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Influence of relative humidity on development of chilling injury of cucumber fruits during low temperature storage

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***Abstract*—** The objective of this study is to evaluate the effect of relative humidity (RH) on development of the chilling injury (CI) symptoms and the shelf life of cucumber fruits. The fruits were stored at 5°C with RH conditions set at high (99%), medium (80%) and low (60%) for 5 days. During storage, gas composition in the chamber was maintained as same as ambient. After storage at 5°C, the fruits were stored at room temperature (24.5°C) for 6 days to check shelf life. Fruits were weighed before and after storage at 5°C to determine weight loss. Skin colour, firmness, electrolyte leakage and malondialdehyde (MDA) content were also evaluated before and after storage at 5°C, as well as every 2 days during shelf life period. The water loss of fruit was suppressed at high RH compared with low or medium RH after storage. Skin colour change (L*, C*, h°) was also maintained at high RH after storage 5°C. In addition, yellowing index increased rapidly of the fruits stored at low RH during shelf life period, while at medium and high RH was supressed at early stage of storage but increased thereafter. No significant different in the firmness was observed after storage at 5°C and during shelf life period. The electrolyte leakage and MDA content which are an indicator of cell membrane damage caused by CI of the fruits stored at low and medium RH increased significantly within 5°C storage period, while at high RH was supressed, however, after storage at 5°C, significant different was not found among them. During shelf life condition, the increase of electrolyte leakage had similar trends with MDA content, which these values increased rapidly of the fruits stored at low RH. While, on the fruits stored at medium RH, the increase of electrolyte leakage and MDA content was observed on the last day of shelf life condition, conversely, these values were supressed of the fruits stored at high RH. These results suggest that controlled RH during distribution process in low temperature is critical due to influence development of CI and acceptable quality of fruit. Storage at high RH not only suppressed the water loss, but also minimized the CI symptoms.

***Keywords*— cucumber fruits, chilling injury, relative humidity, shelf life.**

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INTRODUCTION

The high demand of agricultural products encourages the increasing of their trade values in 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. However, 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, which it has effect directly on lowering fruit respiration, ethylene production, and fruit metabolism. For fresh agricultural produces, some of them are sensitive to chilling temperature. Prolong of storage period may result in chilling injury (CI), whose symptoms are develop when the products removed from chilling to shelf life condition.

Cucumber is chilling sensitive and susceptible to CI for more than 3 days held at temperatures of less than 10°C (Karakas & Yildiz, 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 & 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, whereas maintenance of 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, 2002). Low in RH increases transpiration damage and leads the products to desiccation; conversely, the 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 conducted. Thus, the purpose of study was to evaluate the effect of RH on the development of CI symptoms in cucumber fruit during storage at low temperature. Cucumber fruit was selected in the present study because it is a highly perishable product due to water loss. Fruits were stored at 5°C for 5 days under three RH conditions; low (60%), medium (80%) and high (99%), respectively. After storage at 5°C, fruits were transferred to room temperature (24.5°C) to check shelf life for 6 days. Quality parameter such as weight loss and firmness as physical indices, skin colour as sensory evaluation, and electrolyte leakage and MDA content as the CI indices were evaluated.

MATERIALS AND METHODS

A. Plant Materials and Storage Conditions

Cucumber fruits (*Cucumis sativus* L.) were purchased from Kanesue Supermarket in Gifu, Japan. Thirty fruits for each RH condition were sorted and selected for uniform size and absence of visual defects. They were then putted in acrylic chamber (volume = 0.554 m^3) and stored in an incubator (MIR-154-PJ, Panasonic, Japan) set temperature at 5°C for 5 days. Three RH conditions were established: 60%, 80%, and 99% as low, medium and high RH, respectively. RH in chamber was controlled using RH controller (Japan-Elekit, Japan) equipped with a RH sensor. The RH and temperature change during storage were measured by self-recording thermo recorder (RTR-52, T & D Corporation, Japan). During storage, gas composition in the chamber was maintained as same as ambient. The concentration of O_2 was measured using O_2 controller (MC-86, Ijima Electronic, Japan), while CO_2 used CO_2 controller equipped with a CO_2 probe (GMP221, Vaisala Oyj, Finland) as sensor, a pump-aspirated sampling (GM70, Vaisala Oyj, Finland) for circulation of the sample gas, and an indicator of measurement (M170, Vaisala Oyj, Finland). The outputs from O_2 and CO_2 controller were recorded by touch panel paperless recorder (TR-V550, Keyence Corporation, Japan), which the outputs from O_2 and CO_2 controller were used as input to control a pump making the gas concentration in chamber similar with ambient.

After storage at 5°C for 5 days, the fruits were transferred to room temperature (24.5°C) for 6 days. Skin colour, firmness, electrolyte leakage, and MDA content were evaluated before and after storage at 5°C (5d) and followed every 2 days at shelf life conditions (7, 9, 11 d), whereas weight loss was evaluated only before and after storage at 5°C (5d). The results of quality test were expressed as a percentage (%) i.e. the ratio of the value at time to the value at initial.

B. 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:

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

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

C. Skin Colour

Skin colour was measured using Minolta chromameter (CR-13, Minolta, Japan) to get parameter L^*, a^* and b^*. The measurements of colour were carried out from fives fruits. Four reading were made at equator on the fruit. The results expressed as L^* value correspond to lightness, whereas chorma and hue-angle (h^o) conform to intensity and actual colour 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*| (Hitora et al, 2003).

D. Firmness

Firmness was measured using a Rheo meter (Compac-100 II, Sun Scientific, Japan) from fives of fruits equipped with a 30-mm diameter of plate plunger operated at a depth 1-mm. Stainless steel cork borer was used to produce 17.5-mm diameter and 20-mm thick of discs. The results expressed as F (N) conform to the force exerted on a sample under tension.

E. Electrolyte Leakage

Electrolyte leakage was assessed by the methods described by Saltveit (Salveit, 2002) with some modifications. Mesocarps of cucumber (11-mm diameter) were excised with stainless steel cork borer to produce 4-mm thick discs. The discs were washed in deionized water three times for about 1 min each time, blotted dry and three discs selected were placed in plastic Petri dishes. Three discs were put into a 50-ml centrifuge tubes with 20-ml 0.2 M mannitol and shaken at 100 cycles/min using water bath incubator (Personal-11, Taitec., Japan). Conductivity was measured using conductivity meter (ES-51, Horiba, Japan) 0.5 h after adding the mannitol. The tubes were then frozen, thawed, and weighed. The contents were incubated for 10 min in a 50-ml flask, allows cooling to room temperature, and transferred back to plastic tubes. Deionised water was added to make initial weight and the total conductivity was measured after an additional 0.5 h of shaking. Individual conductivity reading was divided by the total conductivity and multiplied with 100 to convert the readings to the percent of total conductivity.

F. MDA Content

MDA content was determined following method of Hodges et al. (Hodges et al, 1999) with some modifications. 1 g mesocarp tissue of cucumber fruit was homogenized in 10 mL of 80:20 (v/v) ethanol: water along with 0.5 g inert sand using a mortar and pestle followed by centrifugation at 3000 × g and temperature 4°C for 10 min. A 1 mL aliquot of the appropriately diluted sample was either added to 1 mL of 0.65% thiobarbituric acid (TBA) solution containing 20.0% (w/v) trichloroacetic acid (TCA) and 0.01% butylated hydroxytoluene (BHT) or added to a solution containing 20.0% (w/v) TCA and 0.01% BHT.

The samples were then mixed vigorously, incubated at 95°C for 25 min, cooled in an ice bath immediately, and centrifuged at 3000 × g and temperature 4°C for 10 min. Absorbances at 532 nm, 440 nm, and 600 nm were recorded using a UV–vis spectrophotometer. The MDA equivalents were calculated using the following questions:

$$[(A532_{+TBA} - A600_{+TBA}) - (A532_{-TBA} - A600_{-TBA})] = A \quad (2)$$
$$[(A440_{+TBA} - A_{+TBA}) \times 0.0571] = B \quad (3)$$
$$MDA\ equivalents\ (nmol\ ml^{-1}) = \big((A - B)/157000\big) \times 10^6 \quad (4)$$

G. Statistical Analyses

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).

RESULTS AND DISCUSSIONS

A. Weight Loss

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.1). These results were expected, as weight loss of low RH cucumber was one half compared with medium RH and twenty 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 produce after harvest is a serious problem causing shrinkage and loss of weight (Mahajan, et. Al, 2008). Most commodities become unsalable as fresh produce after losing 3-10% of their weight (Ben-Yehosua & Rodov, 2003). In our results, increase of water loss after storage at 5°C of the fruits stored at low and medium RH conditions, which was clearly demonstrated by the higher of its value compared with high RH. It has been reported that increase in weight loss at low temperature might be 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). Aqüero et al. (2011) also 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 produce, the rate of water loss must be as low as possible.

B. Skin Colour

Storage of cucumber fruits to different RH conditions had a different effect on development of external colour retention. During period of storage at 5°C, fruits that held at low and medium RH, showed a significant decrease in lightness. Contrary to the fruits exposed at high RH, significant decrease of lightness did not show. It indicates that a dark green of skin colour because of the severe dehydration.

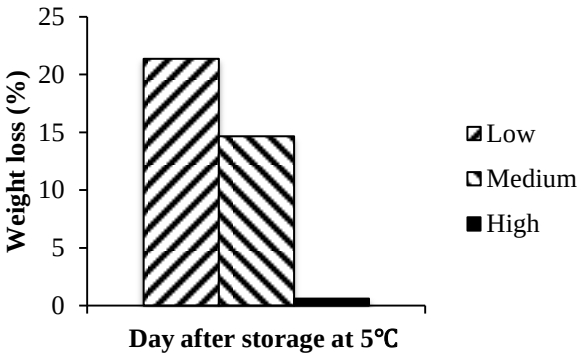

Fig.1 Weight loss of cucumber fruits after stored at 5°C under different RH conditions; low (60%), medium (80%) and high (99%).

Intensity in terms of chroma was also decreased significantly within 5°C storage period of the fruits stored at low RH, while on the fruits stored at medium or high RH significant decrease was not shown. Similar with chroma result, the actual colour of fruit (hue-angle) also decreased significantly at low RH, while at medium or high RH, the decrease of these values were supressed. After storage at 5°C (5 d), cucumber fruits held at low RH showed a lower in skin colour indices compared to those at medium or high RH (Table1), where significant different was shown among them. The dehydration that occurred at low RH caused a deleterious effect on the overall visual quality (Medina et al, 2012). Storage under low RH conditions resulted in substantial degradation in the appearance of cucumber fruits, mainly loss of their lightness, chroma and hue-angel.

Table1
Skin colour indices of cucumber fruits after stored at 5°C for 5 days under different RH condition; low, medium and high RH.

RH	Relative skin colour (%)		
	*L**	*C**	*H°*
Low	91.68b	72.76b	95.37b
Medium	95.79a	91.99a	101.03a
High	98.24a	99.72a	98.51ab

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

Yellowing of the peel is a common postharvest disorder of 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 & Lipton, 1979; Salunkhe & Desai, 1984). During period of storage at room temperature, a sharp increase in YI was observed on the fruits held at low RH, while the cucumber fruits held at medium RH, the YI was remained up to 7 days, after that, the YI increased rapidly. On other hand, the fruits held at high RH, the increase of YI appeared after 9 days of

storage (Fig.2). Pitting, dark watery patches and increase susceptibility to decay are visible symptoms of CI in cucumber fruit. In our results, manifestation of decay was increased rapidly at low RH after fruits were transferred to shelf life condition, which after 7 days of storage, cucumber fruits could not be observed because of decay. Morris and Platenius (Moris & 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.

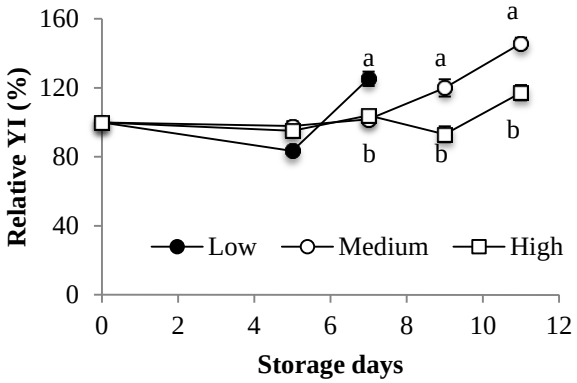

Fig.2 Yellowing index of cucumber fruits during stored at 5°C plus at 24.5°C for 6 days under different RH conditions; low (60%), medium (80%), and high (99%). Vertical bars represent standard error of the mean. Values with different letters for each day were significantly different at P<0.05.

C. Firmness

The firmness of cucumber fruit exposed to different RH conditions decreased gradually during period of storage (Fig.3). Significant decreased in firmness was observed on the fruit stored at low and medium RH within 5°C storage period, but significant decrease was not found at high RH. When the firmness was evaluated after storage 5°C, the firmness was lower on fruits stored to low RH compared to medium or high RH, but no significant different were observed among them. Transferred of fruits to room temperature, the firmness of cucumber stored at high RH did not change significantly during period of storage. In contrasts to the fruits stored at low and medium RH, the firmness decreased during period of storage, however significant different was also not found among them. RH had impact related to fruit softening, which fruit with a greater in mass loss at low and medium RH, lost of firmness more than fruit stored at high RH (Sharkey & Peggie, 1984.

D. Electrolyte Leakage

Increase electrolyte leakage of cucumber fruits during storage at chilling temperature is a qualitative indicator of CI. As shown in (Fig.4), significant increase in electrolyte leakage was shown of fruits stored at low and medium RH during period storage at 5°C, while on the fruits stored at high RH significant increase did not show.

When the electrolyte leakage was observed after storage, high RH cucumbers had a lower electrolyte leakage compared with low or medium RH cucumbers, but significant different did not show among them, neither at low RH nor at medium and high RH. Sharom et. al, (1994) reported that increase in electrolyte leakage was observed after transferred to room temperature.

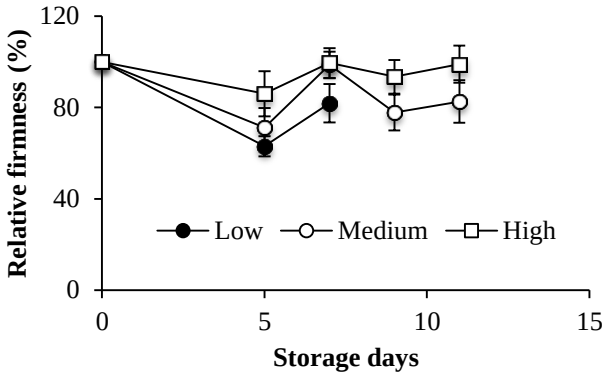

Fig.3 Firmness of cucumber fruits during stored at 5°C for 5 days plus at 24.5°C for 6 days under different RH conditions; low (60%), medium (80%), and high (99%) RH. Vertical bars represent standard error of the mean.

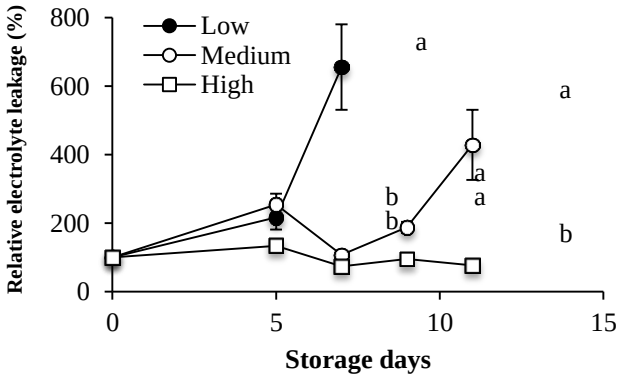

Fig.4 Electrolyte leakage of cucumber during stored at 5°C for 5 days plus at 24.5°C for 6 days under different RH condition; low (60%), medium (80%) and high (99%) RH. Vertical bars represent standard error of the mean. Values with different letters for each day were significantly different at P<0.05.

However, during shelf life condition at room temperature, the electrolyte leakage increased rapidly of the fruits stored at low RH, while on the fruits stored at medium RH, the increase of electrolyte leakage was supressed up to 7 days of storage, after that, electrolyte leakage increased significantly. In contrary, the increase of electrolyte leakage was suppressed on the high RH fruits during shelf life period. Ion leakage has been used an indicator of damage to the plasma membrane, which increased on electrolyte leakage at low RH fruits, is an evidence of membrane deterioration.

E. MDA Content

As the final product of lipid peroxidation, MDA is often used as an index of cell oxidative damage under environmental stress (Shen & Wang, 1997) The MDA of the fruits stored at different RH condition increased significantly within 5°C storage period, however, after storage at 5°C significant different was not found among them.

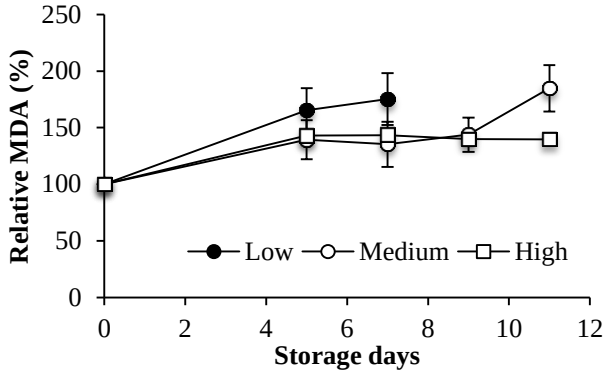

Fig.5 MDA content of cucumber fruits during stored at 5°C for 5 days plus at 24.5°C for 6 days exposed to low (60%), medium (80%) and high (99%) RH. Vertical bars represent standard error of the mean.

After fruits were transferred to room temperature, MDA content increased continuously of fruits stored at low RH, whereas at medium RH, the increase of MDA content was observed at the end of the shelf life condition tested. In contrast to the fruit held at high RH, the increasing of MDA content was supressed during period of storage. The increasing of MDA content after stored at 5°C was associated with chilling-membrane damage. Maintaining high RH during storage at low temperature reduced the expression of CI symptoms.

CONCLUSIONS

In this study, the development of CI symptoms of cucumber fruit was different when exposed to different RH conditions. Storage of cucumber fruit at low or medium RH increased the water loss and accelerated of decay after transferred to self-life condition. On other hand, high RH storage not only reduced fruit moisture loss and subsequently maintained fruit skin color change and firmness but also significantly minimized the expression of CI.

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