EFFECTS OF ASCORBIC ACID IN DELAYING FLORETS SENESCENCE OF BROCCOLI DURING POST-HARVEST STORAGE

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ABSTRACT. The rapid senescence is the main limiting factors of postharvest life in broccoli florets. In this study, effect of different concentration of ascorbic acid (0, 0.5, 1.5 and 2 %) on increasing postharvest life of broccoli florets was investigated. The treated florets was placed in polyethylene bag and transferred to storage with 0°C and 20°C. The characteristics such as total chlorophyll, antioxidant capacity, total phenol and lipid peroxidation was determined during storage. The results showed that during low and high temperature storage, the total chlorophyll and antioxidant capacity content declined and lipid peroxidation increased. The greatest delay in chlorophyll degradation and florets senescence in broccoli during 0°C was found with 1.5 % ascorbic acid treatments that was followed the lowest lipid peroxidation and the highest antioxidant capacity. But broccoli florets treated with 0.5 % ascorbic acid was more efficacious than other concentration at 20°. Overall, ascorbic acid with retarding chlorophyll degradation delayed florets senescence.

KEY WORDS: Broccoli, Ascorbic acid, Shelf life, Lipid peroxidation, Senescence

INTRODUCTION

Broccoli (Brassica oleracea L. var italica) is a floral vegetable that have an important nutritional value due to its content of vitamins, antioxidants and
anti-carcinogenic compounds (Lemoine et al. 2010, Lemoine et al. 2009). Most of the antioxidant has a potential due to the redox properties of phenolic compounds which allow them to act as scavenge reactive oxygen species (ROS) and increase their resistance to environmental stresses (Hodges 2003). Fresh broccoli is highly perishable, with a storage life of 3 to 4 weeks in 0 °C and 95% RH and 2 to 3 days at 20 °C (King & Morris 1994). This high sensitive and perishable caused to high rate of metabolism and consequently a high respiration rate, being extremely sensitive to ethylene. Its deterioration rate appears to be affected by storage temperature (Zhuang et al. 1997).

Plants senescence commonly is show by morphological, biochemical and biophysical deterioration consist of declining protein content, degradation of nucleic acids and lipid fluidity in membranes (Hodges 2003). Furthermore, it was also confirmed that ROS is involved in plant tissues (Dhindsa et al. 1981). Degreening and yellowing of sepals accompanied by chlorophyll breakdown is the main visible symptoms of broccoli senescence (King & Morris 1994, Lemoine et al. 2009). Lipid degradation is another common feature of many tissues undergoing senescence, and lipid peroxidation is usually correlated with tissue deterioration (Zhuang et al. 1997) excess production of ROS (Hodges 2003).

Many researchers has been investigated the effect of different storage methods on shelf life, visual quality and nutritional quality of broccoli and reported that cooling and controlled atmosphere are usually recommended for the storage of broccoli florets (Izumi et al. 1996). However, cooling and controlled atmosphere facilities are not yet available in developing countries (Yuan et al. 2010). Natural antimicrobial compounds are one of the main strategies by dipping treatments of fruits or vegetables in solutions (Rivera et al. 2006). Among them, ascorbic acid (AA) and its derivatives have been used in numerous studies in fruits and vegetables in concentrations ranging from 0.5 to 4% (Soliva-Fortuny et al. 2002). Ascorbic acid is a small water soluble antioxidant molecule (Shalata & Neumann 2001) which contributes to the detoxification of ROS and therefore the usage of AA is associated with resistance plants to oxidative stress and delayed senescence (Farouk 2011). The dip treatment in 0.5% AA increased storage life of salad cut
Effects of ascorbic acid in delaying florets senescence of broccoli

lettuce by about 10% (Bolin & Huxsoll 1991). Ascorbic acid treatment was also effective in delaying red color degradation in lychee (Terdbaramee et al. 2006). In the research of Bolin and Huxsoll (1991) also treatment improved storability of salad cut lettuce. Beneficial effect of the ascorbic acid is attributed to several aspects, such as capturing of oxygen and protection, forming a barrier that prevents oxygen diffusion toward the product, thus, reducing the production of o-quinones and inhibiting the polyphenoloxidase (PPO) (Hodges 2003).

The main objectives of this study were to evaluate the effects of AA treatments on quality maintenance florets and delaying florets senescence of Broccoli during low and high storage temperatures.

MATERIAL AND METHODS

Plant material
Heads of broccoli (Brassica oleracea) were purchased from a local market and transferred immediately to the laboratory, and the experiments were carried out the same day. Head separated into florets and stem and disinfected with chlorinate water (150 mL L-1 as sodium hypochlorite) for 15 min, followed by repeated washing with distilled water. Then, florets were immersed for 5 min at 20 °C in a solution containing ascorbic acid (0.5, 1.5, and 2 %). Distilled water was used as a control. Broccoli florets were dried at room temperature, then three florets of broccoli (include 20 g each florets) were placed in polyethylene bags with overall dimensions 20×20 cm² and 29.2 pmol/s/m²/Pa oxygen transmission rate film and stored three days at 20 °C and at 0 °C for 24 days. The following biochemical characteristics were evaluated immediately after the treatments and periodically with 6 days intervals at 0° C, and during three days at 20 °C. The physiological characteristic such as total phenolic content, antioxidant capacity and lipid peroxidation (MDA) level were measured only in control and those treatments kept in the solution of 1.5% and 0.5% AA, at 0°C and 20°C, respectively. In this treatment chlorophyll degradation was in at least possibly.

Evaluation
Weight loss. Weight loss during postharvest storage was determined on the day of harvest and after the different sampling dates and expressed as percentage loss of original weight (Lemoine et al. 2009).
**Chlorophyll content.** Total chlorophyll content was measured according to Lemoine et al. (2009). Florets were powdered by liquid nitrogen in a refrigerated mill and 0.4 g of the powder obtained was added to 5 mL of acetone/water (80: 20), stirred and then centrifuged at 5000 rpm for 15 min. The supernatant was used to determine the content of chlorophyll. Results were expressed as mg chlorophyll /g FW.

**Total phenolic content.** Total phenolics content were determined by the Folin Cicalteau method as described by Singleton et al. (1999), with minor modifications, according to colorimetric oxidation/reduction reaction of phenols. Polyphenols extraction was carried out by 10 ml 85% methanol added to 1 g fine powder of floret. To 250 μl of extract, 250 μl of sterile distilled water was added, and then 2.5 ml of Folin– Cicalteau reagent and 2 ml of 7.5 % sodium carbonate were added. The samples were shaked for 1.5 to 2 hours. The absorbance of samples was measured at 765 nm by spectrophotometer (PG Instruments T80+ UV, UK). Gallic acid was used for calibration curve. Results were expressed as mg GAE/100 g FW.

**Antioxidant capacity.** The antioxidant activity was measured by the scavenging of 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals according to Brand-Williams et al. (1995) with minor modifications. In the presence of antioxidant the purple color intensity DPPH solution declined and the change of absorbance is followed spectrophotometer at 517 nm. Briefly, a 0.15 mM solution of DPPH in methanol was prepared. 2 ml of this solution was added to 1 ml of methanol extracts of grape fruits. The content of the tubes were mixed and followed to stand for 30 min and absorbance was measured at 517 nm. The antioxidant activity is expressed in the form of the percentage of free radical scavenging.

**Lipid peroxidation (MDA).** Lipid peroxidation (MDA level) was determined by the method of Heath & Packer (1968). Floret sample (0.5 g) was homogenized in 1 mL of 0.1 % trichloracetic acid (TCA). The homogenate was centrifuged at 14000 rpm for 15 min, and then 600 μL of supernatant was added 600 μL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 10000 rpm for 10 min, the absorbance of the supernatant was recorded at 532 nm. The MDA level was calculated according to its extinction coefficient of 155 Mm cm⁻¹.

**Statistical analysis**

The recorded data were statistically analyzed (ANOVA analysis) using the software of SAS, sources of variation were different concentration of ascorbic acid and two storage temperature. Differences of least squared means were considered to be significant at P<0.05.
RESULTS

Weight loss
The changes in the loss weight were shown in Tables 1 and 2. The rapid decrease of loss weight was found in broccoli stored at 20 °C, but low temperature (0 °C) delayed weight decrease (Tables 1 & 2). Furthermore, florets treated with ascorbic acid were better than control and could decrease weight loss. So in this study, the lowest weight decrease was found in florets treated with 1.5% and 0.5% AA during storage at 0 °C and 20 °C, respectively (Tables 1 & 2).

Table 1. Effect of different ascorbic acid (AA) concentration on weight loss (%) of florets broccoli during storage at 0°C *

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5% AA</th>
<th>1.5% AA</th>
<th>2% AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 day</td>
<td>0.98±0.07</td>
<td>0.64±0.05</td>
<td>0.52±0.03</td>
<td>0.87±0.07</td>
</tr>
<tr>
<td>12 day</td>
<td>2.1±0.10</td>
<td>1.13±0.11</td>
<td>0.97±0.11</td>
<td>1.69±0.13</td>
</tr>
<tr>
<td>18 day</td>
<td>3.42±0.21</td>
<td>2.35±0.21</td>
<td>1.91±0.08</td>
<td>2.8±0.13</td>
</tr>
<tr>
<td>24 day</td>
<td>4.6±0.23</td>
<td>3.22±0.2</td>
<td>2.84±0.15</td>
<td>3.95±0.21</td>
</tr>
</tbody>
</table>

*Means of three replicates followed by the same letters were not statistically significant different (P≤0.05).

Table 2. Effect of different ascorbic acid (AA) concentration on weight loss (%) of broccoli during storage at 20°C *

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5% AA</th>
<th>1.5% AA</th>
<th>2% AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>2.12±0.06</td>
<td>0.88±0.02</td>
<td>1.34±0.14</td>
<td>1.635±0.08</td>
</tr>
<tr>
<td>2 day</td>
<td>4.07±0.12</td>
<td>2.13±0.04</td>
<td>2.65±0.14</td>
<td>2.82±0.5</td>
</tr>
<tr>
<td>3 day</td>
<td>6.01±0.14</td>
<td>3.12±0.13</td>
<td>3.97±0.3</td>
<td>5.01±0.27</td>
</tr>
</tbody>
</table>

*Means of three replicates followed by the same letters were not statistically significant different (P≤0.05).

Chlorophyll content
The rapid decrease of chlorophyll was observed only in the case of broccoli heads stored at 20 °C but low storage temperature (0°C) delayed yellowing and reduced loss in chlorophyll content (Figs 1 & 2). Furthermore, the
Figure 1. Effect of different ascorbic acid (AA) concentration on chlorophyll content of broccoli during storage at 0 °C. Vertical bars represent the average values with ±SE (n = 3).

content of chlorophyll decreased significantly during florets senescence in florets kept both in distilled water (control) and AA solution and in both temperature. The greatest delay in chlorophyll degradation and florets senescence in broccoli during 0°C was found with 1.5 % AA treatments but at 20° C broccoli treated with 0.5% AA was more efficacious than other concentration.

**Total phenolic content**

The changes of total phenolic content in broccoli florets during storage at 0°C and 20°C are given in figs 3 and 4. As the results showed, treatment with AA caused an increased significantly (P< 0.05) total phenolic content in both temperature storage, while in control treatment florets is reduced in the end storage at 0°C and 20°C (Figs 3 & 4).
Antioxidant capacity

The changes of antioxidant capacity in broccoli florets during two temperature storage were summarized at figure 5 and 6. It can be observed that ascorbic acid treatment induced antioxidant capacity but in control treatment florets, antioxidant activity decreased in both temperature storage.

Lipid peroxidation (MDA)

MDA concentration significantly increased in florets both AA treated and the control treatment during storage at 0 °C and 20 °C (Figs 7 & 8). At the end of storage (0 °C & 20 °C) the florets that treated with ascorbic acid were shown lower MDA content than control treatment (Figs 7 & 8).
Figure 3. Effect of ascorbic acid (AA) on total phenolic content of broccoli during storage at 0 °C. Vertical bars represent the average values with ±SE (n = 3).

**DISSCUSION**

**Weight loss**
Texture attributes degradation is directly related to water loss, which, in turn, can be evaluated by weight loss measurement (Raffo et al. 2008). It has been shown that storage duration (Javanmardi & Kubota 2006) and temperature and humidity of the storage (Finger et al. 1999) have significant effects on weight loss. Possibly, higher rate of transpiration in room temperature stored florets in compare to lower temperature-stored florets could be the main cause for higher weight loss (Javanmardi & Kubota 2006). Furthermore, Accumulation ROS and ethylene production during storage caused membrane degradation (Hodges, 2003). It is known to be accompanied by changes in membrane permeability change in ability of cell soluble material preservation, increase leakage in cell and finally loss
weight (Mayak 1987). Therefore possibly AA via inhibition of ethylene production (Ieamtim et al. 2008) and cellular membrane destruction and reduce ROS level, had led to decrease loss weight (Shalata & Neumann 2001, Gadallah 2000).

![Figure 4. Effect of ascorbic acid (AA) on total phenolic content of broccoli during storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).](image)

**Chlorophyll content**
Changes of chlorophyll level in photosynthetic cells are good indicator of senescence, occurring in green vegetable after harvested. Chlorophyll loss has been associated by increases in membrane permeability (Zhuang et al. 1995, Farouk 2011) due to increasing membrane lipid peroxidation (Hodges 2003, Zhuang et al. 1995, Farouk 2011). Chlorophyll content degradation was increased with temperature in broccoli florets (Starzyinska et al. 2003) and detected pakchoy leaves (Able et al. 2005). These results demonstrated that the temperature has an important role in florets...
senescence in broccoli (Finger et al. 1999, King & Morris 1994). But in this study the role of AA in increasing antioxidant capacity that is one of the main agents against senescence, should not be ignored. Furthermore, it is found that the application of ascorbic acid delayed chlorophyll degradation (Farouk 2011). This delay might be attributed to efficient scavenging of ROS by antioxidants compounds like AA, that would have destroyed the lipid membrane and chlorophyll pigments (Farouk 2011). Moreover, it has been proposed that AA may retard senescence either by inhibiting ethylene production and decreasing respiration (Ieamtim et al. 2008). Abdel Aziz et al. (2009) also, reported that exogenous application of AA on to several plant species have been shown to retard chlorophyll loss and senescence and protect plants against environmental stress.

![Figure 5. Effect of ascorbic acid (AA) on antioxidant capacity of broccoli during storage at 0°C. Vertical bars represent the average values with ±SE (n = 3).](image-url)
Total phenolic content
Penolic compounds degradation associated with a loss of cellular compartmentation membrane integrity and enzymatic activities because of naturally synthesized enzymes by fruits and vegetables. Especially, PPO were reported as main agents responsible for the degradation of phenolic compounds in plants (Baltacig et al. 2011). With the long time during of storage, cells gradually lose the integrity of the membrane system, which results in the loss of compartmentation and allowing enzymes to act on their substrates (Zhang et al. 2000). However in this study we found that AA treatment cause to increased total phenolic content. Ascorbic acid is extensively used to avoid enzymatic browning of fruit that caused by the reduction of the o-quinones, generated by the action of the PPO, back to the phenolic substrates (Lamikanra & Watson 2001). Furthermore, Kahkonen et al. (2001) reported that ascorbic acid could cause a
synergistic effect with total phenolic content of fruits. Abdel Aziz et al. (2009) reported that application of AA to Gladiolus plants, increased total phenol content.

![Figure 7. Effect of ascorbic acid (AA) on MDA content of broccoli during storage at 0°C. Vertical bars represent the average values with ±SE (n = 3).](image)

**Antioxidant capacity**

Antioxidants are involved in scavenging ROS produced during senescence, and therefore their levels decrease during early day of storage (Hounsome et al. 2009) but under various abiotic stresses and storage of vegetables the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages and therefore reduction antioxidant compounds (Lemoine et al. 2010). It has been reported that phenolic compounds is one of the main effective compounds in the antioxidant activity (Hodges 2003). Thus, treatment with AA could increase the total phenol content and as a result could increase antioxidant capacity.
Altunkaya & Gokmen (2008), also, observed that stored fruits which treated with AA, the level of antioxidant capacity were increased. It was also confirmed that ascorbic acid suppressed free radicals by the formation of ascorbyl radicals (Yamaguchi et al. 1999). The ene-diol structure plays an effective role in scavenging free radicals (Pavet et al. 2005).

Lipid peroxidation (MDA)
Malondialdehyde (MDA) is an end product of lipid peroxidation and has been used as a direct indicator of membrane injury (Yuan et al. 2010). It has been reported that the membrane permeability and the level of MDA content increase during fruit and vegetable senescence (Yuan et al. 2010, Dhindsa et al. 1981). Zhuang et al. (1997, 1995) also showed that postharvest senescence of broccoli is correlated with lipid peroxidation (MDA content), leading to cell-membrane disintegration. Broccoli florets are known to produce ethylene as they senescence and ethylene is known to
enhance membrane lipid degradation in other senescing system (Deschene et al. 1991). Thus, low temperature may inhibit membrane lipid degradation indirectly by inhibiting the action and synthesis of ethylene (Deschene et al. 1991). In this study florets treated with AA maintained the lower MDA content than the control during storage at 0 °C and 20 °C. This protection conferred by ascorbic acid is presumably due to its role in protecting the membrane lipid from peroxidation (Shalata & Neumann 2001, Farouk 2011). Jin et al (2006) also showed that AA treatment of rose flowers decreased MDA content

CONCLUSIONS

Results indicate that the importance of storage temperature and treatment with ascorbic acid on delayed senescence of broccoli. The highest chlorophyll content was observed in florets treated with 1.5% and 0.5% ascorbic acid at 0 °C and 20 °C respectively, which has been associated with increased total phenolic content and antioxidant capacity and retarded lipid peroxidation. It can be concluded that ascorbic acid can retarded the broccoli senescence due to induced and improved antioxidant system.

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REFERENCES


