Postharvest quality response of broccoli florets to combined application of 1-methylcyclopropene and modified atmosphere packaging

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Broccoli is rich in health-promoting compounds such as ascorbic acid and glucosinolates. However, amount of such compounds inevitably decrease along with the storage. This study was conducted to investigate the effects of 1-methylcyclopropene (1-MCP) and Modified Atmosphere Packaging (MAP) treatments alone or in combination on postharvest life and quality of broccoli heads during cold storage. MAP and 1-MCP+MAP significantly reduced weight loss, delayed surface color changes and chlorophyll breakdown, maintained visual quality, ascorbic acid, total phenol, soluble solid contents and titratable acidity compared with control as well as 1-MCP treatment. In 1-MCP plus MAP treated florets, approximate 47% and more chlorophyll and 24% more total phenol contents were found compared to control florets. 1-MCP plus MAP application better performed in maintaining the quality of broccoli florets during 28-day-storage. Overall results revealed that combined treatment can be recommended for commercial broccoli storage at 0 °C.

Key words: Broccoli, 1-MCP, MAP, quality, storage

Introduction

Fresh fruits and vegetables have important nutritional and economic values. Especially the consumption of *Brassica* genus vegetables is recommended to reduce the incidence of human cancer (Rangavajyhyala et al. 1998). Broccoli (*Brassica oleracea* var. *italica*) is a *Brassica* genus vegetable with high nutritional value due to its richness in vitamins, antioxidants, anti-carcinogenic substance and health promoting phytochemicals such as glucosinolates (Nestle 1998, Yuan et al. 2010). But the previous studies revealed that levels of ascorbic acid, glucosinolates and flavonoids have been significantly influenced during harvest, handling and storage (Vallejo et al. 2003, Nath et al. 2011).

Broccoli is a highly perishable vegetable that senesces quickly after harvest and thus its postharvest life is quite short due to yellowing, softening, water loss, decay and off-odor incidences (Forney et al. 2003, Vasconcelos and Almeida 2003). The green color of broccoli is an important quality feature for consumers. The loss of green color in broccoli florets occurs due to chlorophyll breakdown and this is stimulated by exogenously applied and endogenously produced ethylene (Tian et al. 1994, Ku and Wills 1999). Different chemical applications such as aminoethoxyvinylglycine (AVG) or silver ion were used to prevent yellowing (Wang 1977, Aharoni et al. 1985). However, these compounds to prevent yellowing has had limited commercial application as they are not registered for use on broccoli (Ku and Wills 1999). Recently, 1-methylcyclopropene (1-MCP) has been widely used for maintaining postharvest quality of horticultural commodities. 1-MCP is an ethylene action inhibitor that binds to the ethylene receptors and thereby delays ripening of products and extending storage life (Blankenship and Dole 2003). 1-MCP treatment inhibited the increase of respiration ratio and ethylene production (Ku and Wills 1999, Fan and Mattheis 2000), delayed yellowing and extended storage life of broccoli (Forney et al. 2003, Yuan et al. 2010). Yuan et al. (2010) also further emphasized that 1-MCP treatment reduced loss of health-promoting compounds such as glucosinolates or ascorbic acid in broccoli during 5 day-storage at 20 °C. Cefola et al. (2010) found that use of 1-MCP markedly extended the shelf life, reducing postharvest deterioration in broccoli raab florets.

Modified atmosphere packaging (MAP) is a technique used for prolonging the postharvest life of fresh fruits and vegetable. MAP can be defined as an alteration in the composition of gases in and around fresh produce by respiration and transpiration in package (Thompson 2003). Composition of the gas inside the package is changed by respiration of fruits, decreasing O_2 level while CO_2 increases during storage. Storage of broccoli in MAP reduced weight loss and respiration rate, maintained ascorbic acid, total antioxidant activity, glucosinolate contents and visual quality and thus increased postharvest life in cold or ambient storage (Nath et al. 2011, Serrano et al. 2006, Jia et al. 2009).

Manuscript received May 2012

Several researchers have reported that 1-MCP treatment is more effective when used in combination with MAP compared to single use of MAP or 1-MCP in litchi, persimmon, banana and tomato (Jiang et al. 1999, De Reuck et al. 2009, Kaynas et al. 2010, Oz 2011, Sabir and Agar 2011a).

The objective of this study was to investigate the effects of 1-MCP and MAP treatments alone or in combination on postharvest life and quality of broccoli during storage at 0 °C.

Material and Methods

Material

Broccoli heads (*Brassica oleracea* var. *italica* cv. 'Lucky') were obtained from local producers in Tarsus (Mersin Province) and then immediately transferred to Postharvest Laboratory of Horticulture Department, Selcuk University. A total of 240 undamaged and uniformly green heads were sorted randomly dividing into four equal lots.

Treatments and storage

Two lots of broccoli florets were placed in sealed air-tight plastic containers and exposed to 1 µl l^{-1} 1- MCP (0.14% active ingredient). SmartFresh™ powder (AgroFresh, Inc., Dow Agroscinces, Philadelphia, PA, USA) was weighted out into a test tube and afterwards, warm distilled water was added to release 1-MCP gas. After shaking, the tube was placed in the container and then the florets were immediately hermetically sealed and maintained for 12 h at 20 °C. Control florets were kept under the same conditions in containers without 1-MCP. After applications, containers were opened and the florets were led to ventilate. The first lot of the 1-MCP treated florets was stored in MAP (1-MCP+MAP treatment) while the second lot was placed in to open plastic boxes (1-MCP). As to the untreated florets, the first lot was stored in open plastic boxes without any application (as control) while the second was packed with Xtend® (StePac, Israel) MAPs (MAP; CO_2 transmission rate [TR]: 2203 cc m^{-2} day^{-1} and water vapour [WV] TR: 150.0 g m^{-2} day^{-1} at 23 °C). All the samples were stored at 0 °C and 95% RH for 28 days. Initially and 7, 14, 21 and 28 days after storage, samples were analyzed to assess postharvest quality changes as described below.

Color measurement

The surface color of florets (L * a * b* system) was measured with a chromameter (Minolta CR400, Osaka, Japan) during the period of storage. Three broccoli florets from each treatment were assayed and measurements were taken from five different points per floret. The hue angle (h°) was calculated as $h° = \tan^{-1}(b/a)$, when a>0 and b>0, or as $h° = 180° + \tan^{-1}(b/a)$ when a < 0 and b>0 (Lancaster et al. 1997).

Weight loss

The heads were weighted after postharvest treatment and during storage at 7, 14, 21 and 28 days. Results were expressed as percentage of weight loss relative to the initial weight.

Total phenol content

Total phenol content of broccoli florets was determined as previously described by Singleton et al. (1999) with slight modifications. An aliquot (0.1 ml) of broccoli extract was added 6.0 ml distilled water, followed by 0.5 ml Folin–Ciocalteu reagent. Solutions were swirl to mix and incubate 3 min at room temperature. After incubation, 1.5 ml 20% Na_2CO_3 supplemented and the volume was made up 10 ml distilled water. After 2 h incubate at 25 °C, the absorbance was measured at 760 nm and the total phenolic content was calculated using gallic acid as standard. Results were expressed as $mg_{gallic\ acid}$ kg^{-1}.

Ascorbic acid

Broccoli florets were blended with a high speedy warring blender at 3 min. 5 g blended sample was mixed with 45 ml 0.4% oxalic acid and then filtered via filter paper. One milliliter filtrate and 9 ml 2,6- dichlorophenolindophenol sodium salt were mixed then the transmittance value was measured 520 nm using UV spectrophotometer. Blank were prepared in the same way but using 1 ml filtrate and 9 ml distilled water. Results were expressed as mg $100g^{-1}$ (Ozdemir and Dundar 2006).

Total chlorophyll

Total chlorophyll content of broccoli was determined by a spectrophotometric method (Sabir and Agar 2011b). One gram blended broccoli portions were homogenized with 10 ml chloroform:methanol (2:1, v/v) for 1 min. Extracts were filtered with filter paper. The residue was resuspended in 10 ml chloroform:methanol and then filtered. All the filtrates were combined and solutions were supplemented with chloroform:methanol to 25 ml final volume. Total chlorophyll was determined by measuring absorbance of solution in UV spectrophotometer at 663 and 645 nm against chloroform:methanol blank. The total chlorophyll was estimated by the following formula.

Total chlorophyll (mg $100g^{-1}$) = 8.02* (A_{663}) + 20.2* (A_{645})

Titratable acidity (TA) and soluble solid content (SSC)

Broccoli florets were squeezed for SSC and TA determination. SSC was determined using a refractometer (Atago, Tokyo, Japan) and expressed as %. TA was estimated by titrating 5 ml juice with 0.1 N NaOH to a pH end point of 8.1 and expressed as percentage of citric acid.

Visual quality and color

During storage, broccoli heads were evaluated by panelist for visual quality using 1−9 scale described by Yuan et al. (2010) where 9 refers to excellent and fresh appearance, 7 to good, 5 to fair (limit of marketability), 3 to fair (useable but not saleable), and 1 to unusable. Color of broccoli florets was visually scored using 1–5 scale as described by Jia et al. (2009), where 1 refers to dark green, 2 to trace yellow (10% yellow), 3 to slightly yellow (25% yellow), 4 to medium yellow (50% yellow), 5 to completely yellow (100%). The panelists were asked to consider initial color and general head quality (compactness of heads and hardness of stem texture) of the produce while evaluating the samples for each experimental date.

Statistical analysis

Data were analyzed as a factorial experiment in a completely randomized manner with three replication and each replication contained five heads using the JMP statistical software, version 5.1 (SAS Institute Inc., Cary, NC, USA). Sources of variation were storage time, treatments and their interaction. Means were compared by Student's t-test at a significance level of 0.05.

Results

Weight loss

Weight loss of broccoli florets increased during storage period and speed of this increase course was affected by the treatments (Table 1). Weight loss of control and 1-MCP treated broccoli florets rapidly increased and at the end-point the control had lost 46.4% weight whereas the 1-MCP treated samples had lost 51.3% weight. In contrast, modified atmosphere packaging or 1-MCP+MAP significantly prevented weight loss of florets. The least weight loss occurred in MAP samples (10.8%) followed by 1-MCP+MAP (11.2%).

Table 1. Effects of different postharvest treatments on soluble solid content (SSC) and titratable acidity (TA) of broccoli florets during cold storage at 0 °C.

Parameter	Treatments	Days at 0°C				
		Harvest	7	14	21	28
SSC (%) $LSD_{0.05}$=0.43	Control	5.9±0.115	6.5±0.611	8.5±0.458	9.5±0.351	10.1±0.000
	1-MCP	5.9±0.115	7.3±0.252	8.1±0.231	9.1±0.115	8.9±0.000
	MAP	5.9±0.115	6.2±0.058	6.3±0.153	6.2±0.252	6.4±0.404
	1-MCP+MAP	5.9±0.115	6.1±0.100	6.1±0.153	5.9±0.100	6.4±0.436
TA (%) $LSD_{0.05}$=0.05	Control	0.90±0.047	0.75±0.006	0.67±0.072	0.44±0.015	0.45±0.035
	1-MCP	0.90±0.047	0.85±0.012	0.70±0.000	0.74±0.006	0.70±0.031
	MAP	0.90±0.047	0.76±0.010	0.66±0.026	0.54±0.044	0.48±0.020
	1-MCP+MAP	0.90±0.047	0.80±0.006	0.76±0.032	0.71±0.012	0.60±0.021
Weight loss (%) $LSD_{0.05}$=2.42	Control	0.00±0.000	16.5±1.010	29.3±1.075	41.9±1.083	51.3±0.185
	1-MCP	0.00±0.000	15.0±2.894	26.6±3.526	37.1±3.167	46.5±2.596
	MAP	0.00±0.000	3.1±0.212	5.4±0.260	7.2±0.736	10.8±0.570
	1-MCP+MAP	0.00±0.000	3.4±0.130	6.1±0.272	8.5±0.566	11.2±0.805

Each value represents the mean of three replicate samples. ± represent the standard deviation of that mean.

Surface color

Figure 1 illustrates the changes in hue angle that indicates surface color during storage. At the beginning of the storage, hue angle of broccoli florets was measured as 132.0° and this value rapidly decreased during the first 7 days. During storage, 1-MCP+MAP combination significantly delayed hue angle decline compared with single application of 1-MCP or MAP. At the end of storage, the highest hue angle value was measured in 1-MCP+MAP combination (126.7°), followed by 1-MCP (123.9°), MAP (123.2°) and control (121.2°).

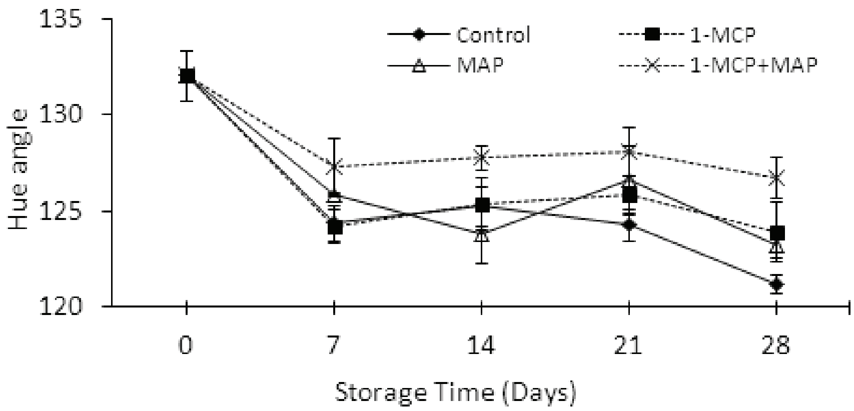

Fig. 1. Effects of different postharvest treatments on hue angle of broccoli florets during cold storage at 0 °C (LSD=1.90, p=0.0112). Each data point represents the mean of three replicate samples. Error bars represent the standard deviation of that mean.

Total phenol content

Total phenol content of broccoli florets in most treatments sharply increased in the first 14 days of storage although it decreased afterwards and again increased up to 28th days, except for control (Fig 2). During storage, the highest total phenol content was determined in 1-MCP or combined treatment. Initial total phenol content of broccoli florets was 413.5 $mg_{gallic\ acid}$ kg^{-1}. At the end of the storage, the least total phenol content was obtained from control florets (514.2 $mg_{gallic\ acid}$ kg^{-1}) while the values of 1-MCP, MAP and 1-MCP+MAP treatments were 630.2 $mg_{gallic\ acid}$ kg^{-1}, 631.3 $mg_{gallic\ acid}$ kg^{-1} and 638.5 $mg_{gallic\ acid}$ kg^{-1}, respectively.

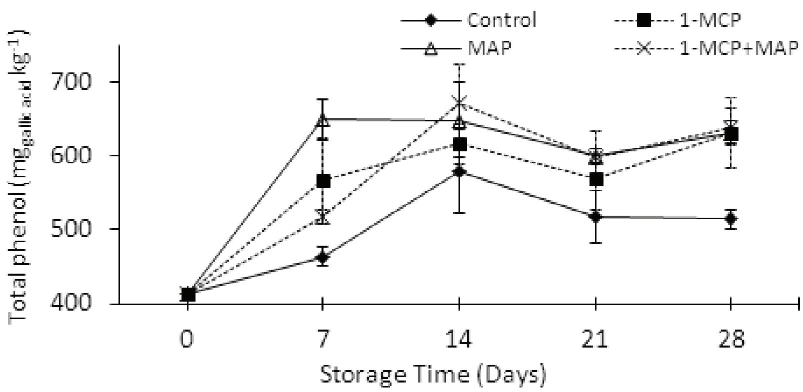

Fig. 2. Effects of different postharvest treatments on total phenol content of broccoli florets during cold storage at 0 °C (LSD=53.85, p=0.0009). Each data point represents the mean of three replicate samples. Error bars represent the standard deviation of that mean.

Total chlorophyll

Total chlorophyll content of broccoli florets decreased in all the treatments during storage at 0 °C. However, chlorophyll degradation rate was significantly delayed by 1-MCP+MAP treatment (Fig. 3). Total chlorophyll amount of florets was 9.79 mg 100 g^{-1} at harvest. At the end of storage, the highest total chlorophyll content was determined in 1-MCP+MAP treatment (5.12 mg 100 g^{-1}) while least amount was detected in control florets (3.88 mg 100 g^{-1}). In 1-MCP+MAP treated florets, 47% more chlorophyll was found compared to control florets. With respect to chlorophyll concentration, combined treatment was more effective than either 1-MCP or MAP treatments.

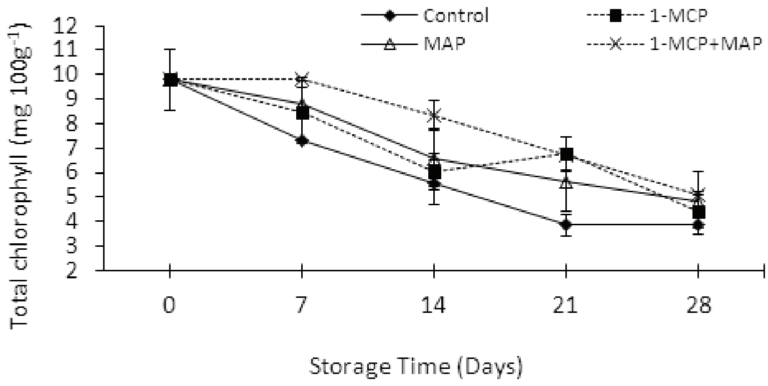

Fig. 3. Effects of different postharvest treatments on total chlorophyll of broccoli florets during cold storage at 0 °C (LSD=1.45, p=0.0417). Each data point represents the mean of three replicate samples. Error bars represent the standard deviation of that mean.

Ascorbic acid

Changes in ascorbic acid amount of broccoli florets are illustrated in Figure 4. During the first 14 days of storage, no significant change was observed in ascorbic acid of florets in treated samples. At harvest, ascorbic acid amount of florets was 52.34 mg $100g^{-1}$. At 28 days, ascorbic acid amount of wrapped florets were 50.18 mg $100g^{-1}$,and 45.22 mg $100g^{-1}$ for MAP and 1-MCP+MAP, respectively. Control and 1-MCP treated florets underwent a significant loss in ascorbic acid and the loss were 11.95 mg $100g^{-1}$ and 11.73 mg $100g^{-1}$, respectively at the end of storage.

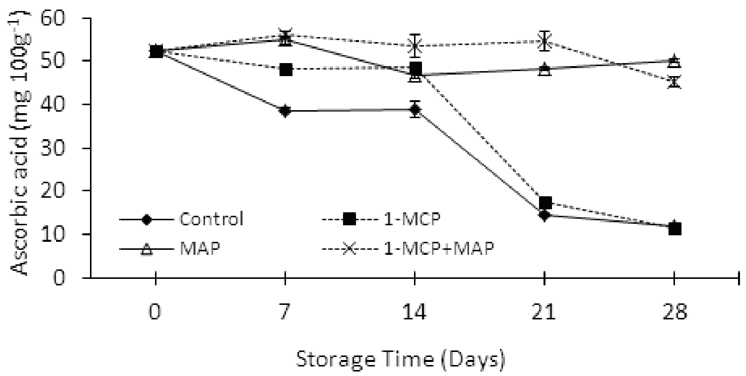

Fig. 4. Effects of different postharvest treatments on ascorbic acid content of broccoli florets during cold storage at 0 °C (LSD=1.78, $p<0.0001$). Each data point represents the mean of three replicate samples. Error bars represent the standard deviation of that mean.

Visual quality and color

Changes of visual quality and color in broccoli florets are illustrated in Figure 5A and B. Visual quality of all the florets decreased during storage and the decrease was most pronounced in control florets. MAP or 1-MCP+MAP significantly preserved the visual quality of florets. In addition, according to the visual quality assessment of the panelists, compactness of heads and hardness of stem texture were maintained in 1-MCP+MAP treated florets. Control florets reached to limit of marketability at 21 d. At the end of storage, the best visual quality score was determined in 1-MCP+MAP (8.0) while control florets (4.3) markedly lost their visual quality feature.

A similar effect was apparent with respect to color of florets, evaluated using 1−5 scale. For 14 days of storage, no change was detected in visual color of florets treated with 1-MCP+MAP and MAP; while control and 1-MCP treated florets indicated slow color degradation. At 28 d, color of control florets reached up to 3.7 score and this value is below the acceptable level. At 21 d score of florets belonging to 1-MCP+MAP treatment was determined as 1.3 by the panelists and the change in these florets was statistically significant (Fig. 5B).

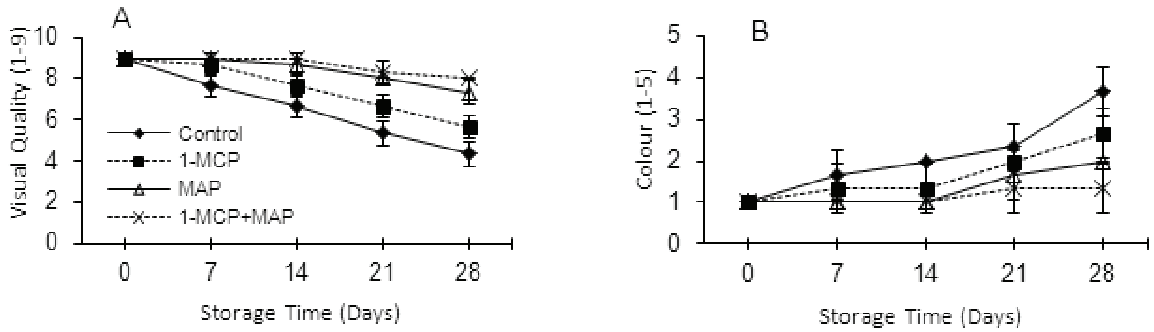

Fig. 5. Effects of different postharvest treatments on visual quality (A) and color (1−5 scale) (B) of broccoli florets during cold storage at 0 °C (LSD=0.71, $p<0.0001$ for A; LSD=0.64, $p=0.0087$ for B). Each data point represents the mean of three replicate samples. Error bars represent the standard deviation of that mean.

Titratable acidity (TA) and soluble solid content (SSC)

Titratable acidity at harvest was 0.90% and this value decreased along with the prolonged storage. During the first 14 days of storage, the changes in TA content of florets were insignificant among the treatments. However, TA contents of MAP or control florets were significantly lower than the others at 21th and 28th days (Table 1). 1-MCP or 1-MCP+MAP treatments effectively maintained the TA levels of florets during storage at 0 °C . At the end of storage, TA values ranged from 0.45% (control) to 0.70% (1-MCP).

SSC content of florets was 5.9% at the beginning of storage and increased along with storage. Such increase occurred sharply in control and 1-MCP treated florets, accompanying with the increase course in weight loss. At 28 days, the highest SSC was determined in control florets (10.1%), followed by 1-MCP (8.9%), MAP (6.4%) and 1-MCP+MAP (6.4%).

Discussion

Yellowing of broccoli florets is an important quality loss during postharvest handling and storage. The increment in the activities of chlorophyll-degrading enzymes such as chlorophyllase, Mg-dechelatase or Mg-dechelation is responsible for senescence (Kaewsuksaeng 2011). Makhlouf et al. (1989) reported that broccoli is defined as climacteric produce due to its respiration ratio and ethylene production increasing during senescence leading to yellowing of florets. In the present study, 1-MCP treatments combined with modified atmosphere package prevented chlorophyll degradation and declined hue angle compared to other treatments including control florets. This effect may explain that 1-MCP+MAP treatment prevent ethylene rising and delay the onset of yellowing. Previous studies have demonstrated that 1-MCP and MAP combination significantly prevented color changes during storage in various horticultural crops such as broccoli (Kasim et al. 2007), litchi (De Reuck et al. 2009), persimmon (Kaynas et al. 2010) and tomato (Sabir and Agar 2011a).

Broccoli is a highly perishable product and its shelf life and visual quality are affected by various postharvest conditions (Jia et al. 2009). In this research, 1-MCP treatment obviously protected both visual quality (1−9 scale) and color (1−5 scale) features of wrapped florets. Combined use of 1-MCP with MAP significantly maintained compactness of heads and hardness of stem texture. Serrano et al. (2006) reported that stem texture was highly correlated with weight loss because tissue dehydration was accompanied by increases in elasticity. Our results also indicated that florets with little water loss had better appearance than florets with a high water loss, and low-loss florets had maximum shelf life.

High weight loss is a significant problem of stored broccoli florets which affect the marketability (Serrano et al. 2006). MAP or 1-MCP+MAP significantly reduced weight loss. Similar results were also reported by Carvalho and Clemente (2004), Tano et al. (2007), Jia et al. (2009), Nath et al. (2011) on MAP used broccoli. Besides, Kasim et al. (2007) reported that combined use of 1-MCP treatment with polyvinylchloride film package reduced weight loss of broccoli heads compared with other films. MAP is an effective tool for reducing weight loss due to its inhibition of water vapour diffusion and thus it generates a water vapour pressure and higher relative humidity inside the package (Serrano et al. 2006, Jia et al. 2009). This effect of MAP is also widely used for successful storage of other vegetables such as tomato (Batu and Thompson 1998, Moretti et al. 2005, Sabir and Agar 2010), cauliflower (Mekwatanakarn 1999), spinach and parsley (Zenoozian 2011) and dill leaves (Sakaldas et al. 2010).

Phenolics and ascorbic acid are compounds involved in the maintenance of antioxidant status (Lemonie et al. 2007). Broccoli is proven as a very important source of phenolic compounds. Bahorun et al. (2004) characterized the broccoli as possessing a significantly high level phenol (higher than 800µg g^{-1}) amongst ten different vegetables. The amounts of nutritional compounds such as total phenol, ascorbic acid and antioxidants were reported to vary among different cultivars, climactic conditions, cultural practices, stage of maturity and postharvest handling and storage (Sivakumar et al. 2012). In this study, total phenol content in treated florets were higher than control florets during storage. This result is in agreement with that of Lemonie et al. (2010) who reported higher total phenol in UV-C+heat air treated florets than control ones.

Vitamin C is one of the most important vitamins in fruits and vegetables for human nutrition and its concentrations in horticultural commodities is fundamentally affected by various factors such as handling and storage conditions (temperature, controlled atmosphere or modified atmosphere packaging and various postharvest applications) (Lee and Kader 2000). Broccoli is rich in health-promoting compounds such as ascorbic acid and the amount of these compounds decrease during storage (Serrano et al. 2006). Present study revealed that MAP or 1-MCP+MAP significantly prevented loss in ascorbic acid content. According to the recent studies conducted on different cultivars with distinct conditions, MAP was significantly effective to prevent ascorbic acid loss in broccoli (Serrano et al. 2006, Carvalho and Clemente 2004). Nath et al. (2011) reported that broccoli heads packaged with polypropylene (PP) film having 10 pin holes showed less ascorbic acid loss than unwrapped samples in refrigerated storage. Similarly, Yuan et al. (2010) reported that 1-MCP treatment significantly minimized loss of ascorbic acid in broccoli florets. However in our study, 1-MCP treatment was ineffective for preventing loss of ascorbic acid in broccoli florets while 1-MCP+MAP resulted in better retention of ascorbic acid during prolonged storage. These results are in accordance with earlier report where positive impacts of combined use of 1-MCP with chitosan coating in Indian jujube fruits (Qiuping and Wenhui 2007) and 1-MCP with controlled atmosphere (3% O_2 + 8% CO_2) in mango (Sivakumar et al., 2012) were investigated.

In conclusion, MAP and 1-MCP combined treatment prevented losses of green color (either chlorophyll breakdown or hue angle decline), ascorbic acid and total phenol content and these florets exhibited significantly less weight loss at 0 °C and 95% RH that control florets. Therefore, combined treatment could be recommended to use for maintaining the postharvest quality of broccoli florets.

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