand using a mortar

and pestle, followed by centrifugation at 3000 × g at 4°C for 10 min. A 1 mL aliquot of the appropriately diluted sample was added either to 1 mL of 0.65% thiobarbituric acid (TBA) solution containing 20% (w/v) trichloroacetic acid (TCA) and 0.01% butylatedhydroxytoluene (BHT) or to a solution containing 20% (w/v) TCA and 0.01% BHT. The samples were then mixed vigorously for 1 min, boiled for 25 min, cooled in an ice bath immediately and centrifuged at 3,000 × g at 4°C for 10 min. Absorbances at 532 nm, 440 nm and 600 nm were recorded using a spectrophotometer (UV1600, Shimadzu, Japan). The MDA equivalents were calculated by the following equations:

$$\left[(A532_{+TBA} - A600_{+TBA}) - \left(A532_{-TBA} - A600_{-TBA}\right)\right] = A \quad (2.2)$$

$$\left[(A440_{+TBA} - A600_{+TBA}) \times 0.0571\right] = B \quad (2.3)$$

$$\text{MDA equivalents } \left(\text{nmol ml}^{-1}\right) = \left((A - B)/157000\right) \times 10^6 \quad (2.4)$$

2.2.7. Statistical Analysis

The results were completely randomized with 5 replications (5 fruits per test). Statistical significance was determined by submitting the means values to analysis of variance and was subsequently compared using Tukey test at the 5% probability level that performed by R software (version 2.15.2 for Windows, R Foundation).

2.3. Results and discussion

The weight loss of cucumber was varied after stored at 5°C, reaching values of 21.35%, 14.65% and 0.62% of fruits stored at low, medium and high RH, respectively (Fig. 2.2). These results were expected as weight loss of low RH cucumber was one half compared with medium RH and 20 times higher than high RH. Weight loss is attributed

to water loss resulting from transpiration. Water loss is an important physiological process that affects the main quality characteristic of fresh commodities. Loss of water from fresh products after harvest is a serious problem causing shrinkage and loss of weight (Mahajan et al., 2008). Most commodities become unsalable as fresh product after losing 3-10% of their weight (Ben-Yehoshua and Rodov, 2003). In our results, the increase of water loss after storage at 5°C under low and medium RH conditions was clearly demonstrated by the higher of its value compared with high RH. Aqüero et al. (2011) reported that weight losses of fresh vegetables can be primarily attributed to: (1) evaporation of a moisture layer that persists on the vegetable surface after harvest; (2) dehydration, that is water loss due to the difference in water vapour pressure between the atmosphere and the foodstuff; (3) respiration, which is consists of carbohydrate breakdown to yield carbon dioxide and water. These results suggest that to extend usable life of fresh products, the rate of water loss must be as low as possible.

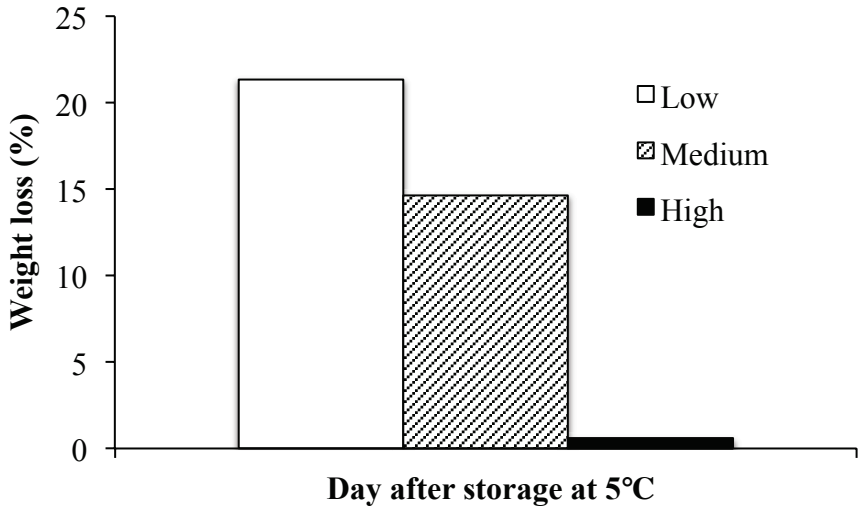

Fig. 2.2. Weight loss of cucumber fruits after stored at 5°C under 3 different RH conditions: 60% (low RH), 80% (medium RH) and 100% (high RH).

Exposed of cucumber fruits under different RH conditions had a different effect on development of external color retention after storage 5°C (Table 2.1). Significant difference in lightness was shown among RH conditions tested after storage at 5°C, particularly, the lightness of fruit stored under high RH was higher than those stored under low and medium RH. These results indicate a dark green of skin color because of the severe dehydration under low and medium RH. Intensity in terms of chroma was lower significantly of fruits stored under low RH compared with fruit stored under medium and high RH. On other hand, the actual color of fruit (hue-angle) was also different significantly among RH conditions tested after storage at 5°C, for which the hue-angle of fruit stored under low RH was lower than those stored under medium and high RH. The dehydration that occurred at low RH causes a deleterious effect on the overall visual quality (Medina et al., 2012). Storage under low and medium RH conditions resulted in substantial degradation in the appearance of cucumber fruits, mainly loss of their lightness, chroma and hue-angel.

Table 2.1. Relative skin color index of cucumber fruit after stored at 5°C for 5 days under 3 different RH conditions: 60% (low RH), 80% (medium RH) and 100% or saturated (high RH).

RH	Relative skin color (%)		
	Lightness	Chroma	Hue-angel
Low	91.6a	72.8a	95.4a
Medium	95.8b	91.7b	101.0b
High	98.2c	99.6b	98.4c

Different letters in the same column were significantly different ($P<0.05$) according to Tukey HSD (Honestly Significant Difference).

Yellowing index (YI) of the skin surface is a common postharvest disorder in cucumber fruit due to storage at ambient temperatures for several days. Cucumber fruits are susceptible to CI at the temperatures lower than the optimum storage temperature with the prolonging storage period and to yellowing at high temperatures (Ryall and Lipton, 1979; Salunkhe and Desai, 1984). A significant increase in YI was observed of the fruit stored at low RH after transfer to 24.5°C, while it was maintained up to day 7 of the fruit stored at medium RH, and then increased significantly thereafter. On other hand, the YI of fruit stored under high RH was maintained up to day 9, and significant increase appeared on day 11 (Fig. 2.3). Pitting, dark watery patches and increase susceptibility to decay are visible symptoms of CI in cucumber fruit (Wang and Qi, 1997; Hakim et al., 1999; Mao et al., 2007b; Yang et al., 2011). In our results, manifestation of decay was increased rapidly of fruit stored at low RH after transferred to 24.5°C. As a result the cucumber fruits only could be observed until day 7. Morris and Platenius (1938) also reported that cucumbers stored at 5°C for 7 days developed severe pitting in 50–60 % RH, while the pitting was prevented in 95–100% RH.

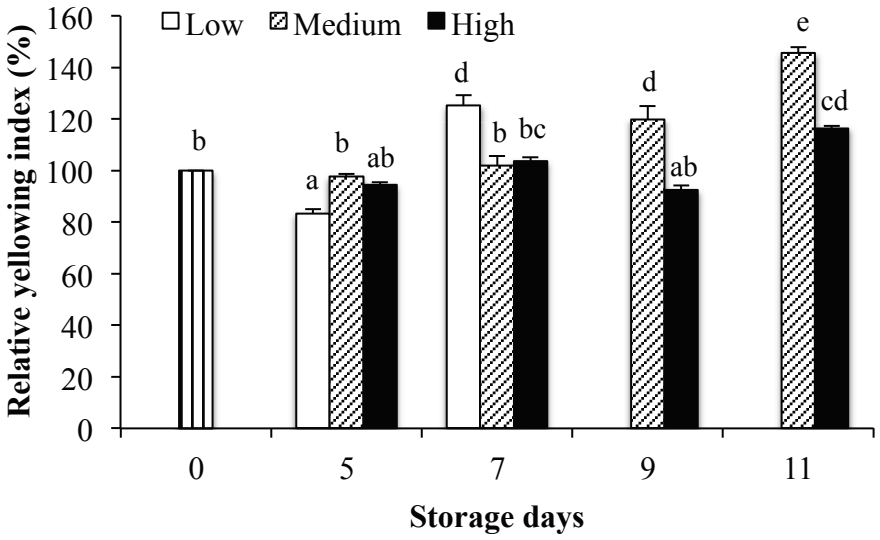

Fig. 2.3. Relative yellowing index of cucumber fruits stored at 5°C for 5 days under 3 different RH conditions: 60% (low RH), 80% (medium RH), and 100% (high RH) followed by 24.5°C for 6 days under ambient air. Vertical lines represent standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

Fig. 2.4 shows the firmness of cucumbers fruits stored at 5°C for 5 days under 3 different RH conditions followed by 24.5°C for 6 days under ambient air. The firmness of fruit decreased after storage at 5°C for all the RH tested, for which significant decrease was shown of fruit stored under low RH. After transferring to ambient air at 24.5°C, the firmness increased at the early stage of storage and then decreased gradually, but significant difference did not show among them. RH had a related effect to fruit softening, which the fruit stored at low and medium RH lose their firmness more than fruit stored at high RH because a greater in water loss (Sharkey and Peggie, 1984).

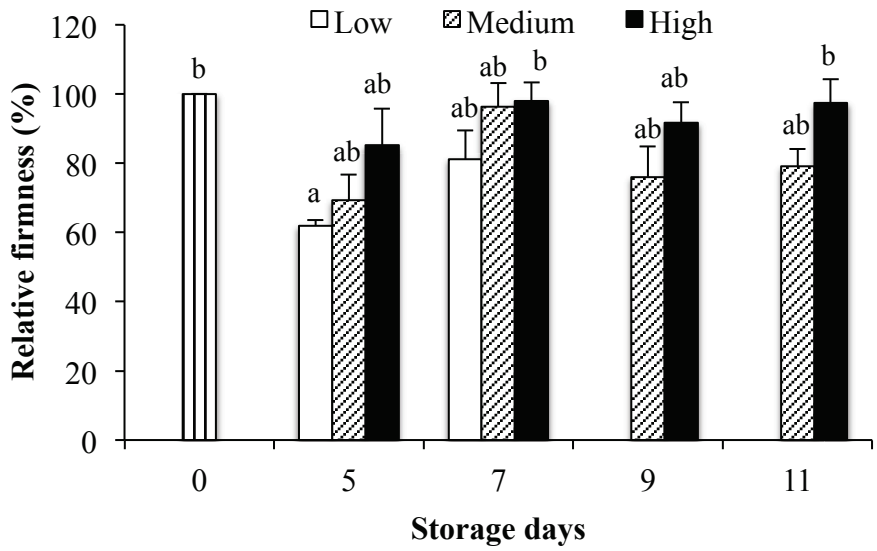

Fig. 2.4. Relative firmness of cucumber fruits stored at 5°C for 5 days under 3 different RH conditions: 60% (low RH), 80% (medium RH), and 100% (high RH) followed by 24.5°C for 6 days under ambient air. Vertical lines represent standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

Increase electrolyte leakage of cucumber fruits during storage at chilling temperature has been reported as a qualitative indicator of CI (Mao et al 2007a; Yang et al., 2011). Fig. 2.5 shows the electrolyte leakage of cucumbers fruit stored at 5°C for 5 day under 3 different RH conditions followed by 24.5°C for 6 days under ambient air. The electrolyte leakage of fruit increased after storage at 5°C for all RH conditions tested, however, significant difference was not found among them after storage. After transferring to room temperature, the electrolyte leakage of fruit stored under low RH increased significantly on day 7, while the fruits stored under medium RH, the electrolyte leakage decreased at the early stage of storage and increased significantly on day 11. On other hand, the electrolyte leakage of fruit stored under high RH also decreased at the early stage of storage, but maintained at the same level thereafter. The decrease of electrolyte leakage at the early stage of room temperature storage is probably due to bulk membrane lipids phase were returned in the liquid-crystalline phase, while bulk-membrane lipid-phase transform from liquid-crystalline phase to solid-gel phase lipids at low-temperature, leading to increase the permeability or leakiness of cellular membranes (Parkin et al., 1989). While, rapid increase of electrolyte of fruit stored under low (on day 7) and medium (on day 11) RH after transferred to room temperature is caused by the increase susceptibility of cucumber fruit to decay.

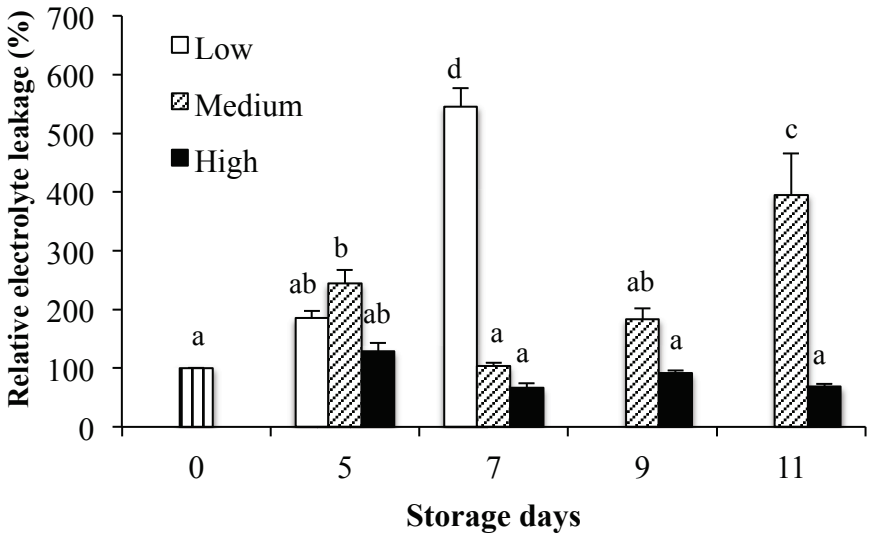

Fig. 2.5. Relative electrolyte leakage of cucumber fruits stored at 5°C for 5 days under 3 different RH condition: 60% (low RH), 80% (medium RH) and 100% (high RH) followed by 24.5°C for 6 days under ambient air. Vertical lines represent standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

As the final product of lipid peroxidation, MDA is often used as an index of cell oxidative damage under environmental stress (Shen and Wang, 1997). Fig. 2.6 shows the MDA equivalent of the cucumbers fruit stored at 5°C for 5 days under 3 different RH conditions followed by 24.5°C for 6 days under ambient air. The change in MDA equivalent shared similar trends with electrolyte leakage. The MDA equivalent increased significantly for all RH conditions tested during the 5°C storage period. After transferring to room temperature, the MDA equivalent of fruit stored under low RH continued to increase until day 7, after that fruits were decay and could not be observed. While, the MDA equivalent was maintained up to day 9 of fruit stored under medium RH, after that the MDA equivalent increased significantly. However, the MDA equivalent of fruit stored under high RH was maintained at the same level during the shelf life period. The increase of MDA under low and medium RH might be caused by higher of the weight loss, which it related to development of CI through cellular breakdown, deterioration of membrane integrity as well as loss of epicuticular wax, which is important in water exchange through cucumber fruit skin (Hakim et al., 1999).

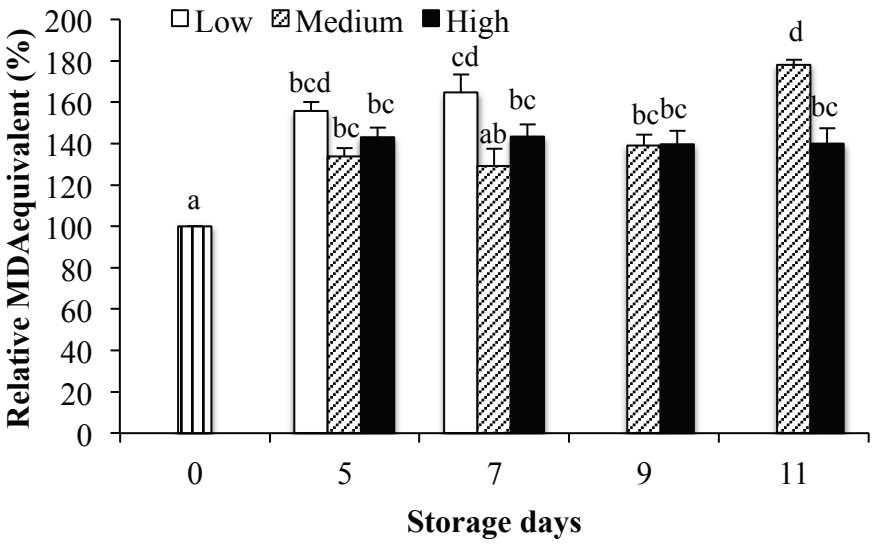

Fig. 2.6. Relative malondialdehyde (MDA) equivalent of cucumber fruits stored at 5°C for 5 days under 3 different RH condition: 60% (low RH), 80% (medium RH) and 100% (high RH) followed by 24.5°C for 6 days under ambient air. Vertical lines represent standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

2.4. Conclusion

In this study, the development of CI symptoms of cucumber fruit differed when they are stored under different RH conditions. Storage of cucumber fruit under low or medium RH increased the water loss and accelerated of decay after transferred to shelf life condition. High RH storage not only reduced water loss and subsequently maintained fruit skin color change and firmness but also significantly minimized the expression of CI. Furthermore, the humidity control must be taken into account for preserving the quality of cucumber fruits during low-temperature storage. The results suggest that application of MAP using a plastic film material having a low water vapour transmission rate and anti microbial growth may be effective for alleviating CI in cucumber fruit.

CHAPTER 3

THE INDIVIDUAL AND COMBINED INFLUENCES OF LOW OXYGEN AND HIGH CARBON DIOXIDE ON CHILLING INJURY ALLEVIATION IN CUCUMBER FRUIT

3.1. Introduction

The consumption of fruits and vegetables in which freshness and safety are guaranteed increases their value in both domestic and overseas markets. The export market usually involves long distances and durations of transportation, and the time to market may be extended by customs and weather restrictions. This condition reduces product quality and often shortens the retail shelf life owing to perishability. For this reason, technology for preventing quality loss during transportation is of primary concern in the international trade in fresh commodities.

Low-temperature storage is the primary tool used for maintaining the quality of perishable commodities during distribution. In general, cold storage retards deterioration by lowering respiration rate, ethylene production and other physiological activities (Wills et al., 2007). However, some commodities are chilling-sensitive and are injured when stored at low temperature (Kader, 2002a), thereby shortening shelf life and reducing market quality, as reported for persimmon fruit (Macrae, 1987).

In actual distribution chain of cucumber fruit, it approximately takes 5 days from harvesting to the table of consumer, and cucumbers fruit were often transported and stored at low-temperature with other kinds of fresh commodities. However, cucumber fruit is also chilling-sensitive and susceptible to chilling injury (CI) if held at

temperatures of <10°C for more than 3 days. The manifestations of CI are surface pitting, dark watery patches and increased susceptibility to decay (Cabrera and Saltveit, 1990). Therefore, various methods have been developed to reduce CI in cucumber fruit. The application of nitric oxide is one of the methods to reduce CI (Yang et al., 2011). Mao et al. (2007a) also revealed that pre-warming before low temperature storage could suppress CI based on the evaluation of electrolyte leakage and malondialdehyde (MDA) equivalent.

Among postharvest technologies available for limiting CI during the storage of chilling-sensitive products at low temperature, modified atmosphere packaging (MAP) is promising, and has been the subject of many studies. Packaging in low-density polyethylene (LDPE) bags delayed the onset of CI in cucumber fruit (Wang and Qi, 1997). MAP also alleviated CI in mango (Pesis et al., 2000), melon (Flores et al., 2004), peach (Fernández-Trujilio et al., 1998) and 'Fuyu' persimmon (Cia et al., 2006). The ability of MAP to reduce CI is thought as reduction in O_2 and elevation of CO_2 inside the package as well as a higher humidity inside packaging. Although MAP has been shown to reduce CI in chilling-sensitive, it is unclear which conditions are the most influential to reduce CI. The response of chilling-sensitive products to low O_2 and high CO_2 is quite different among commodities (Beaudry, 2000; Watkins, 2000). Exposure of fresh produce to levels above its CO_2 limit may cause physiological damage, and storage under the lower O_2 limit induces fermentation (Yearsley et al., 1996), leading to reduced aroma biosynthesis and the possibility of off-flavors.

Identifying the most effective conditions for preventing CI is required for successful MAP design, particularly for long-distance transportation of chilling-sensitive products. Thus, the purpose of this study was to develop a method for enabling

transportation of cucumber fruit at low-temperature without the onset of CI. The individual and combined effects of low O_2 and high CO_2 on CI suppression in cucumber fruit were investigated. Four gas compositions were tested: low O_2, low O_2 with high CO_2, high CO_2 and ambient air as control. Quality parameters including weight loss and firmness as physical indices, skin color as sensory evaluation, and electrolyte leakage as CI indices were determined before and after storage at low temperature. In addition, MDA was measured as a preceding indicator for CI, because CI induces the accumulation of reactive oxygen species (ROS) which cause oxidative damage to the cell membrane lipid, leading to increase MDA (Imahori et al, 2008). In this experiment, assuming a practical distribution chain from farm to table, cucumber fruits were stored at 5 °C under various gas conditions as mentioned above for 5 days, subsequently they were stored at room temperature under ambient air for 6 day to evaluated the progress of CI.

3.2. Materials and methods

3.2.1. Plant materials and storage conditions

Cucumbers fruit (*Cucumis sativus* L.) at commercial maturity were purchased from a wholesale store in Gifu Prefecture, Japan and transported immediately to laboratory. The fruits were sorted and selected on the basis of uniform size and absence of visual defects. About 2 kg of fruits were placed into an acrylic chamber with a volume of 4.8 L for each gas composition tested. The chamber was then placed in an incubator (MIR-154-PJ, Panasonic, Japan) at 5°C for 5 days. Based on information of recommended gas composition for storing cucumber fruits (1–4% O_2, 0% CO_2) (Kader, 2002b), fruits were stored under 4 gas compositions: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with

high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) ambient air as control. Fig. 3.1 shows schematic diagram of experimental apparatus for controlling of atmosphere (CA). Gas was flushed into the chambers continuously from standard gas bottles with a flow rate of 50 mL/min. The gas compositions during storage were monitored by a zirconia O_2 sensor (MC-86, Ijima Electronic, Japan) and a solid state CO_2 probe (GMP221, Vaisala, Finland). Relative humidity (RH) and temperature changes in the chamber were measured using a hygrothermograph (TR-52, T&D Corporation, Japan). RH was approximately 100% during low-temperature storage.

After storage at 5°C, the fruits were transferred to ambient air at 24.5°C and stored for 6 days. Fruits were packaged in polyethylene film bags to prevent water loss. Some small holes were made so as not to occur the gas modifications inside the package. Fruit qualities, such as skin color, firmness, electrolyte leakage and MDA equivalent, were evaluated before and after storage at 5°C and the subsequent storage under ambient air at 24.5ºC. Weight loss was measured before and after storage at 5°C. The flesh was cut into small cubes, frozen in liquid nitrogen quickly and stored in a freezer at −50°C (NF-300SF, Nihon Freezer, Japan) until analysis.

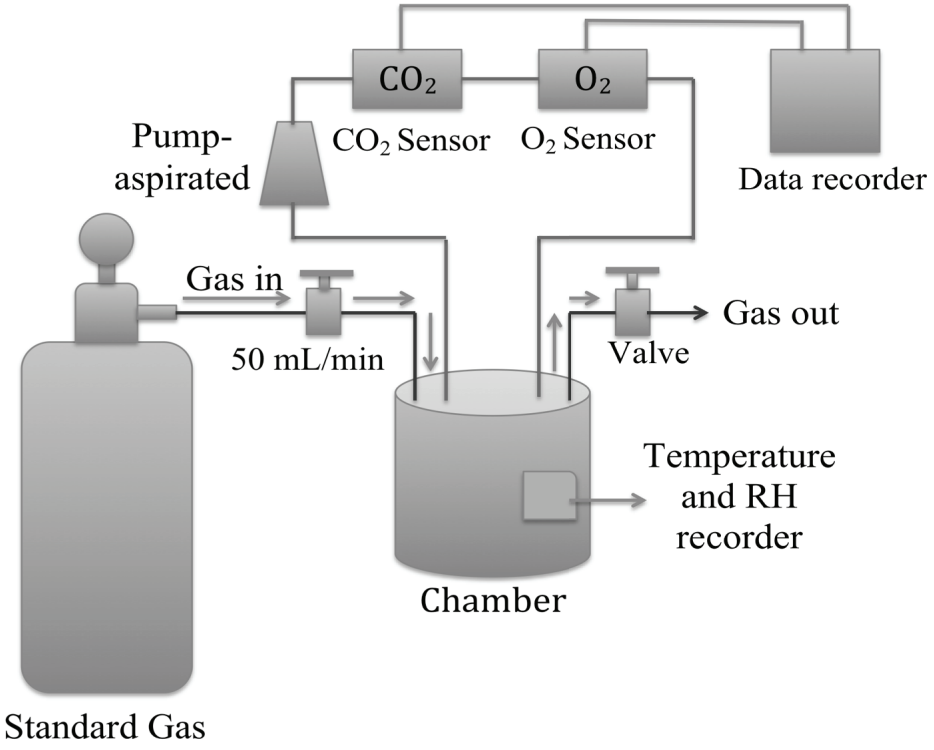

Fig. 3.1. Schematic diagram of experimental apparatus for controlling of atmosphere (CA)

3.2.2. Weight loss

The weight of cucumbers fruit from each experimental condition was measured immediately after arrival at the laboratory and then after removal from refrigerated storage. Weight loss of each fruit in the chamber was calculated as a percentage of initial fresh weight as mentioned in chapter 2.

3.2.3. Skin color

Skin color was measured with a Minolta chromameter (CR-13, Minolta, Japan) yielding parameters L^*, a^* and b^*. The L^* value indicates lightness, a^* indicates chromaticity on a green (-) to red (+) axis, and b^* represent chromaticity on a blue (-) to yellow (+) axis. Color measurements were made for 5 fruits. Four readings were made at the fruit equator. The results expressed yellowing index determined as $L^*|b^*/a^*|$ (Hirota et al., 2003).

3.2.4. Firmness

Firmness was measured for 5 fruits with a rheometer (Compac-100 II, Sun Scientific, Japan) as mentioned in chapter 2.

3.2.5. Electrolyte leakage

Electrolyte leakage was assessed using a method described by Saltveit (2002) with some modifications as described in chapter 2.

3.2.6. Malondialdehyde

Malondialdehyde (MDA) was determined according to the method of Hodges et al. (1999) with some modifications as described in chapter 2.

3.2.7. Statistical analysis

The design was completely randomised with 5 replications (5 fruits per test). Statistical significance was determined by subjecting the mean values to analysis of variance and means were compared by Tukey's test at the 5% level of significance using R 2.15.2 (R Foundation).

3.3. Results and discussion

The percentage of weight loss after low-temperature storage was lower for fruits stored under the controlled gas composition than those stored under the ambient air (Table 3.1). Weight loss is attributed to water loss resulting from transpiration and evaporation from the surface of the fruits. Increased weight loss during low-temperature storage is also associated with the development of CI, which damages membrane integrity (Hakim et al., 1999). Similar findings have been reported for melon; water loss was reduced when stored in MAP for preventing CI (Flores et al., 2004). MAP contributed to reduction in water loss and is known to reduce the development of low-temperature tissue breakdown (Ben-Yehoshua, 1985). Moreover, the reduction of water loss under controlled atmosphere is also thought to reduce transpiration rate. Villaescusa and Gil (2003) concluded that MAP contributed to reduced transpiration at low temperature.

Table 3.1. Weight loss of cucumber fruit after stored at 5°C under 4 different gas compositions: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) control (ambient air).

Gas composition	Weight loss (%)
Low O_2	0.5
Low O_2 with high CO_2	0.5
High CO_2	0.5
Control	2.6

Fig. 3.2 shows changes in the yellowing index of cucumbers fruit stored at 5°C for 5 days under 4 different gas compositions followed by 24.5°C for 6 days under ambient air. The yellowing index of fruits stored under low O_2 did not change during period of storage. While, yellowing index of fruits stored under low O_2 with high CO_2, high CO_2 and ambient air were maintained up to day 9, but on day 11 increased significantly compared with the data on day 0. Low O_2 inhibits the degreening rate of green commodities caused by the loss of chlorophyll, as reported for broccoli (Makhlouf et al., 1989) and Galega kale (Fonseca et al., 2005). This response is probably due to low O_2 limitation of the pheophorbide a oxygenase reaction (Matile et al., 1999). Our results indicated that the yellowing index of cucumber fruit stored in high CO_2 was significantly high on day 11 even if combined with low O_2. It thought to be caused that the CO_2 concentration tested in this study exceeded the limit level for the cucumber fruit storage. In general, visible characteristics of CI in cucumber fruit has been often assessed by surface area scale of surface pitting and dark watery patches (Wang and Qi, 1997; Hakim et al., 1999; Mao et al., 2007b; Yang et al., 2011), however, from our results, these symptoms was not appear after storage at 5°C.

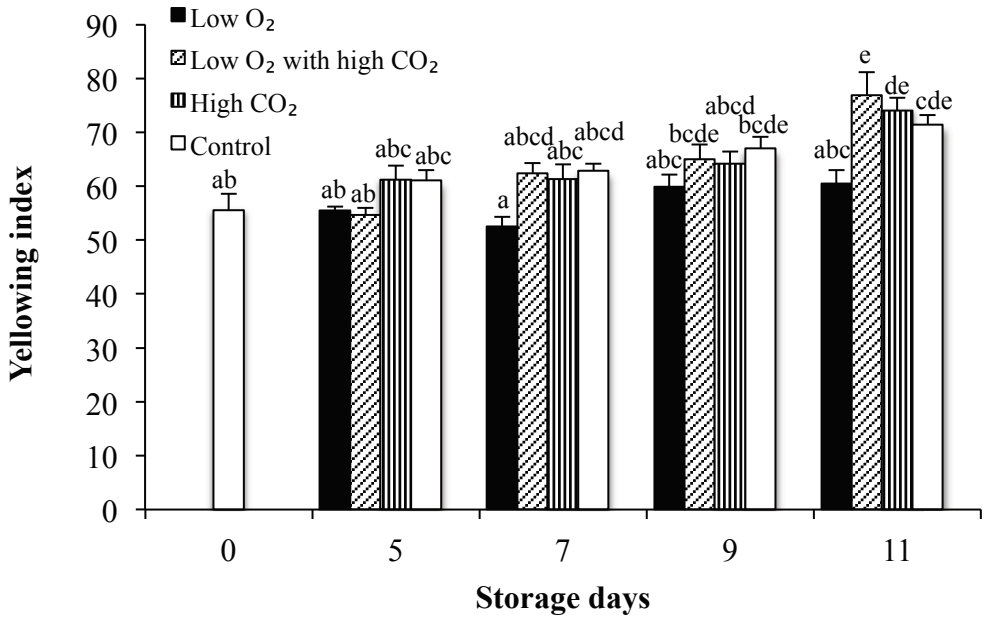

Fig. 3.2. Yellowing index of cucumber fruit stored at 5°C for 5 days under 4 different gas composition: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) control (ambient air) followed by 24.5°C for 6 days under ambient air. Vertical lines represent standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

Fig. 3.3 shows the firmness of cucumbers fruit stored at 5°C for 5 days under 4 different gas compositions followed by 24.5°C for 6 days under ambient air. The firmness decreased after storage at 5°C followed by storage under ambient air at 24.5°C for all gas compositions tested. Although significant difference was not found among all the different gas compositions, however, the average firmness of fruit stored under ambient gas composition was higher than those of fruit stored under controlled gas compositions on day 11. It is thought to be caused by greater water loss in fruit stored under the ambient gas composition, as indicated in the data described above. Drought hardening, caused by higher evapotranspiration, has been observed in plants experiencing water stress (Wilson, 1979).

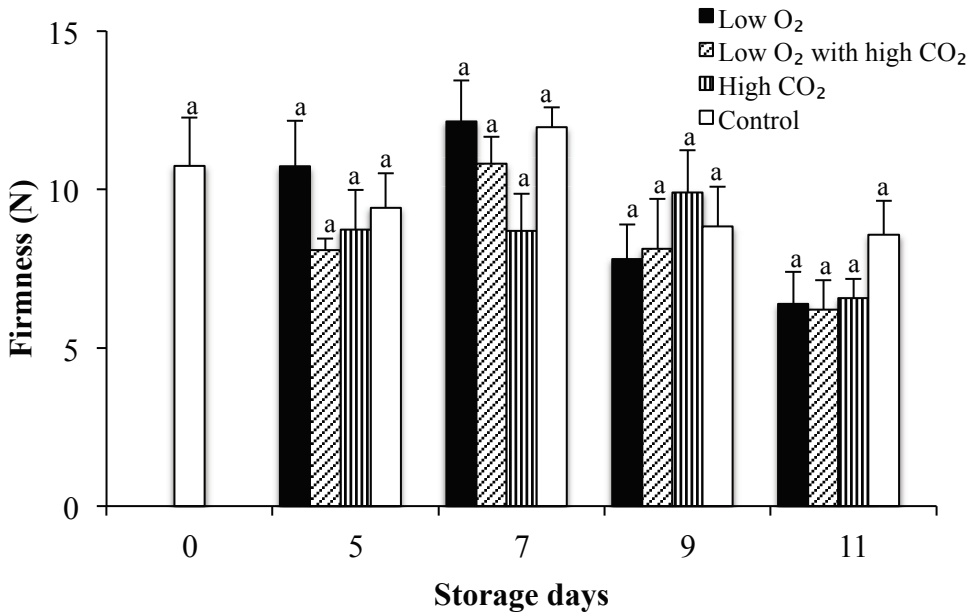

Fig. 3.3. Firmness of cucumber fruit stored at 5°C for 5 days under 4 different gas composition: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) control (ambient air) followed by 24.5°C for 6 days under ambient air. Vertical lines represents standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

Fig. 3.4 shows the electrolyte leakage of cucumbers fruit stored at 5°C for 5 day under 4 different gas compositions followed by 24.5°C for 6 days under ambient air. The electrolyte leakage of fruits stored under high CO_2, low O_2 with high CO_2 and ambient gas composition significantly increased during the 5°C storage period. On the other hand, no significant increase in electrolyte leakage was observed of fruit stored at low O_2. After transferring to room temperature, electrolyte leakage drastically decreased at the early stage of storage and maintained at the same level thereafter.

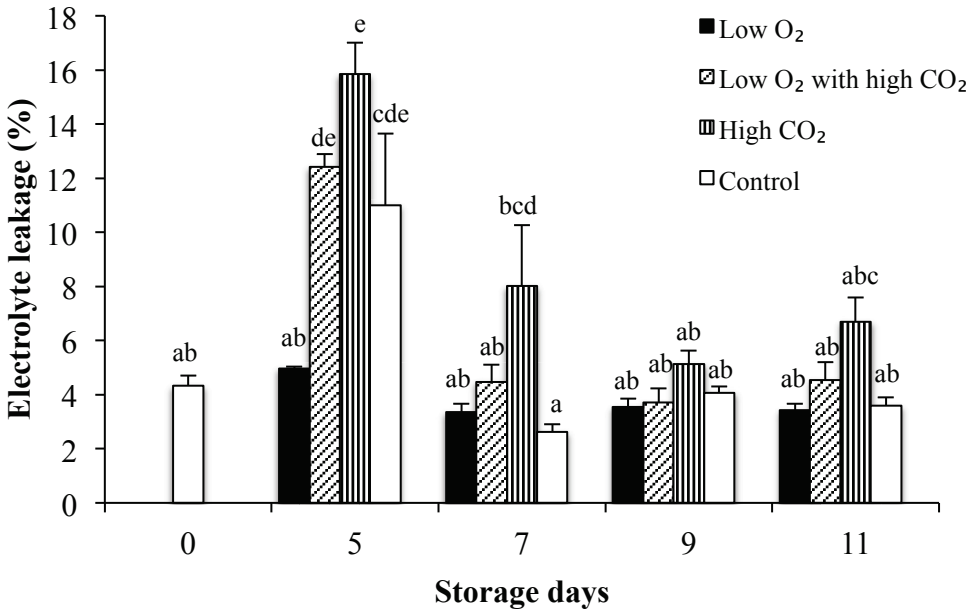

Fig. 3.4. Electrolyte leakage of cucumber fruit stored at 5°C for 5 days under 4 different gas composition: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) control (ambient air) followed by 24.5°C for 6 days under ambient air. Vertical line represents standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

Electrolyte leakage has been used as an indicator of cell membrane damage caused by CI. In many studies on membrane permeability, the rate of electrolyte leakage from chilling-sensitive tissues has been shown to increase during low-temperature storage in cucumber fruit (Mao et al., 2007a; Yang et al., 2011) and tomato (Saltveit, 2002). Increased electrolyte leakage suggests a perturbation of the transport properties of cell membranes that results in an altered cellular environment (Palta, 1990). The present study showed that electrolyte leakage drastically decreased during the early stage of room temperature. In chilling-sensitive products, bulk-membrane lipid-phase transition resulted in the formation of gel-phase lipids at chilling temperatures, leading to increased permeability or leakiness of cellular membranes. Conversely, at a higher temperature, membrane lipids were maintained in the liquid-crystalline phase (Parkin et al., 1989). Therefore, fruit transferred to room temperature did not display increased electrolyte leakage. Hirose (1985) has also found similar results in their previous study in which the effect of interposed warming on the electrolyte leakage from cucumber tissues during cold storage was examined, and they explained the mechanism of these phenomena from the point of view of the reversibility of the cell membrane denaturation. Marangoni et al. (1996) suggested that the measurement of electrolyte leakage for the evaluation of CI on chilling-sensitive tissue should be performed at a chilling temperature, without allowing the tissue to warm up. In fruit stored at low O_2, the membrane lipids were maintained in liquid-crystalline phase at the lower temperature, and, correspondingly, electrolyte leakage did not change during storage.

Fig. 3.5 shows the MDA equivalent of the cucumbers fruit during storage at 5°C for 5 days under 4 different gas compositions followed by 24.5°C for 6 days under ambient air. The MDA equivalent of the fruit stored at the ambient and low O_2 with

high CO_2 gas compositions were maintained at the same level up to day 7, after that, increased significantly until the end of storage. In case of fruit stored at high CO_2, the significant increase of MDA equivalent was observed on day 11. In contrast, the MDA equivalent of fruits stored at low O_2 did not increase significantly compared with that at day 0 during storage.

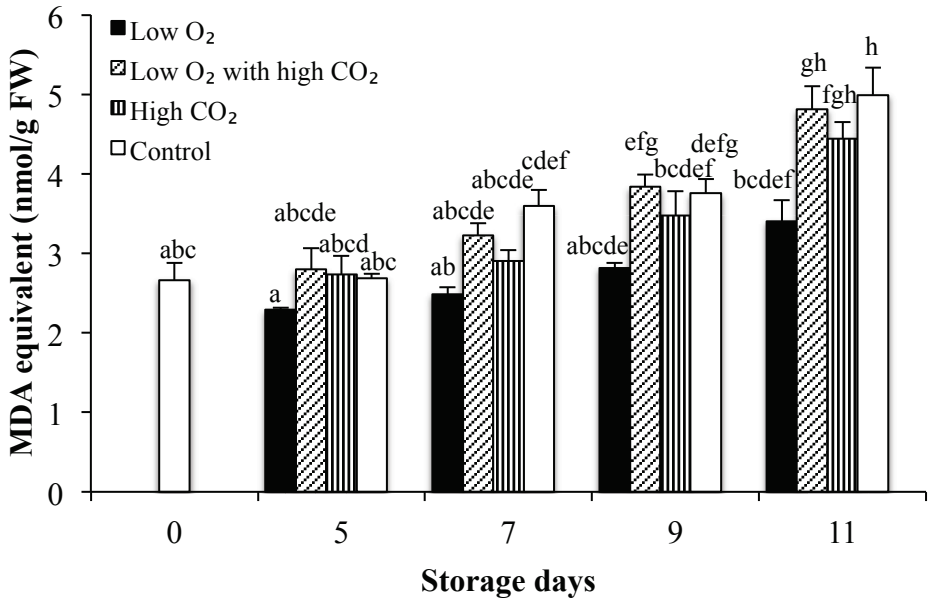

Fig. 3.5. MDA equivalent of cucumbers fruit during storage at 5°C for 5 days under 4 different gas composition: (1) low O_2 (4% O_2 and 0% CO_2); (2) low O_2 with high CO_2 (4% O_2 and 10% CO_2); (3) high CO_2 (21.5% O_2 and 10% CO_2) and (4) control (ambient air) followed by 24.5°C for 6 days under ambient air. Vertical lines represents standard error (n = 5). Values with different letters were significantly different at $P < 0.05$.

A quantitative index of the end products of lipid peroxidation, MDA is often evaluated in studies of plant mechanism under chilling stress. Low temperature induces the production of ROS, which leads to lipid peroxidation, damaged membrane structure, solute leaking and MDA accumulation (Xie et al., 2008). In the present study, as for the fruit stored at low O_2, the MDA equivalent at on and after 5 days storage were not significantly different from that at day 0. Lipid peroxidation is initiated by free radical attack of double bonds in polyunsaturated fatty acids, resulting in the production of a lipid radical, which is an unstable molecule that rapidly reacts with molecular oxygen to produce a lipid peroxyl radical. In addition, the lipid peroxyl radical is unstable and reacts with unsaturated fatty acids to produce lipid peroxide and another lipid radical (Young and McEneny, 2001). Because storage under low O_2 restricts the supply of molecular oxygen in the cell membrane tissue, the transformation of a lipid radical to a lipid peroxyl radical may be inhibited, resulting in a low MDA equivalent. In addition, storage at high CO_2 tended to accelerate the accumulation of MDA equivalent regardless of O_2 concentration. It has been reported that exposure to high CO_2 concentration increased MDA of pears, resulting in enhanced cell membrane damage (Larrigaudiere et al., 2001). Moreover, De Castro et al. (2008) reported that apples stored under high CO_2 concentration exhibited higher concentrations of hydrogen peroxide (H_2O_2) than apples stored under ambient air. H_2O_2 is commonly used as a measure for mitochondrial ROS production generated by the electron transport chain (Murphy, 2009). Storing fruit under high CO_2 may stimulate the electron transport chain, resulting in an enhancement of mitochondrial ROS release.

3.4. Conclusion

In this study, we evaluated the individual and combined effects of low O_2 and high CO_2 in suppressing CI in cucumber fruit. Storage under low O_2 was more effective for preventing CI than storing under low O_2 with high CO_2, high CO_2 and ambient air. These facts indicate that a synergistic effect of low O_2 and high CO_2 does not appear to reduce CI in cucumber fruit. The low O_2 gas condition is adequate for extending the shelf life of cucumbers stored at low temperature. For quality retention during long-distance, low-temperature transportation of cucumbers fruit, the application of MAP using a plastic film material having a very high CO_2 permeability or active MAP with a CO_2 absorber may be effective.

CHAPTER 4

OPTIMAL DESIGN OF MODIFIED ATMOSPHERE PACKAGING FOR ALLEVIATING CHILLING INJURY IN CUCUMBER FRUIT

4.1. Introduction

Cucumber fruits are consumed worldwide as a fresh vegetable. They are frequently transported and stored at low temperature with other kinds of fresh commodities because low temperature is the primary means of preserving the quality in most fresh produce. However, cucumbers are chilling sensitive and injured when exposed to temperatures below 7°C, which lead to visible pitting and increased susceptibility to decay (Hakim et al., 1999).

Modified atmosphere packaging (MAP) is one of the methods used for alleviating chilling injury (CI) in cucumber fruit (Wang and Qi, 1997). MAP is defined as the packaging of a perishable product such that the natural interplay between respiration of the packed product and gas transfer through the packaging material leads to an atmosphere with increased CO_2 and reduced O_2. These atmosphere compositions have been found to be beneficial for preventing CI in chilling-sensitive products by reducing respiration rate, ethylene production, accumulation of ethanol and acetaldehyde, and water loss (Fernández-Trujilio et al., 1998; Flores et al., 2004). In chapter 3, we confirmed that low O_2 conditions suppressed the increase of electrolyte leakage (EL) and malondialdehyde content, the main primary events of CI, in cucumber fruit (Fahmy and Nakano, 2014a).

Given that the change in the gas composition inside a film package is affected by respiration rate and gas interchange through the film, it is necessary to account for these factors in MAP designing. Harmful effects occur if the O_2 concentration inside MAP is out of the proper range. Exposure to O_2 at concentrations below the tolerance limit induces anaerobic respiration, leading to the development of off-flavors owing to the accumulation of acetaldehyde and ethanol, as reported for sweet cherry (Petracek et al., 2002). Knowing the critical low O_2 limit is also important for optimizing the storage atmosphere inside MAP. Therefore, for a successful MAP design, O_2 concentration inside the packaging must equilibrate at just above the critical low O_2 limit, in which the respiration of the packed fresh produce is reduced to the lowest level not leading to the onset of anaerobic respiration.

The selection of a packaging film material such that its gas permeability matches with the respiration rate is also important. To date, a trial-and-error approach has frequently been applied in practice. Fresh produce is packed and stored in various kinds of packaging film material, and then suitable materials are selected based on the measurement of the gas composition in the package and the evaluation of produce quality after packaging and storage. However, this approach is somewhat arbitrary and limited in usefulness because not only the gas permeability of the film but also the weight of the packed fresh produce and the surface area of the packaging affect the gas composition change inside the packaging. To overcome these obstacles, a mathematical model has been developed to predict the gas composition change and applied to many kinds of fresh commodities (Cameron et al., 1994; Joles et al., 1994; Jacxsens et al., 2000; Del-Valle et al., 2009; Finnegan et al., 2013). These models integrate many

variables such as the respiration rate of the product, gas transmission rate through the package, surface area, free volume, and weight of the product.

The mathematical model for MAP design requires the respiration rate of the packed fresh produce, which is affected by the gas composition surrounding the produce, and the temperature. Thus, modeling the respiration rate of products is central to the design of a successful MAP. To date, the Michaelis–Menten equation, which is based on the principles of enzyme kinetics, has been proposed to predict respiration rate as a function of O_2 and CO_2 concentration (Lee et al., 1991) and applied to cherry (Petracek et al., 2002), blueberry (Cameron et al., 1994), raspberry (Joles et al., 1994), broccoli (Lee et al., 1991), apple (Dadzie et al., 1996), Banana (Heydari et al., 2010) and other produce.

MAP systems usually increase CO_2 concentration. However, the response of fruits and vegetables to high CO_2 concentrations is considerably different among commodities (Watkins, 2000). Exposure to high concentrations of CO_2 reduces the respiration rate (Lee et al., 1991; Hertog et al., 1998; Fonseca et al., 2005) and ethylene production (Kubo et al., 1990). In contrast, it also induces the accumulation of acetaldehyde and ethanol (Pesis et al., 2002) and increases malondialdehyde which is products of cell membrane damage (Larrigaudiere et al., 2001). For this reason, differences in the response to CO_2 among commodities must be considered in the design of a successful MAP system.

With respect to alleviating CI in cucumber fruits using MAP, there is little available information, but cucumber fruit is a popular commodity worldwide and improvement in its storability is desired. Wang and Qi (1997) compared the storability at low temperature among cucumber fruits packed in sealed and perforated low-density

polyethylene (LDPE) bags and non-packed fruits and reported that MAP could confer chilling tolerance on cucumber fruits.

Optimal MAP conditions for alleviating CI in cucumber fruits have not yet been established because the efficacy of MAP depends strongly on O_2 and CO_2 concentrations inside the packaging. CI is caused by the lipid peroxidation reaction of cell membrane lipids, in which the role of O_2 is critical. On the other side, excessive of CO_2 gives a harmful effect because it stimulates the respiration of cucumber fruit (Kubo et al., 1989). For the maximum prevention of CI in cucumber fruits using MAP, O_2 concentration in the package must be controlled just above the critical low O_2 limit. Respiratory quotient (RQ) has been used successfully to predict the low O_2 limit in many kinds of fruit. Beaudry et al. (1992) determined the low O_2 limit of blueberry using the concept of the RQ breakpoint. Yearsley et al. (1996) estimated the low O_2 limit as the anaerobic compensation point and the fermentation threshold based on RQ using a mathematical model. As for cucumber fruit, Kannelis et al. (1988) determine the critical low O_2 limit as 0.5% at 12.5°C or 20°C based on RQ and visual quality observation. Kader (2002b) also recommend the atmosphere condition of cucumber fruit as 1%–4% of O_2 and 0% of CO_2 at 8°C–12°C. However, very few information on the critical low O_2 limit of cucumber fruit at chilling temperature is available, nor has a respiration model suitable for MAP design by computer simulation been proposed.

In this study, we aimed to develop a MAP design technique for cucumber fruits stored at low temperature using a mathematical model. The critical low O_2 limit of cucumber fruits was determined by monitoring the respiratory quotient (RQ) with decreasing O_2 concentration in the environment. The respiration rate of cucumber under modified atmospheres at various O_2 concentrations was also measured and modeled.

The relationship among film permeability, surface area of the package, and weight of packed produce, leading to the equilibration of O_2 concentration in the package at the critical low O_2 was determined by application of the mathematical model to the gas composition change in MAP. Finally, in view of the varying responses of chilling-sensitive commodities to high CO_2 concentrations, the effect of CO_2 accumulation inside the package on CI suppression of cucumber fruit was evaluated. MDA, which is used as an indicator of cell membrane damage caused by CI, was assessed.

4.2. Materials and methods

4.2.1. Sample preparation

Cucumber fruit (*Cucumis sativus* L.) at commercial maturity was purchased from a wholesale store in Gifu Prefecture, Japan, and transported immediately to the laboratory. Fruits were sorted and selected for uniform size and absence of visual defects before beginning the experiment.

4.2.2. Determination of low oxygen limit by closed system method

Cucumber fruits (0.5 kg) were placed in an acrylic chamber (4.8 L) with a rubber gas sampling septum. The chamber was flushed and replaced with 5% of O_2 gas generated by a gas mixer connected to high purity O_2 and N_2 high-pressure gas cylinders. It was then placed in an incubator (MIR-154-PJ, Panasonic, Japan) set to 5°C. The changes in gas compositions in the chamber were measured every 1–2 h by gas chromatography (GC) as described later in the section of 5.2.3. A 0.2 mL sample of the headspace gas in the chamber was taken directly using a micro-syringe (MS-GAN100, Ito, Japan) and injected into a GC. The sampling was stopped when the O_2 level inside

the chamber fell below 0.2% owing to fruit respiration. RQ was determined by the closed system method as the ratio of CO_2 production to O_2 consumption following the equations of Fonseca et al. (2002).

$$R_{O_2} = \frac{\Delta C_{O_2}}{100} \times \frac{V_f}{W} \tag{4.1}$$

$$R_{CO_2} = \frac{\Delta C_{CO_2}}{100} \times \frac{V_f}{W} \tag{4.2}$$

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \tag{4.3}$$

where R_{O_2} is the rate of O_2 consumption by the produce (mL kg^{-1} h^{-1}), R_{CO_2} is the rate of CO_2 production (mL kg^{-1} h^{-1}), ΔC_g is the rate of concentration change of the gas g in the chamber (g = O_2, CO_2) (% h^{-1}), V_f is free volume (mL), W is the weight of the product (kg), and RQ is the respiratory quotient. Given that the weight of the product and the free volume of the chamber are constant, RQ at any O_2 concentration may be calculated from the slope of the linear regression line relating the increase in CO_2 concentration to the decrease in O_2 concentration in the chamber, as in Eq. (4.4):

$$RQ = \frac{\Delta C_{CO_2}}{\Delta C_{O_2}}. \tag{4.4}$$

For determining ΔC_{O_2} and ΔC_{CO_2}, sequential sets of 6 data points for O_2 and CO_2 concentration changes were adopted as regression datasets to ensure the accuracy of predicted ΔC_{O_2} and ΔC_{CO_2} and moved in steps of size 1 as in the method for calculating a moving average. Based on the values obtained in Eq. (4.4), the relationship between

RQ and the median values of O_2 concentration of the series of 6 data points was constructed. RQ values were approximately constant under aerobic condition, and at a certain O_2 level, increased abruptly as the O_2 level decreased. The O_2 level just below which RQ increased abruptly compared with RQ under aerobic condition was defined as the critical low O_2 limit (Petracek et al., 2002).

4.2.3. Determination of O_2, and CO_2 concentration by GC

A GC (GC-14A, Shimadzu, Japan) equipped with a thermal conductivity detector was used to determine O_2, and CO_2 concentration. Molecular sieve 5A and a Porapak Q column were used for the separation of O_2, N_2, and CO_2. Helium (He) gas was used as a carrier. The gas chromatogram was analyzed with an integrator (C-R7A plus, Shimadzu, Japan) based on standard curves produced by standard gases with results expressed in percentage of total gas volume.

4.2.4. Measurement of respiration rate by flow-through method

Respiration rate of cucumber fruit were measured using a flow-through method at O_2 concentration ranging from 1.5% to 20%, and 14 sets of data at various O_2 concentrations were obtained. Seven data sets were used to estimate the respiration model parameters and the rest were used to verify the acceptability of respiration model. Fig. 4.1 shows automated system for measurement respiration rate using a flow-through method. Fruits (approximately 2 kg) were weighed and placed into an acrylic chamber (4.8 L) with a gas inlet and outlet. Gas compositions entering the chamber were controlled with a gas mixer (GB-3C, Kofloc, Japan) connected to high purity O_2 and N_2 high-pressure gas cylinders. The respiration chamber was placed in an incubator (MIR-

553, Sanyo, Japan) set to 5°C for 20 h to adapt the samples to testing temperature and O_2 concentration. The gas flow rate through the chamber was monitored with a mass flow meter (SEF-E40, Horiba, Japan) and set at 6000 mL h^{-1}. Both inlet and outlet gas composition were measured by GC as described previously in the section of 5.2.3. Inlet and outlet gas samples were injected into a GC alternately via a 0.5-mL sampling loop attached to a rotating stepping valve, thereby avoiding the use of a syringe. The rate of O_2 consumption and CO_2 production were calculated from the absolute differences in gas concentration between the inlet and outlet following the equation (Fonseca et al., 2002):

$$R_{O_2,\,CO_2} = \left| \frac{\left(y^{in}_{O_2,\,CO_2} - y^{out}_{O_2,\,CO_2} \right)}{100} \right| \times \frac{F}{W}, \tag{4.5}$$

where R_{O_2,CO_2} is the respiration rate for O_2 consumption and CO_2 production of the product (mL kg^{-1} h^{-1}), y_{O_2} and y_{CO_2} are volumetric concentration of O_2 and CO_2 in location h [h = inlet (*in*), outlet (*out*)], respectively (%), W is the weight of the product (kg), and F is flow rate (mL h^{-1}).

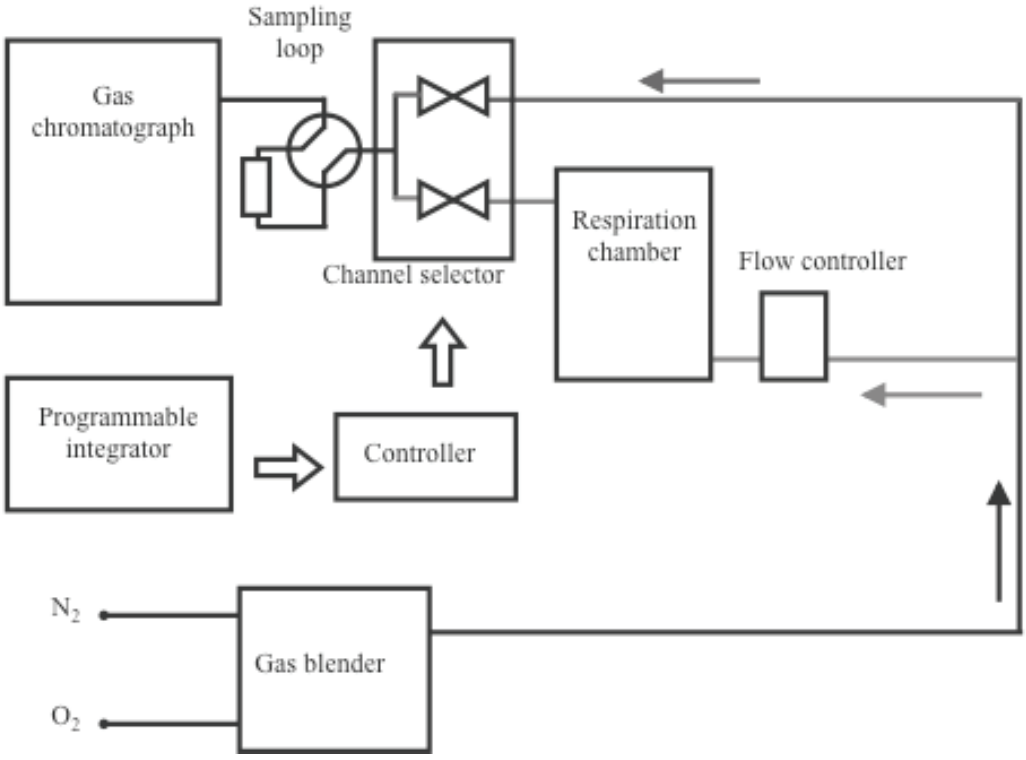

Fig. 4.1. Automated system for measurement respiration rate using a flow-through method.

4.2.5. Respiration model

A Michaelis–Menten equation was proposed for the respiration model as a function of O_2 concentration following the equation (Lee at al., 1991):

$$R_{O_2,CO_2} = \frac{V_{maxO_2,CO_2} \times [O_2]}{K_{mO_2,CO_2} + [O_2]} \tag{4.6}$$

where R_{O_2,CO_2} is the respiration rate for O_2 consumption and CO_2 production by the product (mL kg^{-1} h^{-1}), $[O_2]$ is oxygen concentration (%), K_{mO_2,CO_2} is the apparent Michaelis–Menten constant (% O_2), and V_{maxO_2,CO_2} is the maximum respiration rate (mL kg^{-1} h^{-1}). The model parameters of the Michaelis–Menten equation were estimated by linearization of Eq. (4.6) as in Eq. (4.7):

$$\frac{1}{R_{O_2,CO_2}} = \frac{K_{mO_2,CO_2}}{V_{maxO_2,CO_2}} \frac{1}{[O_2]} + \frac{1}{V_{maxO_2,CO_2}} \tag{4.7}$$

To ensure a more even distribution of error, both sides of Eq. (4.7) were multiplied by $[O_2]$ as in Eq. (4.8):

$$\frac{[O_2]}{R_{O_2,CO_2}} = \frac{K_{mO_2,CO_2}}{V_{maxO_2,CO_2}} + \frac{[O_2]}{V_{maxO_2,CO_2}} \tag{4.8}$$

According to Eq. (5.8), the R_{O_2,CO_2} at $[O_2]$ were transformed to $[O_2]/ R_{O_2,CO_2}$ and then plotted against $[O_2]$. The least-squares method was used to estimate the values of V_{maxO_2,CO_2} and K_{mO_2,CO_2}.

4.2.6. Gas concentration change in packages

The packed cucumber fruit was placed in an incubator (MIR-553, Sanyo, Japan) set to 5°C. The change of gas concentration in the package was measured periodically by GC as described previously in the section of 5.2.3 for 7 days. In case of gas sampling, the upper side of the doubly tape which were pasted on a central part of the packaging surface in advance, was stripped off, and the needle of the micro-syringe was inserted into the package through remained tape. Then, 0.2 mL of the headspace gas was withdrawn for the gas analysis by GC. After that, the pinhole created by the needle was closed right away to prevent gas leakage by pasting the striped side again.

Table 4.1. Packaging configurations for cucumber fruit.

No. Packages	Weight (kg)	Surface area of package ($\times 10^{-3}$ m^2)	Initial free volume (mL)
P1	0.5062	82.43	416.8
P2	0.4743	85.56	462.2
P3	0.4558	85.56	482.1
P4	0.4827	84.82	448.0
P5	0.4688	83.47	377.6

The gas composition change inside the package has been described as following the simultaneous differential equations (Beaudry et al., 1992; Talasila and Cameron, 1997; Chen et al., 2000; Techavuthiporn et al., 2008):

$$\begin{cases} \dfrac{dV_{O_2}^{pkg}}{dt} = K_{O_2} A\left(p_{O_2}^{ext} - p_{O_2}^{pkg}\right) - R_{O_2} W & (4.9) \\ \dfrac{dV_{CO_2}^{pkg}}{dt} = K_{CO_2} A\left(p_{CO_2}^{ext} - p_{CO_2}^{pkg}\right) + R_{CO_2} W & (4.10) \\ \dfrac{dV_{N_2}^{pkg}}{dt} = K_{N_2} A\left(p_{N_2}^{ext} - p_{N_2}^{pkg}\right) & (4.11) \\ \dfrac{dV_{all}^{pkg}}{dt} = \dfrac{dV_{O_2}^{pkg}}{dt} + \dfrac{dV_{CO_2}^{pkg}}{dt} + \dfrac{dV_{N_2}^{pkg}}{dt} & (4.12) \end{cases}$$

where V_g^{pkg} is the volume of the gas g (g = O_2, CO_2, N_2) inside the package (mL), V_{all}^{pkg} is the total volume of the gas composition inside the package (mL), K_g is the film permeance for gas g (mL m^{-2} h^{-1} atm^{-1}), A is the surface area of the packaging film (m^2), p_g^h the partial pressure of gas g in location h [h = external (*ext*), package (*pkg*)] (atm), $R_{O_2}$is the rate of O_2 consumption by the product (mL kg^{-1} h^{-1}), $R_{CO_2}$is the rate of CO_2 production by the product (mL kg^{-1} h^{-1}), W is the weight of the product (kg), and t is time (h). The film permeance K_g for O_2, CO_2 and N_2 was measured at the temperature ranging from 15° to 30°C using an isostatic method in advance, and then their temperature dependences were expressed using the Arrhenius equation $K_g = a\ exp\left({}^{b}/_{T}\right)$, where the parameter a was obtained as 2.846×10^9, 1.274×10^9, and 8.931×10^9 for gas O_2, CO_2, and N_2, respectively, and the parameter b was obtained as -4.969×10^3, -4.291×10^3, and -5.653×10^3 for O_2, CO_2, and N_2, respectively, and T represents temperature (K). However, the prediction of gas permeability at 5°C was out of application of the Arrhenius parameters estimated. On other hand, Nakano and Maezawa (2002) have been conformed the strong linearity of K of LDPE film against the temperature at the range from 5° to 30°C. Based on the assumption that the gas permeance of tested film material in this study have the same temperature dependence,

gas permeance at 5 °C was estimated by extrapolation as 49.61, 254.47 and 13.31 (mL m^{-2} h^{-1} atm^{-1}), for K_{O_2}, K_{CO_2}, and K_{N_2}, respectively. Partial pressure for O_2, CO_2 and N_2 at both external and inside the package were obtained from the volumetric concentrations of each gaseous assuming the total pressure at both locations as 1 atm. Simultaneous differential equations, Eqs. (4.9)–(4.12), were integrated numerically by means of a forward difference method using time intervals of 0.1 h. In calculation, the initial volume of each gas must be given. The initial free volume inside the package was obtained by subtracting the sample volume from the total volume of the package, both of which were measured by a water displacement method before storage. The respiration rate of the package product at any gas composition was calculated from the Michaelis–Menten respiration model as in Eq. (4.6). The packaging configurations for predicting gas concentration inside the packages in this work were shown in Table 4.1. The accuracy of prediction for the gas composition change inside the package was estimated as the root mean square error (RMSE).

4.2.7. Effect of MAP on chilling injury suppression

A weighed 0.5 kg of cucumber fruits were packed in the same bag used in the previous section with and without CO_2 absorber and non-packed fruit. Soda lime (Nacalai Tesque, Inc., Kyoto, Japan) (40 g) was put into a non-woven bag and used as a CO_2 absorber. All samples were placed in an incubator (MIR-154-PJ, Panasonic, Japan) at 5°C for 5 days. The change of gas concentration in the package was measured periodically by GC as described previously in the section of 5.2.3. Gas sampling was conducted as same as section 5.2.6. After storage at 5°C, fruits were taken out from the package and weighed immediately to determine the weight loss which was calculated as

a percentage of initial fresh weight as mentioned previously in the chapter 2. Then all fruits were stored at 24.5°C under ambient air for 3 days to detect the difference of CI development among tested conditions. MDA which has been often used as indicator of CI was determined according to the method of Hodges et al. (1999) with some modifications as described previously in the chapter 2. The MDA test was performed using 5 different fruits for each experimental condition. The results were expressed as the average value and were compared by Tukey's test at the 5% level of significance using R 2.15.2 (R Foundation).

4.3. Results and discussion

Fig. 4.2 shows the relationship between O_2 concentration and RQ of cucumber fruit obtained by the closed system method at 5°C. In measuring RQ values, the O_2 concentration in the chamber decreased from 5% to 0.2% and the CO_2 concentration increased from 0% to 5% in 62 h. The RQ values under the O_2 concentration from 4% to 0.5% ranged from 0.6 to 1.2 with an average value of 0.7. This value corresponds with RQ measured by a closed system method shown to fall between 0.7 and 0.8 (De Wild and Peppelenbos, 2001). When the O_2 concentration fell below 0.5%, RQ increased abruptly, and thus this O_2 level could be defined in this study as the critical low O_2 limit of cucumber fruit at 5°C. But this value was somewhat lower than recommended storage conditions of cucumber fruit, given as 1%–4% of O_2 and 0% of CO_2 at 8°C–12°C (Kader, 2002b). This difference might be caused by the difference of tested temperatures and storage duration. Particularly in temperature, Beaudry et al. (1992) reported that the RQ breakpoint of blueberry decreased gradually from 4.0% to 1.8% as the temperature decreased from 25°C to 5°C. Wang and Long (2014) also

reported the low O_2 limit of sweet cherry as the fermentation induction point based on RQ observation and determined it as <1% and 3%–4% O_2 at 0°C and 20°C, respectively. These observations might be related to O_2 concentration in the internal part of fruit. In case of higher temperature, the cellular O_2 consumption increased, as a result, the O_2 level of the internal part of fruit decreased. To avoid anaerobic respiration, the internal part of fruit requires O_2 from external atmosphere as much as the internal O_2 level decreasing. Therefore, the critical low O_2 limit increases at higher temperature. Similarly, the opposite phenomena will occur in case of low temperature storage.

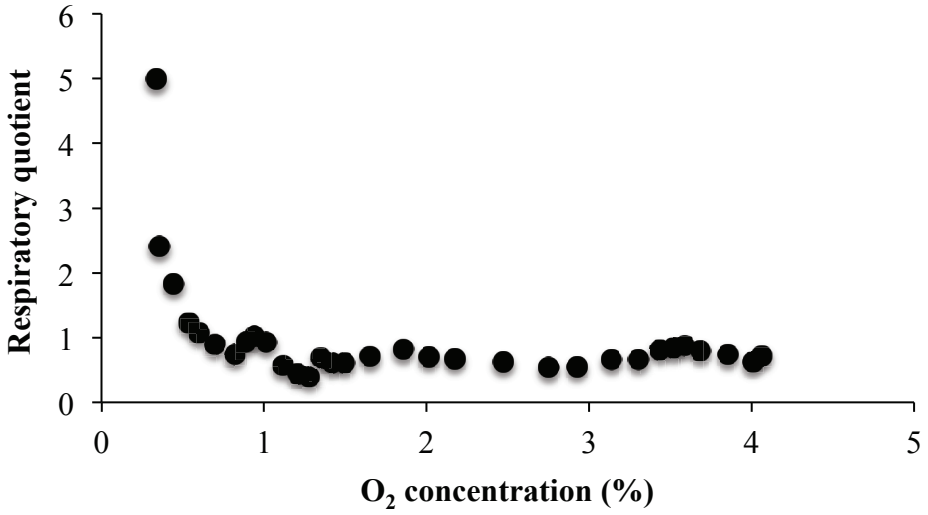

Fig. 4.2. Relationship between O_2 concentration and RQ of cucumber fruit at 5°C.

As for the effect of the storage duration on the critical low O_2 limit, Kuroki et al. (2004) reported that the porosity of cucumber fruit, which affects the characteristic of gas exchange between internal and external of the fruit, decreased with increasing of the storage duration. From this condition, it is speculated that the minimum external O_2 partial pressure enabling to keep the aerobic respiration in whole plant level must increase depending on the storage duration, because O_2 flux from outside to inside of the fruit is limited with progress of the senescence. Apart from that, the difference of tested cultivar and maturity may cause the difference between our estimated critical low O_2 limit and recommended O_2 level described in other literatures, because the physiological response is quite different depending on these factor. Particularly, immature cucumber fruit (approximately 20 cm length) were used in this study, because the commercial maturity is quite young in Japan comparing with other countries.

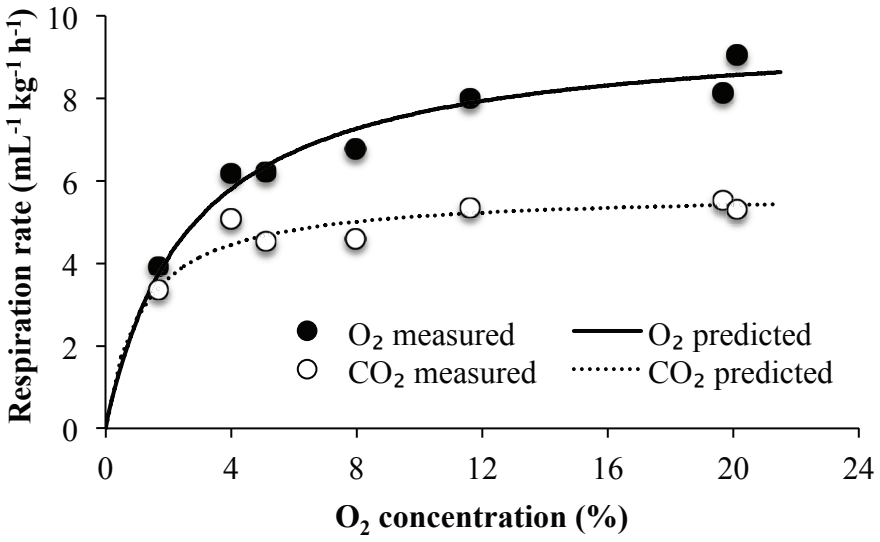

Fig. 4.3. Measured and predicted respiration rates of cucumber fruit at various O_2 concentrations stored at 5°C. Symbols represent measured values by flow-through method (Eq. 4.5). Solid and dotted lines indicate predicted values according to Eq. (4.6).

Fig. 4.3 shows the measured and predicted respiration rates of cucumber fruit under various O_2 concentrations at 5°C obtained by a flow-through method (Eq. 4.5) and respiration model (Eq. 4.6). The predicted value was calculated from the Michaelis–Menten equation based on the principles of enzyme kinetics, as in Eq. (4.6). The rate of O_2 consumption decreased in response to decreasing O_2 concentration. There was a little effect of O_2 concentration in suppressing the rate of O_2 consumption from 20% to 8% of O_2, but a rapid decline was observed from 8% to 1.5% of O_2. However, there was no effect of O_2 concentration in suppressing the CO_2 production rate from 20% to 4% of O_2, and slight decrease was observed from 4% to 1.5% of O_2. Theses results suggest that to preserve the quality of cucumber fruit at 5°C, MAP should be designed so that the O_2 concentration in the package equilibrates below 8%. The estimated model parameters of V_{maxO_2,CO_2} and K_{mO_2,CO_2} are presented in Table 4.2.

Table 4.2 Parameters of the Michaelis–Menten model and root mean square error (RMSE) for Eq. (4.6).

Model parameter	R_{O_2}	R_{CO_2}
V_{max} (mL kg^{-1} h^{-1})	9.718	5.726
K_m (% O_2)	2.699	1.142
RMSE (mL kg^{-1} h^{-1})	0.353	0.297

Fig. 4.4 shows relationship between measured and predicted of the respiration rate as O_2 consumption and CO_2 production to verify the acceptability of the respiration model. Predicted of O_2 consumption rate had a good agreement with measured of O_2 consumption rate and the coefficient of determination (R^2) was 0.89. However, the

relationship between measured and predicted of CO_2 production rate showed weak linearity ($R^2 = 0.44$), because the rate of CO_2 production changed very little compared with the rate of O_2 consumption in response to the change of O_2 concentration resulting uneven in distribution of error. Root mean square error for prediction (RMSEP) was very small, given as 0.521% and 0.509% mL kg^{-1} h^{-1} for O_2 consumption rate and CO_2 production rate, respectively. Based on these facts, the respiration model could accurately predict the respiration rate of cucumber fruit under various O_2 concentrations at 5°C.

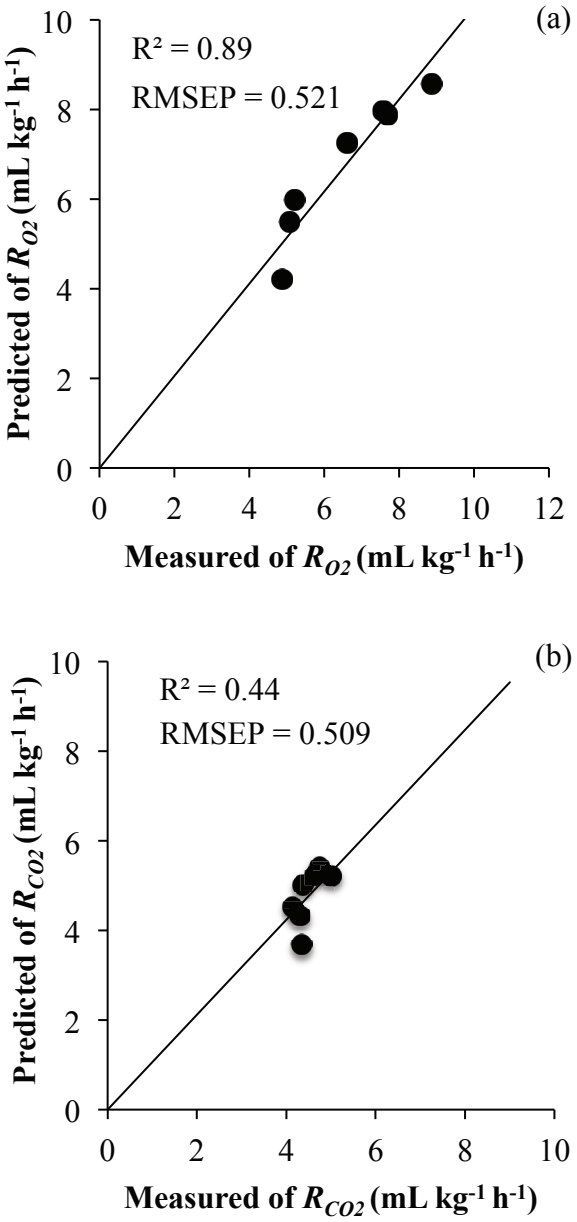

Fig. 4.4. Relationship between measured and predicted of respiration rate as O_2 consumption (a) and CO_2 production (b) to verify the acceptability of the respiration model (Eq. 4.6).

Fig. 4.5 shows the measured and predicted O_2 and CO_2 concentrations inside the package containing cucumber fruit at 5°C. The change of O_2 and CO_2 concentration between 5 packages was different depend on the weight of sample, surface area of film packaging and initial free volume of packages due to influence of the respiration rate of product. The measured O_2 concentration of 5 packages decreased to 1.3%–1.8% in the first 46.5–66.3 h, after which it remained constant until the end of the experiment. The CO_2 concentration of 5 packages increased to 5.7%–6.7% in the first 52–66.3 h, and also stabilized in the range from 5.7%–7.5%. The predicted O_2 concentration decreased slightly faster than the measured one in the transient state and agreed well with measured values in the steady state. In contrast, the predicted value of CO_2 increased rapidly compared with the measured value in the transient state, but stabilized to slightly lower than the measured one in the steady state.

Table 4.3. Root mean square error (RMSE) between measured and predicted gas concentration changes inside the packages

No. Packages	RMSE (%)			
	Transient sate		Steady state	
	O_2	CO_2	O_2	CO_2
P1	5.243	3.577	1.082	0.955
P2	4.609	2.990	0.938	1.123
P3	2.358	2.450	0.556	0.963
P4	3.924	2.965	0.805	1.346
P5	4.391	3.037	1.058	0.973
Average	4.105	3.004	0.888	1.072

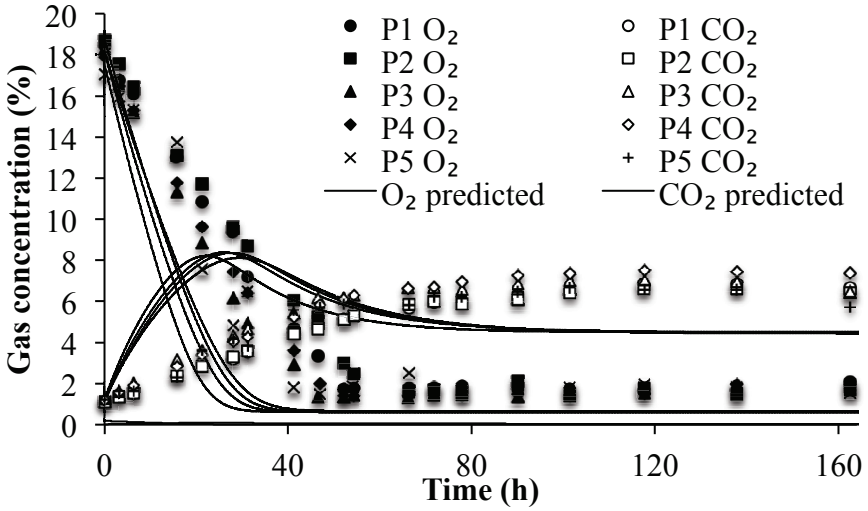

Fig. 4.5. Changes in gas concentration in LDPE film packaging containing cucumber fruit during storage at 5°C. Symbols represent experimental data. Solid lines indicate predicted values according to Eqs. (4.9)–(4.12).

RMSEs between measured and predicted values for O_2 and CO_2 concentration in the package in the transient state (within 46.5–66.3 h for O_2 and 52–66.3 h for CO_2) and in the steady state (after 46.5–66.3 h for O_2 and 52–66.3 h for CO_2) were shown in Table 4.3, which RMSEs in steady state were lower than those at the transient state. This observation indicates that the model for gas concentration change in the package, Eqs. (4.9)–(4.12), including the respiration model, Eq. (4.6), can accurately predict gas composition in the steady state. The difference between measured and predicted values in the transient state may be due to our respiration model, which does not consider the effect of ambient gas concentration change with time on the respiration rate. Nakano et al. (2002) evaluated the response of both O_2 consumption and CO_2 production rate of young soybean and cherry tomato fruits to an abrupt lowering of O_2 concentration from 20% to 5% and showed that O_2 consumption fell rapidly, but CO_2 production only gradually, with the abrupt O_2 reduction. If this response of respiration to gas concentration change is incorporated into the respiration model, correct prediction of the gas concentration change in the package in the transient state should be possible. Apart from that, the respiration rate of fresh produces generally increases when they are injured. CI is one of typical injuries. Eaks and Morris (1956) reported that the respiration rate of cucumber fruits stored at chilling temperature (5 °C) increased after 3 days storage. The respiration model in this study was not considered such an increment caused by CI. If this effect can be incorporated into the respiration model as a function of the development of CI, particular in the steady state, the prediction of gas concentration change will be improved more. Though we did not take the effect of CO_2 on respiration rate into account in respiration model, this will not affect to the prediction accuracy of the gas concentration change in the package. Because Kubo et al. (1989)

have classified the horticultural commodity into the respiratory response to high CO_2, and concluded that the respiration rate only in the fresh commodities producing ethylene was suppressed by high CO_2, but cucumber fruits dose not produce it. They also showed that the respiration rate of cucumber fruits was stimulated, when fruits were exposed in 60% of CO_2. Conversely it has been reported that the CO_2 concentration less than around 10 % has no promoting and inhibition effect on respiration rate of cucumber fruit (Akimoto et al., 2000; Yasunaga et al., 2001). In present study, the maximum average level of CO_2 inside the package of 5 packages was 7.1%, therefore, the effect of CO_2 on respiration is not needed to consider in a range of our study.

Given that our model accurately predicted the gas composition in the package in the steady state, the packaging condition suppressing respiration at the minimum level not leading to anaerobic respiration and inducing maximum quality retention can be estimated on the basis of the critical low O_2 limit obtained in the previous section.

In the steady state, the change of O_2 volume inside the package is zero, therefore Eq. (4.9) can be expressed as follows:

$$\frac{dV_{O_2}^{pkg}}{dt} = K_{O_2} A \left(p_{O_2}^{ext} - p_{O_2}^{pkg} \right) - R_{O_2} W = 0 \tag{4.13}$$

Rearranging Eq. (4.13) to solve for K_{O_2}, yields the following equation:

$$K_{O_2} = \frac{W \times R_{O_2}}{A \left(p_{O_2}^{ext} - p_{O_2}^{pkg} \right)} \tag{4.14}$$

By assuming the critical low O_2 limit (0.5% of O_2) as the optimal O_2 concentration inside the package, optimal K_{O_2} can be expressed as a function of *W*/*A* by substituting R_{O_2} at 0.5% of O_2 and the values of 0.005 for $p_{O_2}^{pkg}$ and 0.205 for $p_{O_2}^{ext}$ into Eq. (4.14). According to Eq. (4.6) and the model parameters listed in Table 1, R_{O_2}= 1.519 mL kg^{-1} h^{-1}. Thus Eq. (4.14) can be rearranged as follows:

$$K_{O_2} = 7.596 \frac{W}{A} \tag{4.15}$$

The relationship between the optimal O_2 permeance of the film material and ratio of the product's weight to the surface area presented in Eq. (4.15) is illustrated in Fig. 4.6. Using this figure, the optimal K_{O_2} can be simply predicted for any product weight and surface area of MAP for cucumber fruit at 5°C.

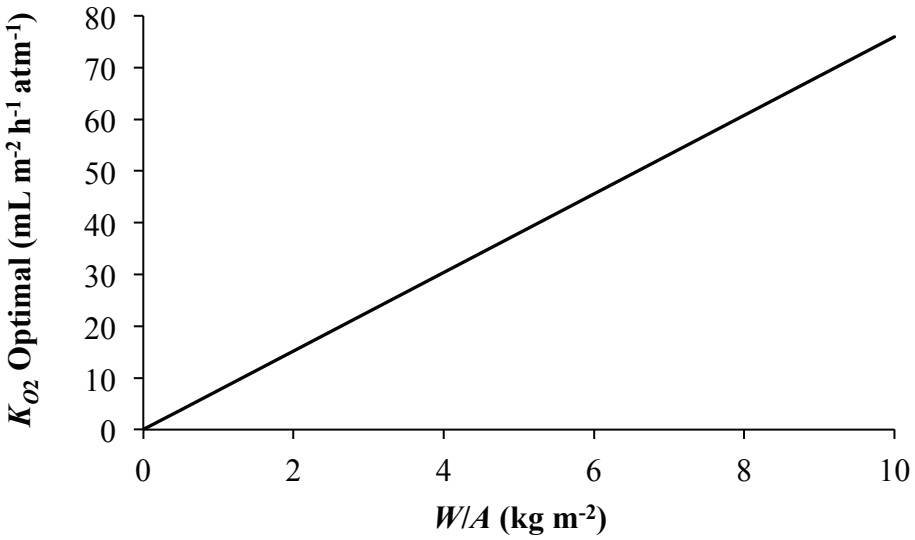

Fig. 4.6. Relationship between and W/A leading to equilibration of O_2 concentration inside the package at 0.5%.

Fig. 4.7 shows MDA equivalent of cucumber fruit packed in an LDPE bag with and without CO_2 absorber, and non-packed stored at 5°C for 5 days followed by 24.5°C for 3 days under ambient air. The MDA equivalent of non-packed fruit was significantly higher than fruit packed in an LDPE bag with and without CO_2 absorber. Comparing the presence or absence of CO2 absorber in the package, the MDA equivalent of fruit packed with a CO_2 absorber was significantly lower than those packed without a CO_2 absorber.

The MDA equivalent has been often used as a preceding indicator of CI development because MDA is a final product of lipid peroxidation associating with CI (Mao et al., 2007a; Yang et al., 2011). The result mentioned above indicates that both packages tested in this study could alleviate CI in cucumber fruit because of low O_2 created in the packaging by product's respiration. But the significant difference was found between them. This might be caused by the difference in CO_2 accumulation level in both packages. In fact, nevertheless O_2 concentration equilibrated at the same level (1.3%) in both packages, for the packaging without a CO_2 absorber, CO_2 concentration increased up to 7.1% in the steady state owing to product respiration. In contrast, in the package with a CO_2 absorber, CO_2 was detected below measurable limit throughout the duration of storage (data not shown). High CO_2 thought to be a stress factor for cucumber fruit. According to Mathooko's review (1996), excessive CO_2 stimulates the mitochondria activity in cucumber fruit through the induction of the various mitochondria enzyme protein. Particularly, the activity of cytochrome c oxidase, which is a terminal enzyme in the electron transport chain, increased at high (> 40%) CO_2 condition (Dostal-Lange and Kader, 1994). The mitochondria electron transport chain is a major source of reactive oxygen species (ROS) in eukaryotic cells, and ROS is well-

known causative substances of lipid peroxidation. MDA is an indicator of CI and also an indicator of cell membrane damage cause by lipid peroxidation. The CO_2 level of the package without a CO_2 absorber (7.1%) did not affected to the respiratory activity in the whole plant level but might affect in the mitochondria level, and stimulate the generation of ROS as a result, the cell membrane is damaged resulting in the increase of MDA.

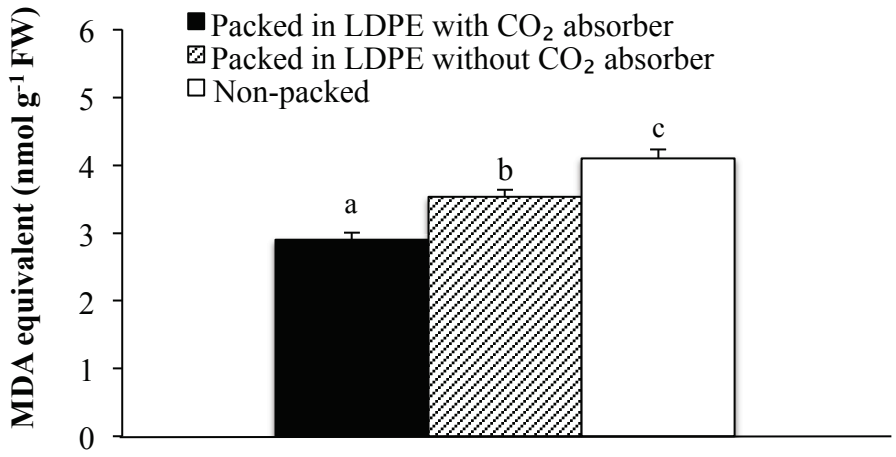

Fig. 4.7. MDA equivalent of cucumber fruit packed in a low-density polyethylene (LDPE) bag with and without a CO_2 absorber and non-packed stored at 5°C for 5 days followed by 24.5°C for 3 days under ambient air. Vertical line represents standard error (n = 5). Values with different letters are significantly different at $P < 0.05$.

Table 4.4. Weight loss of cucumber fruit after stored at 5°C in low-density polyethylene (LDPE) with and without CO_2 absorber and non-packed.

Type of packages	Weight loss (%)
LDPE with CO_2 absorber	1.1
LDPE without CO_2 absorber	0.3
Non–packed	1.1

In addition to high CO_2, soda lime used in this study also takes up water vapor and thereby lowered relative humidity inside the package. For that reason, the higher weight loss was observed in the fruit packed with a CO_2 absorber compared with those packed without it (Table 4.4). Storing cucumber fruit in lower humidity condition accelerated CI development (Fahmy and Nakano, 2013). The increase of weight loss during low-temperature storage also increases susceptibility of cucumber fruit to CI (Purvis, 1994). Considering these knowledges, MDA equivalent of the fruit packed with a CO_2 absorber presented in this study might come form the positive effects of low O_2 and a negative effects of the weight loss. Furthermore, if the material that has no capacity of moisture absorption is used as a CO_2 absorbent, a greater alleviation effect of CI might be obtained.

4.4. Conclusion

In this study, we presented a method based on a mathematical approach for designing MAP to alleviate CI in cucumber fruit. First, to determine the target O_2 concentration in the steady state in MAP, the critical low O_2 limit was estimated to be 0.5% based on the observation of RQ change under low O_2 at 5°C. Next, the respiration rate was fitted with a Michaelis–Menten equation, permitting the prediction of O_2

consumption and CO_2 production rates at any O_2 concentration. These steps yielded an equation able to estimate optimal combinations of packaging parameters including film permeability, product weight, and packaging size able to provide maximum suppression of CI by controlling O_2 concentrations. Accumulation of CO_2 inside the packaging was found to enhance cell membrane damage even under reduced O_2 concentrations. We conclude that for maximum alleviation of CI in cucumber fruits, CO_2 control must be taken into account in MAP design.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

5.1. Conclusion

In the present study, the optimal design of modified atmosphere packaging (MAP) for alleviating chilling injury (CI) in cucumber fruit based on the environmental analysis has been developed. MAP was developed using a mathematical model approach by incorporating the respiration rate and gas mass transfer through film packaging to estimate the optimal O_2 permeance of film packaging, leading to equilibration of O_2 concentration inside the package at the critical low O_2 limit obtained.

First, identifying the most effective conditions for alleviating CI of cucumber fruit has been determined. Storage of cucumber fruit under high relative humidity (RH) not only reduced the water loss but also suppressed the development of CI compared with storage under low and medium RH. Moreover, low O_2 acted as a dominant factor for alleviating CI of cucumber fruits, but combination with high CO_2 did not induce synergistic effects, but it gave negative influences.

Based on identification the most effective condition for alleviating CI in cucumber fruit, a design technique of MAP for cucumber fruits stored at low temperature was developed using mathematical model. To make MAP maintained the quality of fruit at a maximum level, the packaging conditions must be designed with the result that O_2 concentration inside the package equilibrates just above the critical low O_2 limit. The critical low O_2 limit of cucumber fruit was estimated to be 0.5% at 5°C which was determined by monitoring the RQ with decreasing O_2 concentration. Next, the

respiration rate was fitted with a Michaelis–Menten equation, permitting the prediction of O_2 consumption and CO_2 production rates at any O_2 concentration. This equation successfully predicted the respiration rate of cucumber fruit under various O_2 concentration at 5°C. These steps yielded an equation able to estimate optimal combinations of packaging parameters including film permeability, product weight, and packaging size able to provide maximum suppression of CI by controlling O_2 concentrations.

In view of the varying responses of chilling-sensitive commodities to high CO_2, the effect of CO_2 accumulation inside the package on CI suppression of cucumber fruit has been evaluated. The reduction of CO_2 inside the packaging was found to be able to alleviate CI in cucumber fruits more efficiently.

5.2. Future perspective

Since Indonesia produces large amount of chilling-sensitive products, the development technology for alleviating of CI particularly during storage and transportation at low temperature is required to preserve quality and prolong period of storage. In present study, the optimal design of MAP for alleviating CI in cucumber fruit has been developed. By using the proposed procedure presented in this study, it can be applied to determine the optimal condition of MAP for alleviating CI in chilling-sensitive products produced in Indonesia.

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REFERENCES

Akimoto, K., Uchino, T., Nakano, K., Yasanuga, E., Kuroki, S., and Hamanaka, D. (2000). Comparison between fixed and flexible chamber methods for measuring respiratory rate under steady and unsteady states. J. Japan. Soc. Hort. Sci. 69, 646–652.

Ali, Z.M., Chin, L.H., Marimuthu, M., and Lazan, H. (2004). Low temperature storage and modified atmosphere packaging of carambola fruit and their effects on ripening related texture changes, wall modification and chilling injury symptoms. Postharvest Biol. Technol., 33, 181–192.

Aqüero, M.V., Ponce, A.G., Moreira, M.R., and Raura, S.I. (2011). Lettuce quality loss under conditions that favour the wilting phenomenon. Postharvest Biol. Technol., 59, 124–131.

Beaudry, R.M., Cameron, A.C., Shirazi, A., and Dostal-Lange, D.L. (1992). Modified-atmosphere packaging of blueberry fruit: Effect of temperature on package O_2 and CO_2. J. Am. Soc. Hort. Sci., 117, 436–441.

Beaudry, R.M. (2000). Responses of horticultural commodities to low oxygen: Limits to the expanded use of modified atmosphere packaging. HortTechnology, 10, 491–500.

Ben-Yehoshua, S. (1985). Individual seal-packaging of fruit and vegetables in plastic film- a new postharvest technique. HortScience, 20, 32–37.

Ben-Yehoshua, S., Rodov, V. (2003). Transpiration and water stress. In: Bartz, J.A., Brecht, J.K. [eds.], Postharvest Physiology and Pathology of Vegetables, pp. 111–159. Marcel Dekker, New York.

Biale, J.B., Young, R.E., and Olmstead, A.J. (1954). Fruit respiration and ethylene production. Plant Physiol., 29, 168–174.

Cabrera, R.M., and Saltveit, M.E. (1990). Physiological response to chilling temperatures of intermittently warmed cucumber fruit. Ameri. Soc. Hortic. Sci., 115, 256–261.

Cameron, A.C., Beaudry, R.M., Bank, N.H., and Yelanich, M.V. (1994). Modified-atmosphere packaging of blueberry fruit: Modeling respiration and package oxygen partial pressure as a function of temperature. J. Amer. Soc. Hort. Sci., 119, 534–539.

Cia, P., Benato, E.A., Sigrist, J.M.M., Sarantopóulos, C., Oliveira, L.M., and Padula, M. (2006). Modified atmosphere packaging for extending the storage life of 'Fuyu' persimmon. Postharvest Biol. Technol., 42, 228–234.

Chen, X., Hertog, M.L.A.T.M., and Banks, N.H. (2000). The effect of temperature on gas relations in MA packages for capsicums (Capsicum annuum L., cv. Tasty): an integrated approach. Postharvest Biol., Technol., 20, 71–80.

Dadzie, B.K., Banks, N.H., Cleland, D.J., Hewett, E.W. (1996). Changes in respiration and ethylene production of apples in response to internal and external oxygen partial pressures. Postharvest Biol. Technol., 9, 297–309.

Dea, S., Brecht, J.K., Nunes, M.C.N., and Baldwin, E.A. (2010). Occurrence of chilling injury in fresh-cut 'Kent' mangoes. Postharvest Biol. Technol., 57, 61–71.

De Castro, E., Barret, D.M., Jobling, J., and Mitcham, E.J. (2008). Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples. Postharvest Biol. Technol., 48, 182–191.

Del–Valle, V., Hernández-Muñoz, P., Catalá, R., and Gavara, R. (2009). Optimization of an equilibrium modified atmosphere packaging (EMAP) for minimally processed mandarin segments. Food Eng., 91, 474–481.

De Wild, H.P.J., and Peppelenbos, H.W. (2001). Improving the measurement of gas exchange in closed systems. Postharvest Biol. Technol., 22, 111–119.

Dostal-Lange, D., and Kader, A.A. (1994). Mitochondria respiration of "Hass" avocados in response to elevated CO_2 concentrations. HortScience 29, 484.

Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia. (2014). Total production of Indonesian fruits and vegetables, and export and import volume of Indonesian fruits and vegetables. http://hortikultura.pertanian.go.id.

Eaks, I.L., and Morris, L.L. (1956). Respiration of cucumber fruits associated with physiological injury at chilling temperatures. Plant Physiol., 31, 308–314.

Fahmy, K., and Nakano, K. (2013). Influence of relative humidity on development of chilling injury in cucumber fruits during low temperature storage. Asia Pacific. J. Sustainable Agric. Food Energy 1, 1–5.

Fahmy, K., and Nakano, K. (2014a). The individual and combined influences of low oxygen and high carbon dioxide on chilling-injury suppression in cucumber fruit. Environ. Control Biol., 52(3), 149–153.

Fahmy, K., and Nakano, K. (2014b). Optimal design of modified atmosphere packaging for alleviating chilling injury in cucumber fruit. Environ. Control Biol., 52(4), 233–240.

Fallik, E, Temkin-Gorodeiski, N., Grinberg, S., and Davidson, H. (1995). Prolonged low-temperature storage of eggplants in polyethylene bags. Postharvest Biol. Technol., 5, 83–89.

Fernández-Trujilio, J.P., Martínez, J.A., and Artés, F. (1998). Modified atmosphere packaging affects incidence of cold storage disorders and keep 'flat' peach quality. Food Res. Int., 31, 571–579.

Finnegan, E., Mahajan, P.V., O'Connell, M., Francis, G.A., and O'Beirne, D. (2013). Modelling respiration in fresh-cut pineapple and prediction of gas permeability needs for optimal modified atmosphere packaging. Postharvest. Biol. Technol., 79, 47–53.

Flores, F.B., Martínez-Madrid, M.C., Ben-Amor, M., Pech, J.C., Latché, A., and Romojaro, F. (2004). Modified atmosphere packaging confers additional chilling tolerance on ethylene-inhibited cantaloupe Charentais melon fruit. Eur. Food Res. Technol., 219, 614–619.

Fonseca, S.C., Oliveira, F.A.R., and Brecht, J.K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. Food Eng. 52, 99–119.

Fonseca, S.C., Oliveira, F.A.R., Brecht, J.K., and Chau, K.V. (2005). Influence of low oxygen and high carbon dioxide on shredded Galega kale quality for development of modified atmosphere packages. Postharvest Biol. Technol., 35, 279–292.

Forney, C.F., and Lipton, W.J. (1990). Influence of controlled atmospheres and packaging on chilling sensitivity. In: Wang, C.Y. [eds.], Chilling Injury of Horticultural Crops, pp. 257–261. CRC Press, Boca Raton FL.

Hakim, A., Purvis, A.C., and Mullinix, B.G. (1999). Differences in chilling sensitivity of cucumber varieties depend on storage temperature and the physiological dysfunction evaluated. Postharvest Biol. Technol., 17, 97–104.

Hertog, M.L.A.T.M., Peppelenbos, H.W., Evelo, R.G., and Tijskens, L.M.M. (1998). A dynamic and generic model of gas exchange of respiring produce: the effects of oxygen, carbon dioxide and temperature. Postharvest Biol. Technol., 14, 335–349.

Hirose, T. (1985). Effects of pre- and interposed warming on chilling injury, respiratory rate and membrane permeability of cucumber fruit during cold storage. J. Japan Soc. Hortic. Sci., 53, 459–466.

Hirota, T., Tabata, K., Fukushima, A., Inoue, Y., Nakagawa, K. (2003). Effect of holding condition before freezing on quality in immature black soybeans 'Tanbaguro' served as a vegetable. Food Preservation Sci., 29, 11–16.

Hodges, D.M., DeLong, J.M., Forney, C.F., and Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.

Heydari, A., Shayesteh, K., Eghbalifam, N., Bordbar, H., and Falahatpisheh. (2010). Studies on the respiration rate of banana fruit based on enzyme kinetic. Int. J. Agric. Biol., 12, 145–149.

Imahori, Y., Takemura, M., and Bai, J. (2008). Chilling-induced oxidative stress and antioxidant responses in mume (Prunus mume) fruit during low temperature storage. Postharvest Biol. Technol., 49, 54–60.

Joles, D.W., Cameron, A.C., Shirazi, A., Petracek, P.D., and Beaudry, R.M. (1994). Modified-atmosphere packaging of 'Heritage' red raspberry fruit: Respiratory

response to reduced oxygen, enhanced carbon dioxide, and temperature. J. Amer. Soc. Hort. Sci., 119, 540–545.

Jacxsens, L., Devlieghere, F., De Rudder, T., and Debevere, J. (2000). Designing Equilibrium Modifed Atmosphere Packages for Fresh-cut Vegetables Subjected to Changes in Temperature. Lebensm.-Wiss. u.-Technol., 33, 178–187

Kader, A.A. (2002a). Postharvest biology and technology: An overview. In: Kader, A.A. [eds.], Postharvest technology of horticultural crops, 3rd ed. pp. 39–47. University of California, Div. Agric. Nat. Resour., Oakland CA.

Kader, A.A. (2002b). Modified atmosphere during transport and strorage. In: Kader, A. A. [eds.], Postharvest technology of horticultural crops, 3rd ed. pp. 135–144. University of California, Div. Agric. Nat. Resour., Oakland CA.

Kader, A.A. (2005). Increasing food availability by reducing postharvest losses of fresh produce. Acta Hort. 682: 2169–2176.

Kanellis, A.K., Morris, L.L., and Saltveit, M.E. (1988). Responses of parthenocarpic cucumbers to low oxygen storage. J. Amer. Soc. Hort. Sci. 113, 734–737.

Karakaş, B., and Yıldız, F. (2007). Peroxidation of membrane lipids in minimally processed cucumbers packaged under modified atmospheres. Food Chem., 100, 1011–1018.

Kubo, Y., Inaba, A., and Nakamura, R., (1989). Effect of high CO_2 on respiration in various horticultural crops. J. Japan Soc. Hort. Sci. 58, 731–736.

Kubo, Y., Inaba, A., and Nakamura, R. (1990). Respiration and C2H4 production in various harvested crops held in CO_2-enriched atmospheres. Amer. Soc. Hort. Sci., 115, 975–978.

Kuroki, S., Oshita, S., Sotome, I., Kawagoe, Y., and Seo., Y. (2004). Vizualization of 3-D network of gas –filled intercellular spaces in cucumber fruit after harvest. Postharvest Biol. Technol., 33, 255–262.

Larrigaudiere, C., Lentheric, I., Pintó, E., and Vendrell, M. (2001). Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in conference pears. J. Plant Physiol., 158, 1015–1022.

Lee, D.S., Haggar, P.E., Lee, J., and Yam, K.L. (1991). Model for fresh produce respiration in modified atmospheres based on principles of enzyme kinetics. Food Sci., 56, 1580–1585.

Luengwilai, K., Beckles, D.M., and Saltveit, M.E. (2012). Chilling-injury of harvested tomato (Solanum lycopersicum L.) cv. Micro-Tom fruit is reduced by temperature pre-treatments. Postharvest Biol. Technol., 63, 123–128.

Macrae, E.A. (1987). Development of chilling injury in New Zealand grown Fuyu persimmon during storage. New Zealand J. Exp. Agric., 15, 333–344.

Mahajan, P.V., Oliveira, F.A.R., and Macedo, I. (2008). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. Food Eng., 84, 281–288.

Makhlouf, J., Willemot, C., Arul, J., Castaigne, F., and Emond, J.P. (1989). Long-term storage of broccoli under controlled atmosphere. HortScience, 24, 637–639.

Mao, L., Pang, H., Wang, G., and Zhu, C. (2007a). Phospholipase D and lipoxygenase activity of cucumber fruit in response to chilling stress. Postharvest Biol. Technol., 44, 42–47.

Mao, L., Wang., G, Zhu, C., and Pang, H. (2007b). Involvement of phospholipase D and lipoxygenase in response to chilling stress in postharvest cucumber fruits. Plant Sci., 172, 400–405.

Marangoni, A.G., Palma, T., and Stanley, D.W. (1996). Membrane effects in postharvest physiology. Postharvest Biol. Technol., 7, 193–217.

Matile, P., Hörtensteiner, S., and Thomas, H. (1999). Chlorophyll degradation. Annu. Rev. Plant Physiol., 50, 67–95.

Mathooko, F.M., 1996. Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. Postharvest Biol. Technol. 9, 247–264.

McGuire, R.G. (1992). Reporting of objective color measurements. HortScience 27, 1254–1255.

Medina, M.S., Tudela, J.A., Marin, A., Allende, A., Gil, and M.I. (2012). Short postharvest storage under low relative humidity improves quality and shelf-life of minimally processed baby spinach (Spinacia oleracea L). Postharvest Biol. Technol., 67: 1–9.

Meir, S., Naiman, D., Akerman, M., Hyman, J.Y., Zauberman, G., and Fuchs, Y. (1997). Prolonged storage of ‘Hass’ avocado fruit using modified atmosphere packaging. Postharvest Biol. Technol., 12, 51–60.

Morris, L.L., and Platenius, H. (1938). Low temperature injury to certain vegetables. Proc. Amer. Sot. Hort. Sci., 36, 609–613.

Murphy, M.P. (2009). How mitochondria produce reactive oxygen species. Biochem. J., 417, 1–13.

Nakano, K., and Maezawa, S. (2002). Application of the respiration model for young soybean to modified atmosphere packaging systems. J. Japan. Soc. Agric. Machinery. 64, 82–87.

Nakano, K., Nakamura, N., Shiina, T., and Maezawa, S. (2002). Changes in respiration and ethylene production rates by young soybean and cherry tomato fruits exposed to an abrupt decrease in oxygen concentration. J. Japan Soc. Hort. Sci., 71, 710–715.

Nguyen, T.B.T., Ketsa, S., and van Doorn, W.G. (2004). Effect of modified atmosphere packaging on chilling-induced peel browning in banana. Postharvest Biol. Technol., 31, 313–317.

Palma, T., Marangoni, A.G., and Stanley, D.W. (1995). Environmental stresses affect tomato microsomal membrane function differently than natural ripening and senescence. Postharvest Biol. Technol., 6, 257–273.

Palta, J.P. (1990). Stress interaction at cellular and membrane levels. HortSci., 25, 1377–1381.

Parkin, K.L., Maranggoni, A., Jackman, R.L., Yada, R.D., and Stanley, D.W. (1989). Chilling injury. A review of possible mechanisms. Food Biochem., 13, 127–153.

Paulla, R.E., Nishijima, W., Reyes, M., Cavaletto, C. (1997). Postharvest handling and losses during marketing of papaya (Carica papaya L.). Postharvest Biol. Technol. 11: 165–179.

Pesis, E., Aharoni, D., Aharon, Z., Ben-Arie, R., Aharoni, N., and Fuchs, Y. (2000). Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit. Postharvest Biol. Technol., 19, 93–101.

Pesis, E., Dvir, O., Feygenberg, O., Ben Arie, S., Ackerman, M., and Litcher, A. (2002). Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of litchi fruit. Postharvest Biol. Technol., 26, 157–165.

Petracek, P.D., Joles, D.W., Shirazi, A., and Cameron, A.C. (2002). Modified atmosphere packaging of sweet cherry (Prunus avium L., ev. 'Sams') fruit: metabolic responses to oxygen, carbon dioxide, and temperature. Postharvest Biol. Technol., 24, 259–270.

Porat, R., Weiss, B., Cohen, L., Daus, A., and Aharoni, N. (2004). Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biol. Technol., 33, 35–43.

Purvis, A.C. (1994). Interaction of waxes and temperature in retarding moisture loss from and chilling injury of cucumber fruit during storage. Proc. Fla. State Hort. Soc., 107, 257–260.

Ryall, A.L., and Lipton, W.J. (1979). Handling, transportation and storage of fruit and vegetables. Vegetables and melons, p. 587. AVI Publishing Company Inc., Westport, Connecticut.

Salunkhe, D.K., and Desai, B.B. (1984). Postharvest biotechnology of vegetables, p. 194. CRC Press, Inc., Florida.

Saltveit, M.E. (2002). The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. Postharvest Biol. Technol., 26, 295–304.

Saltveit, M.E. (2005). Influence of heat shock on the kinetic of chilling-induced ion leakage from tomato pericarp discs. Postharvest Biol. Technol., 36, 87–92.

Serrano, M., Martinez-Romero, D., Guillén, F., Castillo, S., and Valero, D. (2006). Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Postharvest Biol. Technol., 39, 61–68.

Sharkey, P.J., and Peggie, I.D. (1984). Effects of high-humidity storage on quality, decay and storage life of cherry, lemon and peach fruits. Sci. Hortic., 23, 181–190.

Shen, M., and Wang, M.X. (1997). Advance in research on chilling-resistance mechanisms of plant. Chin. Bull. Bot., 14, 1–8.

Smith, L., and Dawson, P. (2004). Food exporters' guide to Indonesia. Australian Government Department of Agriculture, Fisheries and Forestry, p. xi, Instate Pty, Canberra.

Talasila, P.C., and Cameron, A.C. (1997). Free-volume changes in flexible, hermetic packages containing respiring produce. J. Food Sci., 62, 659–664.

Techavuthiporn, C., Nakano, K., and Maezawa, S. (2008). Prediction of ascorbic acid content in broccoli using a model equation of respiration. Postharvest Biol. Technol., 47, 373–381.

Villaescusa, R., and Gil, M.I. (2003). Quality improvement of Pleurotus mushrooms by modified atmosphere packaging and moisture absorbers. Postharvest Biol. Technol., 28, 169–179.

Wang, C.Y., and Qi, L. (1997). Modified atmosphere packaging alleviates chilling injury in cucumbers. Postharvest Biol. Technol. 10: 195–200.

Wang, Y., and Long, L.E. (2014). Respiration and quality responses of sweet cherry to different atmosphere during cold storage and shipping. Postharvest Biol. Technol., 92, 62–69.

Watkins, C.B. (2000). Responses of horticultural commodities to high carbon dioxide as related to modified atmosphere packaging. HortTechnol. 10, 501–506.

Wills, R., McGlasson, B., Graham, D., and Joyce, D. (2007). Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals, 5th ed. p. 13–27. Univ. New South Wales Press, Sydney.

Wilson, J.M. (1979). Drought resistance as related to low temperature stress. In: Lyons, J.M., Graham, D., Raison, J.K. [eds.], Low temperature stress in crop plants, pp. 47–65. Academic Press, New York.

Wongsheree, T., Ketsa, S., and van Doorn, W.G. (2009). The relationship between chilling injury and membrane damage in lemon basil (Ocimum × citridourum) leaves. Postharvest. Biol., Technol. 51, 91–96.

Woolf, A.B., Cox, K.A., White, A., and Ferguson, I.B. (2003). Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados. Postharvest Biol. Technol., 28, 113–122.

Xie, Z.X., Duan, L.S., Tian, X.L., Wang, B.M., Eneji, A.E., Li, Z.H., 2008. Coronatine alleviates salinity stress in cotton by improving the antioxidative defense system and radical-scavenging activity. Plant Physiol. 165: 375–384.

Yang, H., Wu, F., and Cheng, J. (2011). Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. Food Chem., 127, 1237–1242.

Yasunaga, E., Hamanaka, D., Kuroki, S., Hu, W., Uchino, T., and Akimoto, K. (2001). Effect of gas concentration change on respiration rate of cucumber fruit. J. Japan. Soc. Agric. Machinery, 63, 39–44.

Yearsley, C.W., Banks, N.H., Ganesh, S., and Cleland, D.J. (1996). Determination of lower oxygen limits for apple fruit. Postharvest Biol. Technol., 8, 95–109.

Yearsley, C.W., Banks, N.H., and Ganesh, S. (1997). Temperature effects on the internal low oxygen limits of apple fruit. Postharvest Biol. Technol., 11, 73–83.

Young, I.S., and McEneny, J. (2001). Lipoprotein oxidation and atherosclerosis. Biochem. Soc. Trans., 29, 358–362.

Zagory, D., and Kader, A.A. (1988). Modified atmosphere packaging of fresh produce. Food Technol., 42, 70–77.

Appendix 1. Production of Indonesian fruits in 2010–2013

No	Commodities	Production (Ton)			
		2010	2011	2012	2013
1	Avocado*	224,278	275,953	294,200	280,642
2	Star fruit	69,089	80,853	91,788	81,619
3	Duku	228,816	171,113	258,453	206,889
4	Durian*	492,139	883,969	888,127	859,318
5	Guava *	204,551	211,836	208,151	235,049
6	Water rose apple	85,973	103,156	104,393	114,510
7	Citrus*	1,937,773	1,721,880	1,498,394	1,288,585
8	Pomelo	91,131	97,069	113,375	125,956
9	Mango*	1,287,287	2,131,139	2,376,333	2,092,901
10	Mangosteen*	84,538	117,595	190,287	126,613
11	Jackfruit*	578,327	654,808	663,930	737,571
12	Pineapple*	1,406,445	1,540,626	1,781,894	1,145,806
13	Papaya*	675,801	958,251	906,305	1,006,494
14	Banana*	5,755,073	6,132,695	6,189,043	6,380,471
15	Rambutan*	522,852	811,909	757,336	984,932
16	Salak	749,876	1,082,125	1,035,406	1,018,058
17	Sapodilla*	122,813	118,138	135,322	144,967
18	Passion fruit*	132,011	140,895	134,527	166,834
19	Soursop	60,754	59,844	51,802	69,917
20	Breadfruit*	89,231	102,089	111,766	127,373
21	Apple	190,609	200,173	247,073	362,912
22	Grape	11,700	11,938	10,161	17,013
23	Melon	85,161	103,840	125,447	70,009
24	Watermelon*	348,631	497,650	515,505	479,900
25	Cantaloupe	30,668	62,928	57,917	65,812
26	Strawberry	24,846	41,035	169,796	56,621
Total		15,490,373	18,313,507	18,916,731	18,246,772

* Chilling-sensitive products according to Kader (2002a)

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014

Appendix 2. Production of Indonesian vegetables in 2010–2013

No	Commodities	Production (Ton)			
		2010	2011	2012	2013
1	Shallots	1,048,934	893,124	964,195	959,953
2	Garlic	12,295	14,749	17,630	13,286
3	Green onion	541,374	526,774	596,805	686,813
4	Potato*	1,060,805	955,488	1,094,232	823,856
5	Cabbage	1,385,044	1,363,741	1,450,037	1,355,892
6	Cauliflower	101,205	113,491	135,824	81,175
7	Mustard	583,770	580,969	594,911	636,241
8	Carrots	403,827	526,917	465,527	455,695
9	Radish	32,381	27,279	39,048	49,585
10	Kidney bean	116,397	92,508	93,409	116,162
11	String bean	489,449	458,307	455,562	547,402
12	Chili*	807,160	888,852	954,310	964,121
13	Cayenne*	521,704	594,227	702,214	639,765
14	Paprika	5,533	13,068	8,610	18,171
15	Mushroom	61,376	45,854	40,886	61,589
16	Tomato*	891,616	954,046	893,463	950,109
17	Eggplant*	482,305	519,481	518,787	547,768
18	Green bean*	336,494	334,659	322,097	325,146
19	Cucumber*	547,141	521,535	511,485	615,622
20	Chayote	369,846	428,197	428,061	447,846
21	Kale	350,879	355,466	320,093	406,198
22	Spinach	152,334	160,513	155,070	203,369
23	Melinjo	214,355	217,524	224,333	246,941
24	Petai	139,927	218,625	216,194	217,454
25	Jengkol	50,235	65,830	62,189	45,466
Total		10,706,386	10,871,224	11,264,972	11,415,625

* Chilling-sensitive products according to Kader (2002a)

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014

Appendix 3. Export volume of Indonesian fruits in 2010–2013

No	Commodities	Export volume (Ton)			
		2010	2011	2012	2013
1	Citrus	1,339	1,005	1,315	1,561
2	Apple	86	112	42	81
3	Pear			0	72
4	Grape	148	555	835	596
5	Durian	25		3	0
6	Banana	14	1,735	2,674	5,680
7	Melon & watermelon	271	425	753	503
8	Mango	999	1,485	1,525	1,089
9	Strawberry	374	82	65	54
10	Pineapple	159,009	189,223	198,123	174,096
11	Papaya	111	468	22	26
12	Rambutan	533	496	654	398
13	Duku & starfruit	-	-	1	1
14	Jackfruit	28	4	5	18
15	Mangosteen	11,388	12,603	19,724	7,647
16	Other	22,019	14,818	6,499	6,064
Total		196,341	223,011	232,240	197,886

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014

Appendix 4. Import volume of Indonesian fruits in 2010–2013

No	Commodities	Import volume (Ton)			
		2010	2011	2012	2013
1	Citrus	203,916	231,542	269,167	111,752
2	Apple	199,484	214,245	211,137	131,665
3	Pear	111,276	133,592	151,445	128,000
4	Grape	44,087	59,162	70,889	41,569
5	Durian	24,368	27,149	20,813	4,881
6	Banana	2,779	1,631	2,042	337
7	Melon & Watermelon	1,400	1,180	1,147	28
8	Mango	1,129	989	1,267	119
9	Strawberry	452	564	547	611
10	Pineapple	219	267	327	260
11	Papaya	580	299	70	-
12	Rambutan	23	27	41	-
13	Duku & Starfruit	150	6	1	-
14	Jackfruit	35	66	47	-
15	Mangosteen	13	20	1	-
16	Other	102,791	161,339	185,293	116,239
Total		692,703	832,080	914,233	535,461

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014

Appendix 5. Export volume of Indonesian vegetables in 2010–2013

No	Commodities	Export volume (Ton)			
		2010	2011	2012	2013
1	Garlic	284	214	1,075	1,842
2	Shallots	3,234	13,792	18,980	4,982
3	Onion	34	43	1,475	1,542
4	Potato	6,931	5,867	6,538	6,089
5	Chili	7,928	6,837	10,613	11,009
6	Carrot	5	30	2	2
7	Tomato	626	699	2,799	2,755
8	Pea	2,388	1,482	159	96
9	Mushroom	9,332	7,148	5,272	6,259
10	Corn	306	534	719	818
11	Cabbage	31,941	23,941	58,712	53,672
12	Cauliflower & Broccoli	71	46	71	32
13	Cucumber	74	60	57	61
14	Eggplant	949	1,433	1,596	1,425
15	Other	74,003	71,822	92,598	37,746
	Total	138,106	133,948	200,667	128,330

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014

Appendix 6. Import volume of Indonesian vegetables in 2010–2013

No	Commodities	Import volume (Ton)			
		2010	2011	2012	2013
1	Garlic	361,289	419,090	471,105	446,577
2	Shallots	73,270	160,467	123,315	96,139
3	Onion	56,352	78,681	74,343	37,722
4	Potato	53,250	104,704	122,508	107,900
5	Chili	20,200	28,887	27,896	23,145
6	Carrot	33,692	41,868	66,369	18,599
7	Tomato	10,325	10,639	13,090	12,614
8	Pea	14,478	22,120	20,856	22,713
9	Mushroom	3,081	3,373	5,974	4,227
10	Corn	2,447	3,285	3,542	3,250
11	Cabbage	1,228	2,179	2,281	952
12	Cauliflower & broccoli	285	269	953	569
13	Cucumber	40	40	150	137
14	Eggplant	1	-	0	-
15	Other	221,430	298,682	326,316	220,240
Total		851,368	1,174,284	1,258,699	994,784

Source: Directorate General of Horticultural, Ministry of Agriculture Republic of Indonesia, 2014.