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Effect of 1–MCP Concentration on the Quality of Broccoli (*Brassica Oleracea* L., var. *Italica*) during Storage

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The effect of 1–methylcyclopropene (1–MCP) on the quality of broccoli was investigated in this study. Broccoli was treated with air (control) and 1–MCP (0.5, 1.0, 1.5, 2.5 and 5.0 μL/L) at 15˚C for 15 h, and then stored at 15˚C for 10 days in low density polyethylene (LDPE) film. The inhibition of postharvest degradation by 1–MCP treatment was remarkable. The color changes, weight loss and ethylene production of broccoli were inhibited. Furthermore, 1–MCP treated broccoli had higher content of ascorbic acid, total phenolic compounds, and carotenoids than the control sample. These results indicated that 1.0 and 2.5 μL/L of 1–MCP treatment was the most effective in extending the shelf life of broccoli with maintaining its quality.

Key words: broccoli, 1–MCP, ethylene production, quality, respiration

INTRODUCTION

Broccoli is one of the most popular vegetables in the world and is known as a fresh vegetable with high nutritional value of vitamins, antioxidants and anti–cancer substances (Podsedek, 2007). Floret yellowing is a major quality deterioration factor of broccoli during transportation and storage. The yellowing of florets occurred significantly after 2 to 3 days at 20˚C and completely yellowing in 4 days (Wang, 1977) and is caused by sepal chlorophyll degradation, but the pedicel and stem do not turn yellow after harvest (Clarke *et al*., 1994). It is necessary to retain the green color as long as possible for the quality maintenance. Delaying senescence phenomena is the main goal of postharvest handling of broccoli (Page *et al*., 2001). Oxidative metabolic processes are mainly related to the physicochemical changes in fresh fruit and vegetables after harvest. Respiration changes the chemical components and quality deterioration of fresh vegetables. Broccoli deteriorates quickly due to its relatively high respiration rate when stored at room temperature (Gillies and Toivonen, 1995). Suppression of respiration rate during post–harvest is effective in maintaining the freshness and quality of broccoli.

1–methylcyclopropene (1–MCP) is effective at very low concentrations and does not leave residues on fruit and vegetables. It has been considered non–toxic for humans and the environment (Luo *et al*., 2007). 1–MCP is an ethylene perception inhibitor and effectively controls the negative effects of ethylene on fresh fruit and vegetables (Watkins, 2006). The inhibitory effect of 1–MCP on the ripening of fruit and vegetables is influenced by internal factors such as species, cultivar, maturity and external factors: treatment temperature, duration and delays between harvest and 1– MCP treatment (Watkins, 2006, 2008). Yuan *et al*. (2010) treated broccoli with 1–MCP at concentrations ranging from 0.1 to 10 μL/L at 20˚C for 6 h and found 2.5 μL/L to be an appropriate concentration; Fernández–León *et al*. (2013) treated broccoli with 0.6 μL/L 1–MCP at 1˚C for 24 h but found no effect on extending shelf life; Fan *et al*. (2000) concluded that treatment with 1.0 μL/L of 1–MCP for 12 h at 10˚C was effective; Gong *et al*. (2003) treated broccoli with 1.0 μL/L of 1–MCP at 20˚C for 14 h and could extend shelf life. The treatment time depended on the temperature during the experiment, and increased as the temperature decreased (Watkins, 2006). If we can define the treatment index (TI) as "concentration × temperature × time", the previous studies were carried out in the range of 30 to 3000 μL/L ˚C h and 1–MCP was effective at TI above than 120 μL/L ˚C h. In this study, broccoli was treated with 0.5, 1.0, 1.5, 2.5, and 5.0 μL/L of 1–MCP at 15˚C for 15 h. It means that TI varied from 112.5 to 1125 μL/L ˚C h.

Postharvest quality retention of broccoli may be better with a combination of 1–MCP and controlled atmosphere (CA) than with 1–MCP alone (Fernández–León *et al*., 2013). Phuong *et al*. (2018) found that the quality of broccoli treated with 1–MCP and modified atmosphere packaging (MAP) was higher than that of broccoli treated with 1–MCP. Low density polyethylene (LDPE) film was the most appropriate film for broccoli among the four types of films were used for broccoli as polypropylene (PP), micro–perforated polypropylene (PP hole), oriented polypropylene (OPP), and low density polyethylene (LDPE) (Phuong *et al*., 2018). Therefore, LDPE film was applied to broccoli storage in this study.

Although there are extensive studies on the effects of 1–MCP on broccoli quality parameters (Fan &

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Mattheis, 2000; Ku & Wills, 1999), there are no extensive studies on the effects of 1–MCP on physiological and bioactive compounds (Yuan *et al*., 2010). Therefore, the aim of this study was to investigate the effect of 1–MCP on quality changes of broccoli during storage at 15˚C, especially on physiological and bioactive compounds.

MATERIALS AND METHODS

Plant material

Broccoli (*Brassica oleracea* L., var. *italica*), 'Grandome' cultivar, was obtained from a wholesale store in Fukuoka, Japan. It was precooled at 3˚C in 3 h and transported to the laboratory within 1 h and selected before the experiment as uniform and good in appearance with a weight of 330±72 g and a diameter of 15±2 cm.

1–MCP treatment

Broccoli was treated with 1–MCP (Rohm and Haas China Inc., Beijing, China) at different concentration as follows: 0.0, 0.5, 1.0, 1.5, 2.5 and 5.0 μL/L of 1–MCP at 15˚C for 15 h (TI = 0, 112.5, 225, 337.5, 562.5 and 1125 μL/L ˚C h, respectively). For each 1–MCP treatment, 12 broccoli heads placed in a plastic box (54 cm × 64 cm × 65 cm) contained a beaker with 150 mL of distilled water and 1–MCP, and then the plastic box was immediately sealed with a lid and kept at 15˚C for 15 h. After treatment, all samples were packed in LDPE bags (20 μm in thickness, 20 cm × 30 cm, Marumoto Co., Japan) and stored at 15˚C with relative humidity (RH) of 55%. Three replicates were done with three broccoli per replicate. The samples were taken randomly every 3 or 4 days to analyze color change, weight loss, total carotenoids, chlorophyll a, chlorophyll b, ascorbic acid, respiration rate, ethylene production and total phenolic compound.

Color

Color parameters such as L^*, a^*, and b^* were measured in the color space CIELab using a colorimeter (CR–20, Konica Minolta, Japan). Measurements were taken at nine different locations on each broccoli head. The results were expressed as L^*, a^*, b^* and ΔE values.

Weight loss

Individual broccoli was weighted on the harvest day and after the different sampling days for each treatment. Weight loss was expressed as a percentage of fresh weight (FW).

Total carotenoids and chlorophyll content determination

A 1–g of broccoli was ground with 15 mL of acetone–hexane (4:6) and homogenized at 20 000 rpm for 2 min using the homogenizer. The mixture was centrifuged at 10 000 rpm and 4˚C for 10 min. Total chlorophyll and carotenoid contents in the supernatant were determined by reading the absorbance at 400~800 nm using a spectrophotometer (UV–530, JASCO, Japan). Chlorophyll a showed maximum absorbance at 663 nm, chlorophyll b at 645 nm, total carotenoids at 505 nm and 453 nm, and the contents of these pigments was calculated. The content of total carotenoids was expressed in mg/100 g FW. The chlorophyll content was the sum of chlorophyll a and chlorophyll b, and estimated as mg chlorophyll/100 g FW. All measurements were performed in triplicate.

The concentrations of total chlorophyll and carotenoids were calculated using the following formulas:

Chlorophyll a (mg/100 g) = $[(12.64 \times A_{663}) - (2.99 \times A_{645})] \times 10.1 \times (100/0.1) \times (1/1000)$

Chlorophyll b (mg/100 g) = $[(-5.6 \times A_{663}) + (23.26 \times A_{645})] \times 10.1 \times (100/0.1) \times (1/1000)$

Total chlorophyll = chlorophyll a + chlorophyll b

Carotenoids (mg/100 g) = $0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$

Where A_{453}, A_{505}, A_{645} and A_{663} were absorbance at 453 nm, 505 nm, 645 nm and 663 nm each other.

Ascorbic acid determination

Fresh broccoli (5 g) was homogenized with 30 mL of 5% metaphosphoric acid using a mortar (T25, Homogenizer Ultra–Turrax, IKA, China). The mixture was centrifuged at 3 500 rpm for 10 min at 20˚C (Centrifuge 5922, Kubota, Japan) and the filtrate was collected by filtering through filter paper. Ascorbic acid was measured using a reflectometer (RQflex 20, KANTO KAGAKU, Japan). The content of ascorbic acid was expressed in mg/100 g FW.

Respiration rate and ethylene production

Broccoli was weighed and sealed in the gas–tight glass desiccator, and incubated at 15˚C for 2 h. A 1–mL gas sample was withdrawn from the valve with a gas tight syringe and injected into the gas chromotograph (GC) (GC–390, Shimadzu, Japan). The gas samples were separated in columns for CO_2, O_2 and N_2 and analyzed with a thermal conductivity detector (TCD). Helium gas was used as a carrier gas. The temperatures in the injector, column and detector were set to at 120, 30 and 120˚C, respectively. The results of respiration rate were expressed in mg/kg.h. Ethylene production was measured using a handheld VOC gas detector (Tiger, ION Science Ltd., UK) and the ethylene production was expressed in μL C_2H_4/kg.h.

Total phenolic compounds

Approximately 200 mg of freeze–dried broccoli florets was homogenized twice in 10 mL of 80% ethanol. The mixture was incubated in a water bath at 80˚C for 3 min and then cooled for 5 min. It was then placed in an ultrasonic bath at 20˚C for 30 min. The mixture was then centrifuged at 3 000 rpm for 10 min at 20˚C and filtered through a filter paper. The filtrate was diluted to 20 mL by adding distilled water. The extract was used to determine the total phenolic compounds by a method modified from Singleton *et al*. (1999). A 0.1–mL of the extract was added to 7.9 mL of distilled water and 0.5 mL of Foline–Ciocalteu reagent. After 3 min at 25˚C, 1.5 mL of 20% Na_2CO_3 solution was added and incubated

in darkness at 25°C for 1.5 h. The absorbance at 725 nm was measured using a spectrophotometer (UV–530, JASCO, Japan) and the total phenolic compounds were calculated using gallic acid as a standard. The total phenolic compounds were quantified using gallic acid by preparing standard curves at concentrations of 0~0.025 mg/mL. The results of total phenolic compounds were expressed as mg gallic acid equivalent (GAE) per gram of FW (mg GAE/g FW).

Statistical analyses

All results were reported as mean values and their standard deviations. Data were analyzed using one–way ANOVA model test, and differences in means between treatments were determined by Duncan multiple range test at $P < 0.05$. The SAS package program (version 11.5) was applied to analyzed data.

RESULTS AND DISCUSSION

Effect of 1–MCP on respiration rate and ethylene production

Respiration rate is a key factor to evaluate the effect of 1–MCP treatment on the physicochemical changes of fruit and vegetables during storage. An increasing respiration rate accelerates metabolism and reduces fruit and vegetable quality (Ma *et al*., 2009; Tian, 1997). 1–MCP has recently been applied to broccoli to reduce respiration rate during storage and in the market (Fernández–León *et al*., 2013; Fan *et al*., 2000). 1–MCP could delay broccoli aging during postharvest because it inhibits the synthesis of ethylene, a senescence accelerator (Ku and Wills, 1999; Able *et al*., 2002; Gong and Mattheis, 2003). The results of respiration measurements of broccoli are shown in Table 1. The respiration rate was significantly affected by 1–MCP treatment after 10 days of refrigerated storage ($P < 0.01$). The respiration rate of 1–MCP treated broccoli decreased rapidly from day 0 to day 3 of storage and was significantly lower than that of the control ($P < 0.05$). The respiration rate of control and 1–MCP treated broccoli reached its peak on the 7th day of storage. After reaching the peak in both treatments, the respiratory rate decreased on the 10th day. However, the respiratory rate of control broccoli was significantly higher than that of 1–MCP–treated broccoli at the same time ($P < 0.01$). The respiratory rates of broccoli treated with 1.5 and 2.5 μL/L 1–MCP were higher than those of broccoli treated with 0.5, 1.0, and 5.0 μL/L 1–MCP (141.93 mg CO_2/kg.h, 130.46 mg CO_2/kg.h, and 133.07 mg CO_2/kg.h, respectively), but lower than those of the control samples (121.88 mg CO_2/kg.h and 128.19 mg CO_2/kg.h, respectively). The respiratory rate of the control sample was 146.5 mg CO_2/kg.h, which was about 17% higher than that of the sample treated with 1.5 μL/L of 1–MCP (121.88 mg CO_2/kg.h). In the present study, 1–MCP treatment decreased the respiratory rate and delayed the peak respiration. As a result, the respiration rate of 1–MCP treated broccoli was effectively suppressed, thus maintaining its quality during storage.

Ethylene production accelerates the senescence of the broccoli florets. A direct indicator of broccoli senescence was the peak of ethylene production during storage (Ma *et al*., 2014). The results of ethylene production of control and 1–MCP samples are shown in Table 1. The ethylene production of broccoli treated with 1–MCP was markedly lower than that of control broccoli stored at 15°C at all time points. Ethylene production was significantly suppressed by 1–MCP. The ethylene production of both control and 1–MCP treated broccoli reached its peak on the 7th day of storage, and the inhibitory effect of 1–MCP on ethylene production was dependent on the dose of 1–MCP used (Table 1). The ethylene production on the 10th day of storage was statistically equivalent in 1.0, 1.5, 2.5 and 5.0 μL/L 1–MCP treatments. Makhlouf *et al*. (1989) proposed that broccoli is a climacteric vegetable because the respiration rate and ethylene production increase during senescence and the florets turn yellow. In the present study, respiration rate and ethylene production increased slightly when the florets turned yellow. The 1–MCP treatment effectively inhibited ethylene production and respiration rate. This result confirms that broccoli is a climacteric vegetable group. In conclusion, 1–MCP treatment at a concentration of 1.0 μL/L inhibited the respiration rate and ethylene production of broccoli during postharvest storage at 15°C. This result was consistent with the results of Ku

Table 1. Respiration rate and ethylene production of broccoli subjected to 1–MCP during storage

Treatment	Respiration rate (mg CO_2/kg.h)				Ethylene production (μL C_2H_4/kg.h)			
	Days of storage							
	0	3	7	10	0	3	7	10
Control	173.79	130.84^{a}	181.82^{a}	146.55^{a}	0.17	0.51^{a}	1.64^{a}	0.99^{a}
1MCP 0.5	173.79	124.58^{a}	160.94^{b}	141.93^{a}	0.17	0.44^{ab}	1.20^{b}	0.73^{b}
1MCP 1.0	173.79	116.78^{ab}	152.77^{c}	130.46^{b}	0.17	0.37^{b}	1.07^{bc}	0.51^{c}
1MCP 1.5	173.79	115.94^{ab}	143.40^{d}	121.88^{c}	0.17	0.35^{b}	0.71^{d}	0.49^{c}
1MCP 2.5	173.79	119.31^{ab}	142.21^{d}	128.19^{bc}	0.17	0.26^{c}	0.65^{d}	0.46^{c}
1MCP 5.0	173.79	105.06^{b}	154.65^{c}	133.07^{b}	0.17	0.35^{b}	0.92^{c}	0.57^{c}
F–test	*NS*	*	**	**	*NS*	**	**	**

*Means within the same column followed by different letters are significantly different. *P ≤ 0.05, **P ≤ 0.01, NS – non significance.*

and Wills (1999), Fan and Mattheis (2000), Able *et al.* (2002). However, Ku and Wills (1999) found that a higher concentration of 1–MCP resulted in a longer shelf life of broccoli florets, suggesting that a lower concentration range (1.0~10 μL/L) should be used to avoid toxicity. Able *et al.* (2002) showed that the optimum concentration of 1–MCP for extending the shelf life of broccoli was 12 μL/L 1–MCP. These results indicated that the most effective concentration of 1–MCP for maintaining broccoli quality was 1.0 to 5.0 μL/L 1–MCP. This difference might be due to the variety and temperature used.

Effect of 1–MCP treatment on color changes

The change in floret color from green to yellow is a direct indicator of the senescence of broccoli. The color change of broccoli during storage was evaluated using the color parameters L^*, a^*, b^* and ΔE. The L^* value represents the brightness of the sample, while the a^* and b^* values represent the change in chromaticity coordinates of the sample. ΔE represents the magnitude of the color difference between the reference and the sample. The results of the color change are recorded in Fig. 1, Fig. 2 and Table 2. The increase in L^* and a^* values during storage indicated a change from dark green to light yellow, while the increase in b^* value indicated an increase in the degree of yellowing. In control broccoli, color parameters (L^*, a^*, b^* and ΔE) increased significantly ($P < 0.05$) at the end of storage. The increase in color parameters was related to the yellowing of broccoli florets. 1–MCP treatment effectively inhibited the yellowing process of broccoli florets. As shown in Fig. 1, a significant difference in color change was clearly observed between control and 1–MCP treatments after 10 days of storage at 15°C.

The L^* value of broccoli on day 0 of storage was 43.79. The L^* value of broccoli increased slightly during storage at 15°C (Fig. 1). The highest L^* values for control and 0.5 μL/L 1–MCP treated broccoli were observed after 10 days of storage (51.64 and 50.10, respectively). The lowest L^* values were observed for 1.0, 1.5, 2.5 and 5.0 μL/L 1–MCP treated broccoli (46.12, 46.83, 46.23 and 47.67, respectively). There was no significant difference in the L^* values of broccoli treated with 1.0, 1.5, 2.5 and 5.0 μL/L 1–MCP after 10 days of storage. The increasing trend of L^* values was in agreement with the findings of Tian (1996) and Yuan *et al.* (2010).

The ΔE values were correlated with total chlorophyll content of broccoli florets (Tian *et al.*, 1994). The ΔE values of broccoli gradually increased during storage. This increase in the ΔE value was associated with yellowing of the florets as shown in Fig. 1. In our study, ΔE values of control and 0.5 μL/L–treated broccoli increased

Table 2. a^* value and b^* value of broccoli subjected to 1–MCP during storage

Treatment	a^* value				b^* value			
	Days of storage							
	0	3	7	10	0	3	7	10
Control	–6.11	–6.36	–5.96	–4.53^{a}	14.94	14.99	15.73^{ab}	22.31^{a}
1MCP 0.5	–6.11	–6.31	–5.91	–5.83^{b}	14.94	14.59	15.91^{a}	20.19^{b}
1MCP 1.0	–6.11	–6.22	–6.26	–6.02^{b}	14.94	14.42	15.01^{abc}	14.75^{d}
1MCP 1.5	–6.11	–6.45	–6.20	–5.92^{b}	14.94	14.89	14.25^{c}	14.18^{d}
1MCP 2.5	–6.11	–6.16	–5.91	–5.48^{b}	14.94	14.20	14.49^{c}	14.63^{d}
1MCP 5.0	–6.11	–6.56	–5.72	–5.92^{b}	14.94	14.25	14.82^{bc}	16.98^{c}
F–test	*NS*	*NS*	*NS*	**	*NS*	*NS*	*	**

*Means within the same column followed by different letters are significantly different. *P ≤ 0.05, **P ≤ 0.01, NS – non significance.*

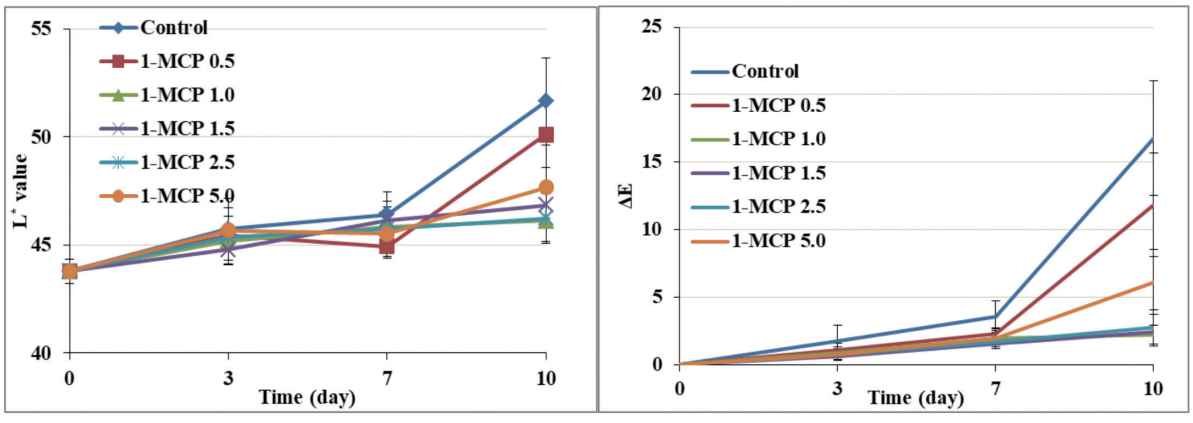

Fig. 1. Effect of 1–MCP treatment on L^* (left) and ΔE (right) of broccoli during storage.

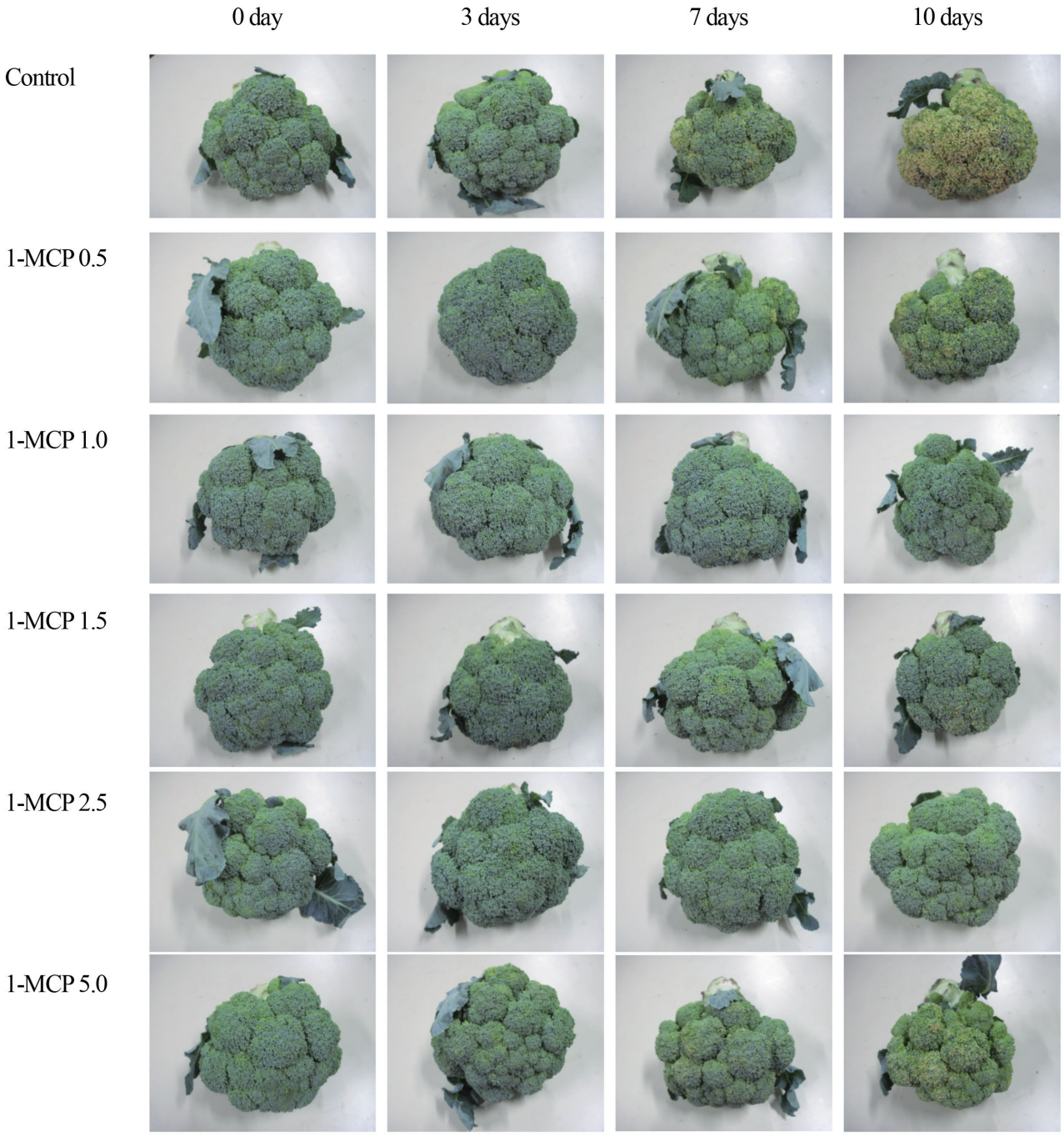

Fig. 2. Effect of 1– MCP treatment on the appearance of broccoli during storage.

slightly during the first 7 days of storage at 15˚C (Fig. 1), but then increased rapidly. The ΔE values of 1–MCP–treated broccoli were significantly lower than that of the control ($P < 0.01$) after 10 days at 15˚C. Treatment with 1–MCP at concentrations of 1.0 to 5.0 μL/L inhibited yellowing of the florets for 10 days after storage. Broccoli treated with 1.0, 1.5 and 2.5 μL/L of 1–MCP showed significantly lower levels of ΔE values than the other treatments. The highest ΔE value was observed in the control broccoli stored at 15˚C for 10 days (16.8). The lowest ΔE value was found in 1.0 μL/L 1–MCP– treated broccoli (2.23) (Fig. 1). The ΔE values of 1.0, 1.5 and 2.5 μL/L 1–MCP treated broccoli were not significantly different after 10 days of storage at 15˚C. The color changes of broccoli have been related to the chlorophyll degradation and yellowing process during storage (Eason et ail, 2007; King and Morris, 1994). Consistent with our results, 1–MCP treatment delayed yellowing and chlorophyll degradation in broccoli florets (Fernández–León *et al*., 2013; Yuan *et al*., 2010).

The values of a^* and b^* of fresh broccoli were –6.11 and 14.94, respectively (Table 2). a^* values increased in all treatments during storage. There was no significant difference between a^* and b^* values in all treatment during the first 7 days of storage at 15˚C. However, there was a significant difference in a^* and b^* values between

control and 1–MCP treatments after 10 days of storage at 15°C. The values of a^* and b^* were statistically higher in control (–4.53 and 22.32, respectively) than in 1.0 μL/L 1–MCP treated broccoli (–6.02 and 14.75, respectively). No significant difference in a^* values was observed in 1–MCP treatments (0.5, 1.0, 1.5, 2.5 and 5.0 μL/L) during the first 10 days of storage. There was no significant difference in b^* values among broccoli treated with 1.0, 1.5 and 2.5 μL/L of 1–MCP. After 10 days of storage, significant yellowing of florets occurred in control and broccoli treated with 0.5 μL/L of 1–MCP. The decrease in green color and increase in yellow color at the end of cold storage was higher in the control samples than in the 1–MCP treated samples. The yellowing may have caused a mark increase in the values of a^*, b^* and ΔE. However, broccoli treated with 1.0, 1.5, 2.5 and 5.0 μL/L of 1–MCP maintained its own color, the increase in a^* and b^* values meant a decrease in green and an increase in yellow in broccoli, which was related to the yellowing of the florets during storage (Eason *et al*., 2007; King & Morris, 1994). According to these results, broccoli treated with 1–MCP has a brighter green color than control broccoli stored at 15°C. This retention of color may be related to the fact that 1–MCP reduced the respiration rate and ethylene production of broccoli during storage, thus delaying water loss and chlorophyll degradation. 1–MCP delayed broccoli senescence and retained chlorophyll (Yuan *et al*., 2010). Our study supports the evidence that 1–MCP treatment inhibited the chlorophyll degradation, yellowing and quality deterioration for broccoli.

Effect of 1–MCP treatment on chlorophyll, total carotenoids and weight loss

The senescence of broccoli after harvest accelerates yellowing process and loss of green color due to chlorophyll degradation (Yamauchi and Watada, 1998). As shown in Table 3, the chlorophyll content in fresh broccoli was 5.36 mg/100 g FW, and 1–MCP treatment had a significant effect on maintaining the chlorophyll content of broccoli. The contents of chlorophyll of control and 1–MCP–treated broccoli fluctuated during storage. The degradation rate of chlorophyll of 1–MCP treated broccoli was significantly lower than that of control broccoli. The chlorophyll content of broccoli treated with 0.5 and 5.0 μL/L 1–MCP was about 85.45%~95.89% of the initial chlorophyll content, whereas the chlorophyll content of control broccoli was only 68.47% of the initial value after 10 days of storage at 15°C. In our experiment, the chlorophyll content of broccoli treated with 1.0 and 2.5 μL/L of 1–MCP increased at the end of storage, while the chlorophyll content in broccoli treated with 1.5 μL/L 1–MCP maintained the level of the initial day. The 1–MCP treatment represented an effect of slowing down the process of green loss and yellowing of broccoli (Fan and Mattheis, 2000; Gong and Mattheis, 2003). The results supported that 1.0 μL/L of 1–MCP effectively inhibited chlorophyll degradation, yellowing, and quality deterioration of broccoli. This result was in agreement with other studies on the effect of 1–MCP on broccoli (Gong and Mattheis, 2003). Yuan *et al*. (2010) reported that broccoli treated with 2.5 μL/L 1–MCP maintained 67% of its initial chlorophyll content after 5 days of storage at 20°C. Yuan *et al*. (2010) reported that broccoli treated with 2.5 μL/L 1–MCP maintained 67% of its initial chlorophyll content after 5 days of storage at 20°C. Fernández–León *et al*. (2013) reported that broccoli treated with 0.6 μL/L 1–MCP maintained 28.78% of its initial chlorophyll content after 27 days of storage at 1~2°C.

The content of total carotenoids in control and 1–MCP broccoli during refrigerated storage is summarized in Table 3. The content of total carotenoids in raw broccoli was 1.77 mg/100 g FW. This result was in agreement with the results obtained by Fernández–León *et al*. (2013). The carotene content of control and 1–MCP–treated broccoli increased gradually, but the carotene level of 1–MCP–treated broccoli were higher than that of control broccoli after 10 days of storage at 15°C. The percentage increase in carotene ranged from 40.11% to 89.83% in 1–MCP–treated broccoli compared to 22.03% in control broccoli stored at 15°C for 10 days. The content of carotene was 1.4 or 1.9 times higher in 1–MCP–treated broccoli than in control broccoli after 10 days of storage (2.16 mg/100 g FW in control and 2.48 mg/100 g FW to 3.36 mg/100 g FW in 1–MCP samples). This increase in chlorophyll content may be a natural response to the

Table 3. Chlorophyll, total carotenoids and weight loss of broccoli subjected to 1–MCP during storage

Treatment	Chlorophyll (mg/100 g)				Total carotenoids (mg/100 g)				Weight loss (%)		
	Days of storage										
	0	3	7	10	0	3	7	10	3	7	10
Control	5.36	3.56^{d}	6.83^{a}	3.67^{d}	1.77	1.46^{c}	2.91^{b}	2.16^{d}	0.28	0.59^{a}	0.82^{a}
1MCP 0.5	5.36	3.92^{cd}	5.92^{ab}	5.14^{bc}	1.77	1.56^{c}	2.59^{bc}	2.74^{bc}	0.25	0.45^{c}	0.81^{ab}
1MCP 1.0	5.36	6.55^{a}	6.42^{a}	6.04^{ab}	1.77	4.60^{a}	3.49^{a}	3.36^{a}	0.23	0.54^{ab}	0.71^{abc}
1MCP 1.5	5.36	6.09^{ab}	5.14^{bc}	5.51^{bc}	1.77	2.39^{b}	2.38^{cd}	2.68^{bc}	0.23	0.49^{bc}	0.68^{c}
1MCP 2.5	5.36	4.90^{bc}	4.60^{c}	7.01^{a}	1.77	1.90^{c}	2.05^{d}	3.09^{ab}	0.24	0.52^{bc}	0.69^{bc}
1MCP 5.0	5.36	3.33^{d}	4.60^{c}	4.58^{cd}	1.77	1.49^{c}	2.10^{d}	2.48^{cd}	0.23	0.46^{c}	0.74^{abc}
F–test	*NS*	**	**	**	*NS*	**	**	**	*NS*	**	*

*Means within the same column followed by different letters are significantly different. *P ≤ 0.05, **P ≤ 0.01, NS – non significance.*

inhibition of 1–MCP. This increase was greater in the 1–MCP sample than in the control sample, and there was no statistically significant difference in the total carotenoid content of the 1–MCP sample stored at 15°C after 10 days of storage. In the present study, an increasing trend in total carotenoids was observed during storage. This result was different from the results obtained by Yuan *et al*. (2010) and Fernández–León *et al*. (2013) for control and 1–MCP samples. The reason for the different results could be due to differences in cultivar, treatment period, and treatment temperature. Further study is needed to clarify the reason.

A common storage problem of fresh fruit and vegetables is weight loss, which affects the commercial market for these crops. The weight loss of broccoli steadily increased in all treatments during storage and the results are shown in Table 3. The weight loss of the control increased rapidly during storage, reaching 0.82% after 10 days of storage. Conversely, the weight loss of broccoli treated with 1–MCP decreased significantly; however, the weight loss values of samples treated with different concentrations of 1–MCP were different. The results showed that broccoli treated with 1.5 and 2.5 μL/L of 1–MCP decreased to less than 0.69% of the initial fresh weight after 10 days of refrigerated storage, while broccoli treated with 1.0, 5.0, and 0.5 μL/L of 1–MCP increased this value to 0.71%, 0.74%, and 0.81 This value increased to 0.71%, 0.74%, and 0.81%, respectively.

Effect of 1–MCP treatment on the contents of ascorbic acid and total phenolic compounds

The total phenolic compounds and ascorbic acid content of broccoli are shown in Table 4. Ascorbic acid is one of the most important nutritional components found in vegetables. The ascorbic acid content of broccoli gradually decreases during storage and the rate of ascorbic acid loss can be controlled by storage methods (Serrano *et al*., 2006; Vallejo *et al*., 2003). The ascorbic acid content of fresh broccoli on 0 day of storage was 107.80 mg/100 g FW. This result was similar to the results reported by Yuan *et al*. (2010). The ascorbic acid content of all treatments decreased linearly during storage. The ascorbic acid content of control broccoli decreased more rapidly than that of 1–MCP treated broccoli. In the control sample, the ascorbic acid content decreased by about 47.99% (107.80 mg/100 g FW to 56.07 mg/100 g FW) during 10 days of storage, whereas in 1.0 μL/L 1–MCP–treated broccoli, the ascorbic acid content decreased by about 32.53% (107.80 mg/100 g FW to 72.73 mg/100 g FW). However, the content of ascorbic acid was not significantly different among 1.0, 1.5 and 5.0 μL/L 1–MCP–treated samples after 10 days of cold storage. The results showed that broccoli treated with 1–MCP maintained a higher ascorbic acid content than the control group during the postharvest storage period. In addition, the decreasing trend of ascorbic acid content was consistent with the results shown in other studies (Ma *et al*., 2010; Fernández–León *et al*., 2013; Yuan *et al*., 2010).

The results of the changes in total phenolic compounds of all samples during refrigerated storage are shown in Table 4. The content of total phenolic compounds in fresh broccoli on day 0 of storage was 27.73 mg GAE/g FW and this result was in agreement with the results reported by Koh *et al*. (2009). Slight variation in the content of total phenolic compounds in broccoli during storage was observed. The content of total phenolic compounds in broccoli treated with 0.5 and 1.0 μL/L 1–MCP increased slightly during cold storage (28.01 mg GAE/g FW and 28.95 mg GAE/g FW at 10 days of storage, respectively). The increase in total phenolic compounds in broccoli may be a natural response to inhibition by 1–MCP treatment. This increase was observed in 1.5 and 2.5 μL/L 1–MCP–treated samples on day 7 of storage (28.10 mg GAE/g FW and 28.01 mg GAE/g FW, respectively). Thereafter, a decrease in the content of total phenolic compounds was observed. At the end of refrigerated storage, the contents of total phenolic compounds of 1.5, 2.5 and 5.0 μL/L 1–MCP–treated broccoli were statistically similar to that of fresh samples (27.74 mg GAE/g FW, 27.47 mg GAE/g FW and 27.11 mg GAE/g FW, respectively).

In the control broccoli, large fluctuations were observed during refrigerated storage and a sharp decrease in the content of total phenolic compounds was

Table 4. Total phenolic and ascorbic acid of broccoli subjected to 1–MCP during storage

Treatment	Total phenolic compounds (mg/g FW)				Ascorbic acid (mg/100 g FW)			
	Days of storage							
	0	3	7	10	0	3	7	10
Control	27.73	26.79^{b}	27.01^{b}	25.09^{c}	107.80	72.87^{b}	68.67^{d}	56.07^{c}
1MCP 0.5	27.73	27.69^{ab}	27.96^{ab}	28.01^{b}	107.80	97.27^{a}	76.27^{ab}	64.53^{b}
1MCP 1.0	27.73	28.59^{a}	28.84^{a}	28.95^{a}	107.80	94.20^{a}	74.20^{bc}	72.73^{a}
1MCP 1.5	27.73	27.90^{ab}	28.10^{ab}	27.24^{b}	107.80	96.47^{a}	77.67^{a}	72.07^{a}
1MCP 2.5	27.73	27.10^{b}	28.01^{ab}	27.47^{b}	107.80	95.53^{a}	71.73^{cd}	67.13^{ab}
1MCP 5.0	27.73	28.73^{a}	27.63^{b}	27.11^{b}	107.80	94.13^{a}	75.46^{ab}	70.53^{a}
F–test	*NS*	*	*	**	*NS*	**	**	**

*Means within the same column followed by different letters are significantly different. *P ≤ 0.05, **P ≤ 0.01, NS – non significance.*

observed at the end of refrigerated storage (25.09 mg GAE/g FW). The content of total phenolic compounds in the 1–MCP sample was higher than that of the control sample. The higher respiration rate of control broccoli than 1–MCP–treated broccoli may be the reason for the lower total phenolic content of control broccoli than 1–MCP sample (Izumi *et al*., 2006). This may be due to the higher metabolism of control than 1–MCP sample, which may result in the degradation of total phenolic compounds (Vallejo *et al*., 2003). This result was in agreement with the results reported by Costa *et al*. (2005).

As the above results, it was concluded that 1–MCP treatment with 1.0 μL/L (TI = 225 μL/L ˚C h) was the most effective in extending the shelf life of broccoli. Yuan *et al*. (2010) and Gong *et al*. (2003) found the effect of 1–MCP at TI = 300 μL/L ˚C h and TI = 280 μL/L ˚C h, respectively. In addition, Fan *et al*. (2000) showed that 1–MCP treatment with 1.0 μL/L of 1–MCP at 10˚C for 12 h (TI = 120 μL/L ˚C h) was effective. In our study, we have also observed the slight effect of 1–MCP treated with 0.5 μL/L at 15˚C for 15 h (TI = 112.5 μL/L ˚C h). There might be a threshold around this TI value, but further research is needed to quantify the TI.

CONCLUSIONS

1–MCP treatments were effective in delaying broccoli aging and inhibiting respiration rate, ethylene production and yellowing compared with the control samples. The results showed that the overall appearance and physicochemical parameters of 1–MCP treated broccoli were higher than those of control broccoli. These data indicated that low concentrations of 1–MCP strongly inhibited the loss of green color, decreased respiration, decreased ethylene production in broccoli when stored at 15˚C and delayed the yellowing that occurs with broccoli aging. No adverse effects were observed from the use of 1–MCP, and at concentrations of 1.0 μL/L, 1–MCP treatment was found to be the most effective in inhibiting broccoli senescence, inhibiting respiration rate, ethylene production, and yellowing.

AUTHOR CONTRIBUTIONS

Nguyen Thi Hang Phuong designed the study, conducted the experiments, analyzed the data and wrote the paper. Toshitaka Uchino, Fumina Tanaka and Fumihiko Tanaka supervised the work and revised the manuscript.

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