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Broccoli (*Brassica oleracea* L., cv. Keirin) is a highly perishable produce and its postharvest life and quality greatly depend on storage conditions, such as temperature, atmosphere composition, relative humidity and packaging films. The effect of modified atmosphere packaging (MAP) on the quality of broccoli heads was investigated in the present study. Broccoli was packaged with four different types of films: polypropylene (PP), micro–perforated polypropylene (PP hole), oriented polypropylene (OPP) and low density polyethylene (LDPE), and then stored at 15ºC for 13 days to study the effects of modified atmosphere packaging (MAP) on the maintenance of quality and functional properties by comparison with non–packaged broccoli as a control. Results indicated that deterioration occurred quickly in the control broccoli due to a relatively high rate of metabolism and a consequent high respiration rate and ethylene production, manifested mainly by weight loss, yellowing and chlorophyll degradation. Also, a rapid decrease in ascorbic acid and total phenolic concentration was observed. Conversely, in those broccoli packaged under MAP, especially for LDPE, all changes related with loss of quality were significantly reduced and delayed with time. Moreover, levels of total carotenoids and total phenolic compounds remained during the whole period. Thus, broccoli packaged with LDPE film had prolonged storability up to 10 days at 15ºC with high quality, this period being only 4 days in control broccoli.

Key words: broccoli, MAP, LDPE, quality, package

INTRODUCTION

Broccoli contains a high level of bioactive compounds, such as ascorbic acid, total phenolic compounds, total carotenoids. Freshly harvested broccoli, however, deteriorates and senesces rapidly after harvest when left at ambient temperatures because of its high respiration rate. Controlling the respiration of broccoli is very important for delaying senescence as indicated by, for example, the softening and yellowing of the florets and leaves. Wrapping broccoli with appropriate plastic films, which generate a modified atmosphere (MAP), may be a good tool to maintain broccoli quality. MAP is a technique for controlling internal atmosphere, taking into account the natural process of respiration of the commodity and the gas permeability of the package that holds the product. The combination of both processes leads to an increase of CO_2 and a reduction of O_2. However, the choice of film is a key factor in order to obtain optimum modification of the atmosphere and to avoid extremely low levels of O_2 and high levels of CO_2, which could induce anaerobic metabolism with possibility of off–flavor generation, and the risk of anaerobic microorganism proliferation (Beaudry, 2000; Lange, 2000; Watkins, 2000). For broccoli, the use of MAP is to reduce the respiration rate, maintains sensory attributes and increases the shelf life (Barth *et al*., 1993; Gillies *et al*., 1997; Toivonen and DeEll, 2001; Jones *et al*., 2006; Rangkadilok *et al*., 2002; Serrano *et al*., 2006). However, the literature about the changes in functional properties during cold storage is scarce, and especially how it is affected by MAP.

In this work, the effects of different modified atmospheres by using four distinct types of films on the change in several parameters related to broccoli quality and its functional properties were studied during 13 days at 15ºC. The MAP were performed with polypropylene (PP), micro–perforated polypropylene (PP hole), oriented polypropylene (OPP) and low density polyethylene (LDPE), while non–wrapping broccoli were used as a control to study the quality deterioration process by measuring headspace gas concentration, color change, weight loss, respiration rate, ethylene production, contents of ascorbic acid, chlorophylls, total carotenoids and total phenolic compounds.

MATERIALS AND METHODS

Plant material

Fresh broccoli (*Brassica oleracea* L., cv. Keirin) was harvested from farm in Fukuoka, Japan, placed in a cardboard box, precooled at 3ºC in 3 h and transported to the laboratory within 1h. Broccoli heads were sorted on arrival to select only undamaged broccoli of uniform green color for use in experiments.

Sample preparation

Broccoli heads were wrapped with four different types of films at the same thickness and the same size (20 μm and 23 cm × 30 cm, respectively): polypropylene (PP), micro–perforated polypropylene (PP hole), oriented polypropylene (OPP) and low density polyethyl-

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ene (LDPE). The unwrapped broccoli served as the control. All bags were sealed by a thermal sealer and then stored in incubation chamber with 55% RH at 15°C for 13 days. There were three replicates per treatment with three broccoli heads per replicate. Samples were taken randomly every 4 or 5 days to analyze weight loss, color change, chlorophyll A, chlorophyll B, total carotenoids, total phenolic compounds, ascorbic acid, respiration rate, ethylene production and headspace gas composition.

Color

Color attributes (L*, a*, b*) were measured by a color reader (Konica Minolta CR–20, Japan). The color reader was calibrated with white calibration board. Measurements were taken on nine random points on the broccoli. Results were expressed as L*, a*, b* and delta E.

Weight loss

Individual broccoli was weighed at the beginning of the experiment and at each sampling day during the storage period. Results were expressed as percentage of weight loss relative to the initial weight.

Total carotenoids and chlorophyll content determination

Florets (1 g) were ground and soaked in 15 mL of acetone–hexane (4:6) and homogenized with the homogenizer at 20000 rpm for two minutes. The supernatant was separated automatically and the absorbance was read at 400–800 nm on JASCO UV–530 spectrophotometer. It was recorded that Chlorophyll A showed the maximum absorbance at 663 nm, chlorophyll B at 645 nm and total carotenoids at 505 nm and 453 nm and the contents of these pigments were calculated. Total carotenoids content was estimated as mg/100 g FW. Chlorophyll content was represented as the sum of chlorophyll A and chlorophyll B, and expressed as mg chlorophyll/100 g FW.

Ascorbic acid determination

Five grams of fresh broccoli florets were homogenized (Homogenizer Ultra–Turrax, T25, IKA, China) with 30 mL of 5% m/v metaphosphoric acid. The mixture was centrifuged at 3500 rpm for 10 min at 20°C (Centrifuge Kubota 5922, Japan). The supernatant was recovered and filtered through filter paper. Ascorbic acid determination was done by using a RQflex device. Results were expressed as mg/100 g FW.

Respiration rate and ethylene production

Fresh broccoli was put into a fixed volume sealed glass desiccator for 2 h at 15°C, and then the respiration rate was measured in triplicate using gas chromatograph (GC–390). Briefly, 1 mL of gas was collected from headspace of each desiccator by a gas–tight hypodermic syringe and analysed by using gas chromatograph (GC–390). Respiration rate was expressed as mg/kg.h of CO_2 evolved. Ethylene production was measured using a Tiger device and the results were expressed as μL C_2H_4/kg.h.

Headspace gas composition

Oxygen and carbon dioxide headspace concentrations of packaged broccoli were measured using gas chromatograph (GC–390). A syringe was inserted into the package through a rubber seal placed on the bag. The volume taken from the package headspace for gas analysis was about 1 mL. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single measurement of the headspace gas composition. Three replicates were made at each sampling time. Results were expressed as oxygen concentration (%) and carbon dioxide concentration (%) inside the bags.

Total phenolic compounds

Freeze–dried broccoli (200 mg) was suspended twice with 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. After cooling, the mixture was moved to ultrasonic treatment for 30 min at 20°C. Then, the extract was centrifuged at 3000 rpm for 10 min at 20°C and filtered through a Whatman No. 42 filter paper. The supernatant was transferred into a volumetric flask and diluted to 20 mL with distilled water. A 0.1 mL of supernatant was mixed with 7.9 mL distilled water and 0.5 mL of FolineCiocalteu reagent. After 3 min of incubation at 25°C, 1.5 mL of 20% w/v Na_2CO_3 solution was added and the reaction mixture was incubated for 1.5 h in darkness at the same temperature. The absorbance was measured at 725 nm on UV/VIS spectrophotometer (JASCO–UV–530). A standard curve was constructed to quantify phenolic compounds, using gallic acid at concentrations of 0–0.025 mg/mL. The total phenolic contents were performed in triplicate and results were expressed as mg gallic acid equivalent (GAE) per gram of fresh weight (mg GAE/g FW).

Statistical analyses

Statistical analysis was performed using the SAS package program version 11.5. Data was analyzed by analysis of variance (ANOVA model one–way). Sources of variation were storage duration and treatments. The means were compared by the least significant differences (LSD) test at a significance level of 0.05. The values are reported as means with their standard deviations for all results.

RESULTS AND DISCUSSION

Effect of packaging on the headspace gas composition

The O_2 and CO_2 concentrations in the package headspace for samples packaged into PP, PP hole, OPP and LDPE film are shown in Table 1. A decrease in the headspace O_2 concentration and an increase in the headspace CO_2 concentration with time were observed for MAP in the four films, although gas composition inside the packages was significantly different depending on the film used. The O_2 and CO_2 concentrations inside the bag at day 0 were 20.05% and 0.18%, respectively. Differences in trends shown in the Table 1 are related to differences in barrier properties of films. During the 13 days of cold storage at 15ºC, the concentration of O_2 dropped significantly from 20.05% to 4.60% in PP and to 5.79% in LDPE after 4 days, followed by a steady decrease to 3.19% in PP and 4.49% in LDPE after 13 days. On the other hand, the concentration of CO_2 increased quickly from 0.18% to 16.01% in PP and to 3.13% in LDPE after 4 days, followed by a gradual increase to 21.01% in PP and 3.78% in LDPE after 13 days. Similarly, the concentration of O_2 decreased slightly from 20.05% to 16.32% in PP hole and to 15.81% in OPP after 4 days, and reached almost 15% in PP hole and 14% in OPP at the end of 13 days storage, while CO_2 increased rapidly from 0.18% to 4.33% in PP hole and to 4.58% in OPP after 4 days, and reached to 7.34% in PP hole and to 6.63% in OPP at the end of 13 days storage (Table 1). The relatively higher CO_2 level and lower O_2 level in PP compared to LDPE during 13 days of storage at 15ºC indicated that a stronger modified atmosphere occurred in PP film than in LDPE film. The higher were the barrier properties of a packaging, the faster was the decrease in the headspace O_2 concentration and the increase in the headspace CO_2 concentration (Del Nobile *et al.*, 2008).

Broccoli can benefit from 1% to 2% O_2 and 5% to 10% CO_2 atmospheres at low temperatures. However, a low O_2 level (0.5–2%) or CO_2 concentrations in excess of 10%, combined with temperature fluctuations, may result in the production of off–odor containing sulfur, that reduce shelf–life of packaged broccoli (Forney and Rij, 1991). As a consequence, broccoli packaged in PP film induced off–odor due to low level of O_2 (3.19%) and high level of CO_2 (higher than 21%) that led to anaerobic conditions, whereas the odor of broccoli packaged in LDPE, PP hole and OPP film was normal.

Effect of packaging on respiration rate and ethylene production

There were significant effects of packaging on respiration rate, ethylene production of broccoli during storage period. As shown in Table 2, the changes in the respiration rates and ethylene production of broccoli during storage are clearly described according to the different types of films and compared with the control. The respiration rate of the control broccoli decreased rapidly from 323.52 mg CO_2/kg.h to 163.23 mg CO_2/kg.h after 4 days, whereas packaged broccoli showed significantly lower respiration rates of 135.66 mg CO_2/kg.h for PP, 141.27 mg CO_2/kg.h for PP hole, 146.11 mg CO_2/kg.h for OPP, and 97.39 mg CO_2/kg.h for LDPE after 4 days. And then respiration rates of all samples increased slightly and reached a peak on 8 days with 200.36 mg CO_2/kg.h for control broccoli, 150.08 mg CO_2/kg.h for PP, 164.58 mg CO_2/kg.h for PP hole, 153.43 mg CO_2/kg.h for OPP, and 121.08 mg CO_2/kg.h for LDPE after 8 days. After reaching the peak the respiration rate of broccoli dropped at the end of storage period with 162.3 mg CO_2/kg.h for control broccoli, 138.18 mg CO_2/kg.h for PP, 150.40 mg

Table 1. Concentration of gas inside the packaging of broccoli during storage

Treatment	Concentration of CO_2 (%)				Concentration of O_2 (%)			
	Days of storage							
	0	4	8	13	0	4	8	13
PP	0.18	16.01^{a}	19.79^{a}	21.01^{a}	20.05	4.60^{c}	4.09^{c}	3.19^{d}
PP hole	0.18	4.33^{b}	6.80^{b}	7.34^{b}	20.05	16.32^{a}	15.12^{a}	14.97^{a}
OPP	0.18	4.58^{b}	5.37^{c}	6.63^{c}	20.05	15.81^{a}	14.87^{a}	13.95^{b}
LDPE	0.18	3.13^{c}	3.50^{d}	3.78^{d}	20.05	5.79^{b}	5.31^{b}	4.49^{c}
F–test	*NS*	**	**	**	*NS*	**	**	**

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

Table 2. Respiration rate and ethylene production of broccoli subjected to packaging during storage

Treatment	Respiration rate (mg CO_2/kg.hr)				Ethylene production (μL C_2H_4/kg.hr)			
	Days of storage							
	0	4	8	13	0	4	8	13
Control	323.52	163.23^{a}	200.36^{a}	162.33^{a}	0.17	0.55^{c}	2.15^{a}	1.29^{a}
PP	323.52	135.66^{c}	150.08^{c}	138.18^{c}	0.17	1.04^{a}	1.41^{b}	0.99^{c}
PP hole	323.52	141.27^{bc}	164.58^{b}	150.40^{b}	0.17	0.35^{d}	1.49^{b}	1.11^{b}
OPP	323.52	146.11^{b}	153.43^{c}	140.31^{bc}	0.17	0.63^{b}	1.01^{c}	0.73^{d}
LDPE	323.52	97.39^{d}	121.08^{d}	101.93^{d}	0.17	0.29^{e}	0.53^{d}	0.41^{e}
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.*

CO_2/kg.h for PP hole, 140.31 mg CO_2/kg.h for OPP, and 101.93 mg CO_2/kg.h for LDPE. During storage, the respiration rate of LDPE packaged broccoli was well controlled and significantly reduced while comparing with the other packaged broccoli within 13 days. This finding indicated that LDPE film more effectively decreased the respiration rate of broccoli after harvest.

Ethylene productions are summarized in Table 2. The ethylene peak that occurs during storage is direct index of senescence in broccoli (Ma *et al.*, 2014). Thus, it is essential to suppress ethylene production in broccoli during postharvest storage in order to maintain quality (Ma *et al.*, 2012). As indicated in Table 2, overall ethylene production was significantly higher in control broccoli rather than in packaged broccoli. The significant differences in ethylene production were observed among the packages studied in this work. Higher values of ethylene production were found in broccoli wrapped with PP and PP hole film. On the other hand, levels of ethylene productions of LDPE packaged broccoli were significantly lower than those of other packaged broccoli. Ethylene production in both the control and packaged broccoli stored at 15ºC reached a peak at 8 days but the level of ethylene production in LDPE packaged broccoli was 4.0 fold lower than that in the control. At the end of storage, the level of ethylene production in control broccoli was more than three–fold higher than level of ethylene production in LDPE packaged broccoli. This indicates that LDPE film could suppress ethylene production in broccoli during postharvest storage.

Effect of packaging on chlorophyll and total carotenoids

The degradation of chlorophyll is the main reason for the yellowing and can determine the shelf life of broccoli. As shown in Table 3, the chlorophyll content in fresh broccoli was 8.75 mg/100 g FW. After 4 days of storage at 15ºC, the chlorophyll content increased slightly for all treatments. From 4 days of cold storage until 13 days at 15ºC, there was a reduction in chlorophyll contents. This reduction being less in packaged samples than in the control samples. During storage at 15ºC, control samples showed a rapid decline in chlorophyll content, losing more than 56% of the initial content, only 3.82 mg/100 g FW remained after 13 days of storage. In contrast, PP packaged samples presented higher levels of chlorophyll, losing only 13% of the initial content, 7.61 mg/100 g FW remained at the same storage period (Table 3). Levels of chlorophyll were similar in PP hole samples, OPP samples and LDPE samples after 13 days, losing about 33% of the initial chlorophyll content, about 5.85 mg/100 g FW remained.

Table 3 summarizes the total carotenoids content in control and packaged samples during cold storage compared with fresh sample. The content of total carotenoids in fresh broccoli was 3.48 mg/100 g FW. The content of total carotenoids increased for all samples after 4 days of storage. This could be a response to the stress caused by the controlled atmosphere storage (Bennett & Wallsgrove, 1994). After 8 days storage at 15ºC a decrease in the total carotenoids content was observed in PP wrapped broccoli (2.45 mg/100 g FW). At the end of cold storage, the level of total carotenoids in PP hole samples, OPP samples and LDPE samples remained similar to that of the fresh sample (3.79 mg/100 g FW, 3.52 mg/100 g FW and 3.11 mg/100 g FW, respectively). The level of total carotenoids in control samples and PP samples decreased at the end of storage, and the loss percentages in the PP samples reached as much as 49%, only 1.76 mg/100 g FW remained after 13 days. The level of total carotenoids in control samples was 2.47 mg/100 g FW, losing only 29% of the initial content.

These findings indicated that MAP significantly postponed the degradation of chlorophyll and total carotenoids in broccoli during storage. The results obtained are in agreement with other studies on modified atmosphere packaged broccoli such as Serrano *et al.* (2006), Fernández–León *et al.* (2013), Caleb *et al.* (2016).

Effect of packaging on weight loss, ascorbic acid and total phenolic compounds

As shown in Table 4, there were significant effects of packages on the weight loss, ascorbic acid content and total phenolic compounds in all the samples stored at 15ºC. Weight loss is a common storage problem of broccoli which affects commercial marketing of this

Table 3. Chlorophyll and total carotenoids of broccoli subjected to packaging during storage

Treatment	Chlorophyll (mg/100 g FW)				Total carotenoids (mg/100 g FW)			
	Days of storage							
	0	4	8	13	0	4	8	13
Control	8.75	9.06^{b}	7.44^{b}	3.82^{c}	3.48	4.00^{b}	4.11^{ab}	2.47^{bc}
PP	8.75	12.28^{a}	8.48^{b}	7.61^{a}	3.48	4.21^{b}	2.45^{c}	1.76^{c}
PP hole	8.75	11.38^{ab}	10.31^{a}	5.87^{b}	3.48	4.08^{b}	4.77^{a}	3.79^{a}
OPP	8.75	10.0^{4ab}	6.75^{b}	5.75^{b}	3.48	3.68^{b}	3.70^{b}	3.52^{a}
LDPE	8.75	9.23^{b}	10.93^{a}	5.91^{b}	3.48	5.78^{a}	$4.27a^{b}$	3.11^{ab}
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.*

Table 4. Weight loss, ascorbic acid content and total phenolic compounds of broccoli subjected to packaging during storage

Treatment	Weight loss (%)			Ascorbic acid (mg/100 g FW)				Total phenolic (mg/g FW)			
	Days of storage										
	4	8	13	0	4	8	13	0	4	8	13
Control	22.34[a]	36.51[a]	47.80[a]	120.07	95.60[bc]	107.20[a]	77.20[a]	27.98	25.85[c]	25.09[c]	25.26[c]
PP	0.30[b]	0.68[b]	1.00[b]	120.07	87.27[c]	51.13[c]	0[c]	27.98	28.12[a]	27.65[a]	25.50[c]
PP hole	0.24[b]	0.45[cd]	0.65[b]	120.07	100.40[ab]	91.80[ab]	59.13[b]	27.98	26.88[b]	26.62[b]	27.05[b]
OPP	0.25[b]	0.37[d]	0.63[b]	120.07	98.93[ab]	85.67[b]	62.93[b]	27.98	27.87[a]	25.30[c]	26.09[c]
LDPE	0.35[b]	0.61[bc]	0.93[b]	120.07	107.20[a]	95.13[ab]	78.20[a]	27.98	28.01[a]	27.14[ab]	27.94[a]
F–test	**	**	**	*NS*	**	**	**	*NS*	**	**	**

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

crop. The loss of weight of control broccoli increased sharply during storage, with final values of 47.80% after 13 days of storage. Conversely, the weight losses for those wrapped in films were significantly reduced, but differences were found among the films used. Thus, broccoli packaged with PP hole and OPP films lost below 0.65% of their initial fresh weight after 13 days of cold storage, while this value increased up to 1.0% for broccoli packaged in PP and LDPE film (Table 4). The effect of MAP on reducing weight loss is likely due to the limitation of water vapor diffusion by plastic films and, in turn, generating a water vapor pressure and higher relative humidity inside the package. Our results also showed that the modified atmosphere created by MAP greatly reduced the weight loss of broccoli, as was also reported in earlier study (Serrano *et al.*, 2006).

Broccoli is rich in content of ascorbic acid, which is an important antioxidant. Ascorbic acid is a main nutritional constituent of broccoli and is also considered as a primary quality index of broccoli during storage. The ascorbic acid content found in fresh broccoli (120.07 mg/100 g FW) was in accordance with the values reported in the literature (Fernández–León *et al.*, 2013). The reduction trend of ascorbic acid content was also observed in all samples stored at 15ºC during 13 days of storage period (Table 4). It was found that the content of ascorbic acid in control was 77.20 mg/100 g FW while the contents of ascorbic acid in LDPE wrapped samples, OPP wrapped samples and PP hole wrapped samples were 78.20 mg/100 g FW, 62.93 mg/100 g FW, and 59.13 mg/100 g FW, respectively at the end of storage. After 13 days, the content of ascorbic acid in PP wrapped broccoli was only 0 mg/100 g FW, which equated to a reduction of 100%. This fact is explained by occurring anaerobic conditions inside the PP film that might increase the metabolism and therefore the degradation of ascorbic acid rapidly. The present study showed that MAP was not effective in retention of ascorbic acid during postharvest storage. This decrease of ascorbic acid content during cold storage is in agreement with work published by other authors, such as Fernández–León *et al.* (2013), Serrano *et al.* (2006).

The total phenolic contents in control and MAP samples during cold storage are summarized in Table 4. The content of total phenolic compounds in fresh broccoli was 27.98 mg GAE/g FW. The slight fluctuations in total phenolic contents of broccoli were observed during cold storage. The content of total phenolic compounds in control broccoli decreased gradually, with a final value of 25.26 mg GAE/g FW over a 13 day storage at 15ºC. This decrease was also observed for OPP samples with the value of 26.09 mg GAE/g FW. Broccoli wrapped in PP films and LDPE films increased in the contents of total phenolic compounds after 4 days; after this time a decrease was observed. At the end of cold storage, the total phenolic content in LDPE samples remained similar to that of the Fresh sample (27.94 mg GAE/g FW). On the other hand, for PP samples a sharp decrease was observed after 13 days (25.50 mg GAE/g FW), while the content of total phenolic compounds in OPP samples was 27.05 mg GAE/g FW. This fact is explained by the high respiration rate of broccoli stored under air (Izumi *et al.*, 1996) that might increase the metabolism and the degradation of phenolic compounds (Vallejo *et al.*, 2003a). The same behaviour was also observed by other authors (Serrano *et al.*, 2006; Fernández–León *et al.*, 2013).

Effect of packaging on color changes

The most important factor for marketing of broccoli is retention of the green color. This is a key determinant of consumer preference. The color changes are related to the yellowing process of broccoli inflorescences and to the degradation of chlorophylls (Eason *et al.*, 2007; King & Morris, 1994). Changes of surface color during broccoli yellowing were measured through color parameters L*, a*, b* and delta E. The L* value is a measure of the lightness of the sample, the values of a* and b* describes the change in the chromaticity coordinates of the sample while delta E represents the size of color difference between the reference and sample objects.

Yellowing occurred in control broccoli during storage at 15ºC. PP and LDPE films inhibited the yellowing and quality deterioration of broccoli. A significant difference in color and visual quality was found after 8 days of storage at 15ºC between MAP treatments and unwrapped control. Similarly, a significant difference in color among MAP treatments was observed after 8 day of storage. Best retention of color and visual quality were found in LDPE samples, followed by PP samples (Fig. 1, Fig. 2

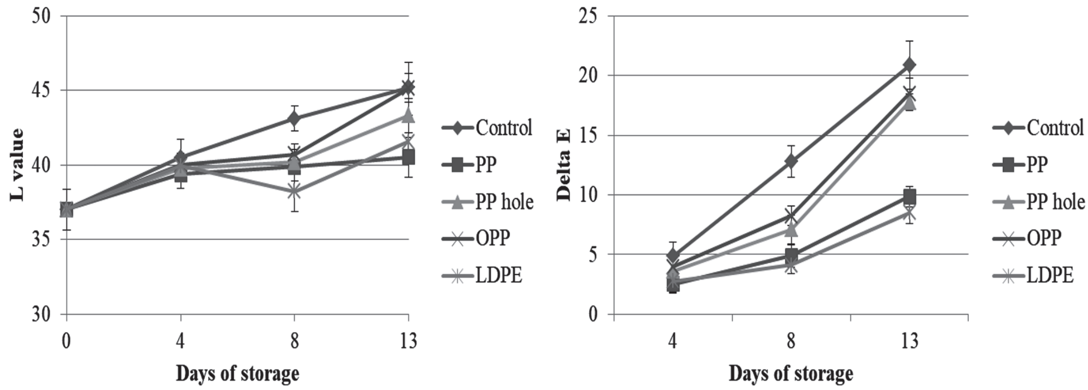

Fig. 1. Effect of packaging on L* value and delta E of broccoli color during storage.

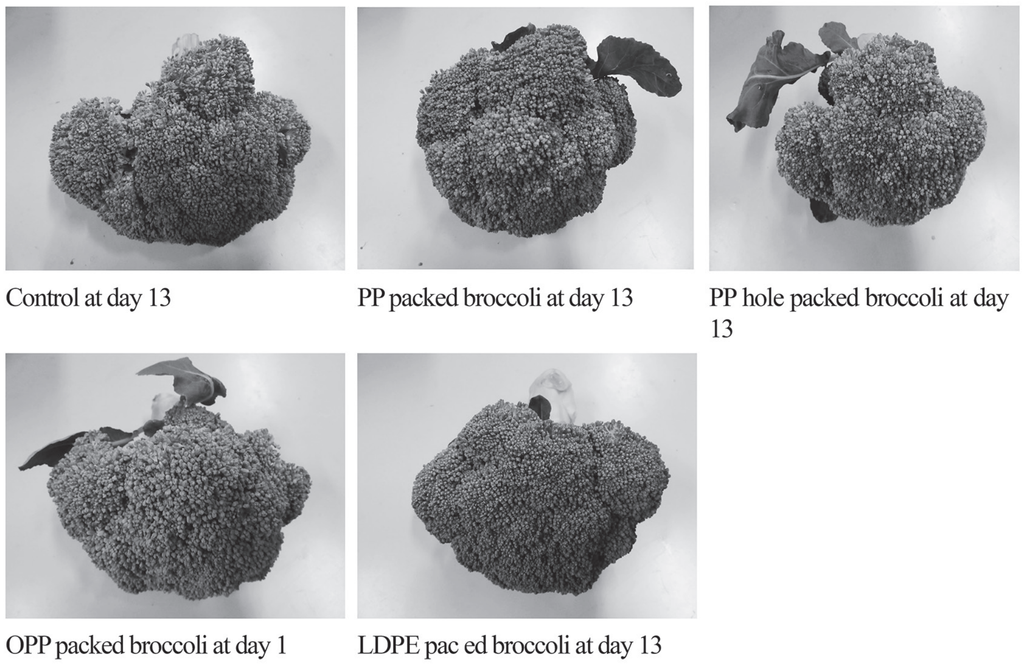

Fig. 2. Visual aspect of control broccoli and MAP broccoli after 13 days at 15°C.

and Table 5). The L* value of the fresh broccoli was 37.48. The L* value of broccoli gradually increased during storage at 15°C. All individual color parameters (L*, a* and b*) significantly increased in unwrapped control broccoli during storage, which was related to the yellowing process of broccoli inflorescences. In fact, L* value increased drastically from 37.03 to almost 45.10 in control broccoli and OPP packed broccoli after 13 days of storage. On the contrary, a slight increase was observed for PP hole packaged broccoli (43.32), while no significant increment in L* values were found for broccoli heads packed in LDPE and PP films at the end of storage (41.56 and 40.53, respectively).

Delta E values are highly correlated with total chlorophyll content of broccoli florets (Tian *et al.*, 1994). Delta E generally increased during the storage, similar to L* values. The increase in the delta E value was related to the yellowing of florets (Fig. 1). In our study, delta E value of control broccoli increased quickly during storage period (Fig. 1), while delta E of PP hole packed broccoli and OPP packed broccoli increased slightly within the first 8 days at 15°C, but increased rapidly thereafter. Wrapping broccoli with LDPE and PP films prevented yellowing over the entire 13 day period. The delta E values in LDPE and PP packed broccoli were significantly ($P < 0.05$) lower than that of control broccoli and PP hole and OPP packed broccoli after 13 days at 15°C. The highest delta E value after 13 days of storage at 15°C was measured in control broccoli (20.85). The lowest delta E value was found in LDPE packed broccoli (8.53) (Table 5).

In case of a* value and b* value, the values of a* and b* in fresh broccoli at day 0 were –3.13 and 5.84, respectively. These values generally increased during the storage. No significant differences were observed for a* value in control and PP hole packed broccoli after 13 days.

Table 5. Color changes in a* and b* value of broccoli subjected to packaging during storage

Treatment	a* value				b* value			
	Days of storage							
	0	4	8	13	0	4	8	13
Control	–3.13	–2.94	–1.58[a]	–0.91[a]	5.84	7.02[a]	10.99[a]	16.32[a]
PP	–3.13	–3.12	–2.97[cd]	–2.63[c]	5.84	6.00[bc]	7.74[d]	11.73[c]
PP hole	–3.13	–3.00	–2.68[bc]	–1.33[a]	5.84	6.59[a]	9.34[bc]	15.53[ab]
OPP	–3.13	–2.76	–2.55[b]	–1.86[b]	5.84	6.46[ab]	9.81[b]	14.96[b]
LDPE	–3.13	–3.30	–3.09[d]	–2.98[c]	5.84	5.87[c]	8.71[c]	9.69[d]
F–test	*NS*	*NS*	**	**	*NS*	**	**	**

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

Similarly, no significant differences were observed for a* values in PP and LDPE films. However, significant differences were observed for b* values in PP and LDPE films after 13 days. Unwrapped broccoli (control) showed the highest values of a* and b* (–0.91 and 16.32, respectively), whereas broccoli wrapped in LDPE films showed the lowest values of a* and b* (–2.98 and 9.69, respectively) after 13 days. Higher value a* indicates a decrease in green color of the vegetable. The values of a* and b* increase means a decrease in green color and an increase in yellow color, respectively, due to the yellowing of broccoli during storage (Eason *et al*., 2007; King & Morris, 1994). According to these results, broccoli wrapped with LDPE and PP films has a bright green coloration than control broccoli and broccoli wrapped with PP hole and OPP. Broccoli is a commodity that benefits from storage under increased CO_2 and reduced O_2 concentration atmospheres. The MAP storage conditions tested in this research work, positively affected the maintenance of the outer quality parameters of broccoli, especially for LDPE and PP films, as obtained by other authors (Serrano *et al*., 2006; Fernández–León *et al*., 2013).

CONCLUSIONS

The results of this study showed that packaging broccoli with LDPE films was the most effective film for reducing weight loss, respiration rate, ethylene production, delaying the yellowing and chlorophyll degradation and maintaining total carotenoids and total phenolic compounds in broccoli. Unwrapped control broccoli became rapidly unmarketable by day 8 because of yellowing. Both PP hole packed broccoli and OPP packed broccoli became rapidly unacceptable after 13 days because of yellowing. Whereas, packing broccoli with PP film caused symptoms of off–odors when opening the bag due to anaerobic metabolisms. After 13 days of storage, we considered only the packaged broccoli with LDPE film to be marketable, with appropriate quality for consumption and maintenance of their health–promoting compounds.

AUTHOR CONTRIBUTIONS

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

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