

Effect of Different CO₂ Deastringency Application Timing on Fruit Quality Attributes and Physiological Disorders in Cold-Stored ‘Sangjudungsi’ Persimmon Fruit

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Abstract

The effects of different application timing of CO₂ deastringency treatment on fruit quality attributes and physiological disorders of ‘Sangjudungsi’ persimmon fruit during cold storage were evaluated. Treatment with an application of 95% CO₂ at harvest ensured the complete removal of astringency after one or two months of cold storage. The deastringency treatment greatly reduced flesh firmness, and early CO₂ application caused the lowest flesh firmness at the end of cold storage. The soluble solids content (SSC) was also decreased after CO₂ application, while fruit treated later with CO₂ had lowest SSC, compared with the other CO₂ application timings. Weight loss and respiration rate both increased in deastringency-treated fruit. Peel color variables, as measured using the CIELab color space, were also affected by the treatment. The changes in peel color variables were more detectable in the calyx-end regions than in the equatorial regions. Fruit treated with CO₂ one-month after harvest were observably different in peel color variables. Flesh browning was also detected in fruit treated one-month after harvest. The decrease in astringency following CO₂ treatment led to increased incidence of flesh browning and fruit softening at the end of the cold storage period. Peel blackening was also detected in early deastringency-treated fruit at the end of cold storage. This study suggested that earlier CO₂ application may decrease flesh firmness and increase the incidence and severity of physiological disorders in ‘Sangjudungsi’ persimmon fruit during cold storage.

Additional key words: flesh firmness, fruit softening, peel blackening, peel color variables, soluble solids content, soluble tannin

Introduction

The persimmon (*Diospyros kaki* Thunb.) plant is widely cultivated in many tropical and sub-tropical regions. Persimmon fruit can be classified into astringent and non-astringent types, depending on the level of soluble tannin content present in the flesh at harvest (Kim and Ko, 1997). Astringency refers to

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the sensation where the binding of soluble tannins to salivary proteins results a rough 'sandpapery' feel in the mouth (Novillo et al., 2015). Therefore, the removal of astringency is required for astringent persimmon cultivars prior to their commercialization. The taste of astringency-removed persimmons is driven by consumer demand, and they are considered to have better quality than that of non-astringent persimmons (Nam et al., 1998).

Traditionally, astringent persimmons are either consumed in over-ripe fruit stage, or artificially ripened by the application of exogenous ethylene (Munera et al., 2017). However, these astringency applications provoke a dramatic loss of flesh firmness and ultimately contribute to fruit softening. For these reasons, several postharvest deastringency technologies have been developed to remove astringency, while preserving the flesh firmness of the fruit. Numerous deastringency technologies have been suggested and are discussed in the previous reports, many of which are based on fruit physiological changes. These technologies include ethanol treatment (Kato, 1984; Chung et al., 2017), warm water treatment (Guan et al., 2015; Chung et al., 2015), CO₂ treatment (Novillo et al., 2015; Munera et al., 2017), and modified atmosphere packaging (Pesis et al., 2006). Of these technologies, astringency removal by CO₂ application (90 - 95% CO₂) is the most effective industrial approach, and commonly adapted as a commercial deastringency technology due to the high fruit storability and marketability, compared with other technologies (Taira et al., 1992; Cheng et al., 2015). The biochemical mechanism of CO₂ application is based on exposing the fruit to anaerobic conditions in which acetaldehyde reacts with soluble tannins that become insoluble at the end of cold storage, and results in fruit that have higher firmness following long-term cold storage (Taira et al., 1992; Arnal and Del Rio, 2003).

Previous reports have mostly focused on the effects of CO₂ treatment on the physiological and structural changes of fruit quality attributes that occur in many different astringent cultivars after the application of the treatment, and following cold storage. Maturity stage and the timing of application affect the level of astringency reduction in astringent persimmon fruit (Harima et al., 2003; Salvador et al., 2007; Besada et al., 2008). Nonetheless, deastringency treatment by CO₂ before storage was observed to negatively affect fruit quality attributes after application, resulting in a loss of firmness, and an increase in the incidence of physiological disorders during cold storage and shelf life (Salvador et al., 2008). Furthermore, previous studies on the use of CO₂ treatment for the removal of astringency only explored application timing of CO₂ treatment during harvest. As such, there have not been any reports regarding application timing of deastringency treatments during cold storage.

Therefore, the objective of this study was to evaluate the effects of different CO₂ deastringency application timing on fruit quality attributes and the incidence of physiological disorders in cold-stored 'Sangjudungsi' persimmon fruit.

Materials and Methods

Plant Materials

'Sangjudungsi' persimmon (*Diospyros kaki* Thunb.) fruit were collected on October 13, 2017 from a commercial orchard in Sangju, Korea. The harvested fruit were transported to the postharvest quality management laboratory at the Department of Horticultural Sciences, Kyungpook National University in Daegu, Korea. The fruit used in the study were carefully sorted to be of uniform size, and free from mechanical damage or infection.

CO₂ Deastringency Treatment and Cold Storage

For astringency removal, 95% CO₂ gas was used as a deastringency treatment, and the fruit were divided into four treatment groups: untreated controls, those treated with CO₂ at harvest, those treated with CO₂ 1 month after harvest, and those treated with CO₂ after 2 month of storage. The selected fruit groups were then separately exposed to CO₂ treatment at 20°C and 90% relative humidity (RH) for 2 days under the standard conditions in an enclosed 48 L container by passing a stream of air containing 95% of CO₂ through the container (Novillo et al., 2014). Untreated fruit were not subjected to CO₂ treatment. All treated fruit were stored together with untreated fruit at -1°C and 90% RH. Physiological quality attributes were evaluated immediately after cold storage and fruit quality assessments were conducted at one-month intervals for up to three months of cold storage.

Assessment of Fruit Quality Attributes

For assessment of physiological and fruit quality attributes, flesh firmness, soluble solids content (SSC), ethylene production rate, respiration rate, fresh weight loss, peel color variables, physiological disorders, sensory evaluation, and tannin content index (TI) were evaluated, and replicates of 15 fruit per treatment were used for all measurements. Flesh firmness was taken from three readings of the equator regions of each fruit using a Rheo-meter (Compac-100II, Sun Scientific Co., Tokyo, Japan). The juice from each fruit was then used to determine SSC using a refractometer (PR-201 α , ATAGO Co., Tokyo, Japan). To measure ethylene production and respiration rate, three fruit from each treatment were put into a 1.6 L enclosed container for 1 hour, then 1 mL of the headspace gas was withdrawn and injected into a gas chromatograph (GC-2010, Shimadzu Co., Kyoto, Japan) equipped with an activated column and flame detector. The injector and detector temperature were run at 200°C and 100°C for ethylene production and respiration rates, respectively, with the oven temperature set at 90°C. Flesh weight loss was recorded throughout the storage period.

Peel color variables were measured using the CIELab method with three readings on the equator and calyx-end regions of individual fruit, using a chromameter (CR-200, Minolta Co., Tokyo, Japan). The three CIELab parameters are L^* , a^* , and b^* . L^* defines lightness or color density, with 100 as absolute white and 0 as absolute black; a^* defines color in term of red (positive values) to green (negative values); b^* defines color in terms of blue (negative values) to yellow (positive values), according to McGuire (1992). Physiological disorders, such as fruit softening, peel blackening, wilting, and decay, were assessed throughout the storage period. The scores for severity rate of the disorders were 0 = 0%, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100% of peel coverage.

Tannin content index (TI) was determined according to the method described by Salvador et al. (2008). Fruit were cut in half horizontally and pressed in Whatman #4 filter paper (Whatman International Limited, Maidstone, Kent, England). These papers were immersed individually into a 5% (w/v) FeCl₃ solution for 1 minute, and then dried for 3 minutes. The intensity of soluble tannin was visually recorded by the distribution of tannin-Fe ion complexes, which are blue-black colored against the white paper. The flesh browning index was visually measured by cutting the fruit in longitudinal and cross sections. Then a five-point scale for the tannin index data was determined from 0 (no astringency) to 5 (high astringency), and the flesh browning index was subjectively scored from 0 (no browning) to 5 (maximum browning) based on a visual observation of the surface of the flesh area.

Statistical Analysis

All statistical analyses were performed with SPSS Statistics software (IBM SPSS Statistics 23, IBM Corp., Armonk, NY, USA). All data were subjected to a two-way ANOVA ($p < 0.05$).

Results

Flesh firmness is used to determine the physiological responses of fruit ripening. Untreated fruit were significantly harder than those treated with CO₂ ($p < 0.05$, Fig. 1A). Application of the CO₂ deastringency treatment resulted in a decrease in flesh

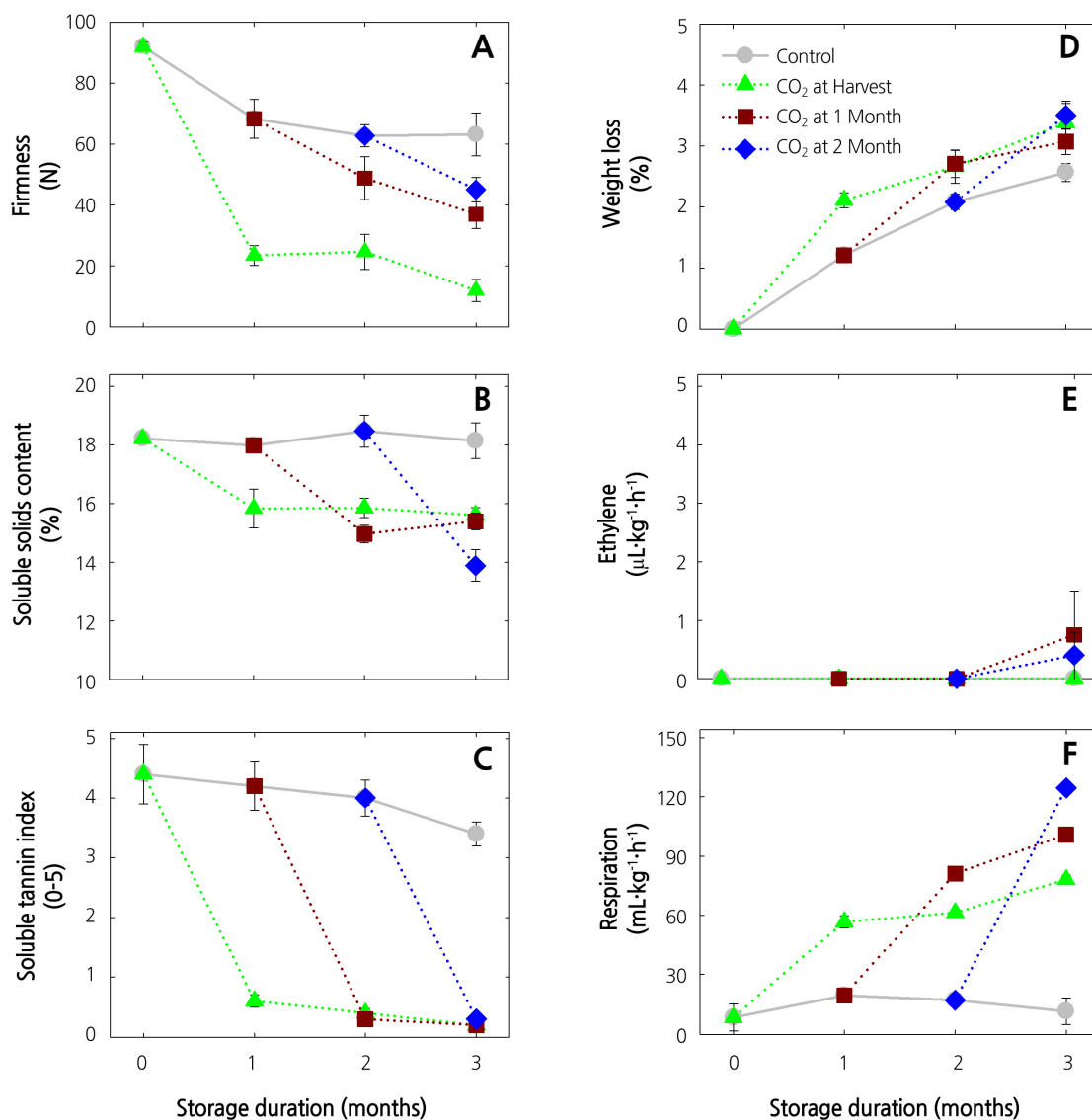


Fig. 1. Changes in flesh firmness (A), soluble solids content (B), soluble tannin index (C), fresh weight loss (D), ethylene production rate (E), and respiration rate (F) in 'Sangjudungs' persimmon fruit after different application timing of CO₂ deastringency removal at harvest, one or two month during cold storage, and then stored for up to 3 months at -1°C. All values are expressed as means ± standard error ($n = 15$).

firmness both after treatment and during cold storage. CO₂-treated fruit were much softer at harvest than after one or two months of cold storage ($p < 0.05$, Fig. 1A). SSC also decreased immediately after CO₂ application. SSC of all CO₂-treated fruit were lower than that of untreated fruit throughout the storage period ($p < 0.05$). Of the CO₂ treatments, the fruit treated after two months of cold storage were lowest in SSC both after CO₂ application and during cold storage ($p < 0.05$, Fig. 1B).

The loss of fresh weight both after CO₂ treatment and during cold storage was tracked throughout cold storage. CO₂ application increased fresh weight loss for all CO₂-treated fruit, compared with control fruit. However, a statistically significant difference was not found among the CO₂-treated fruit at the end of cold storage (Fig. 1D). Ethylene was neither produced at the early storage period, nor significantly affected by any CO₂ treatments at the end of cold storage (Fig. 1E).

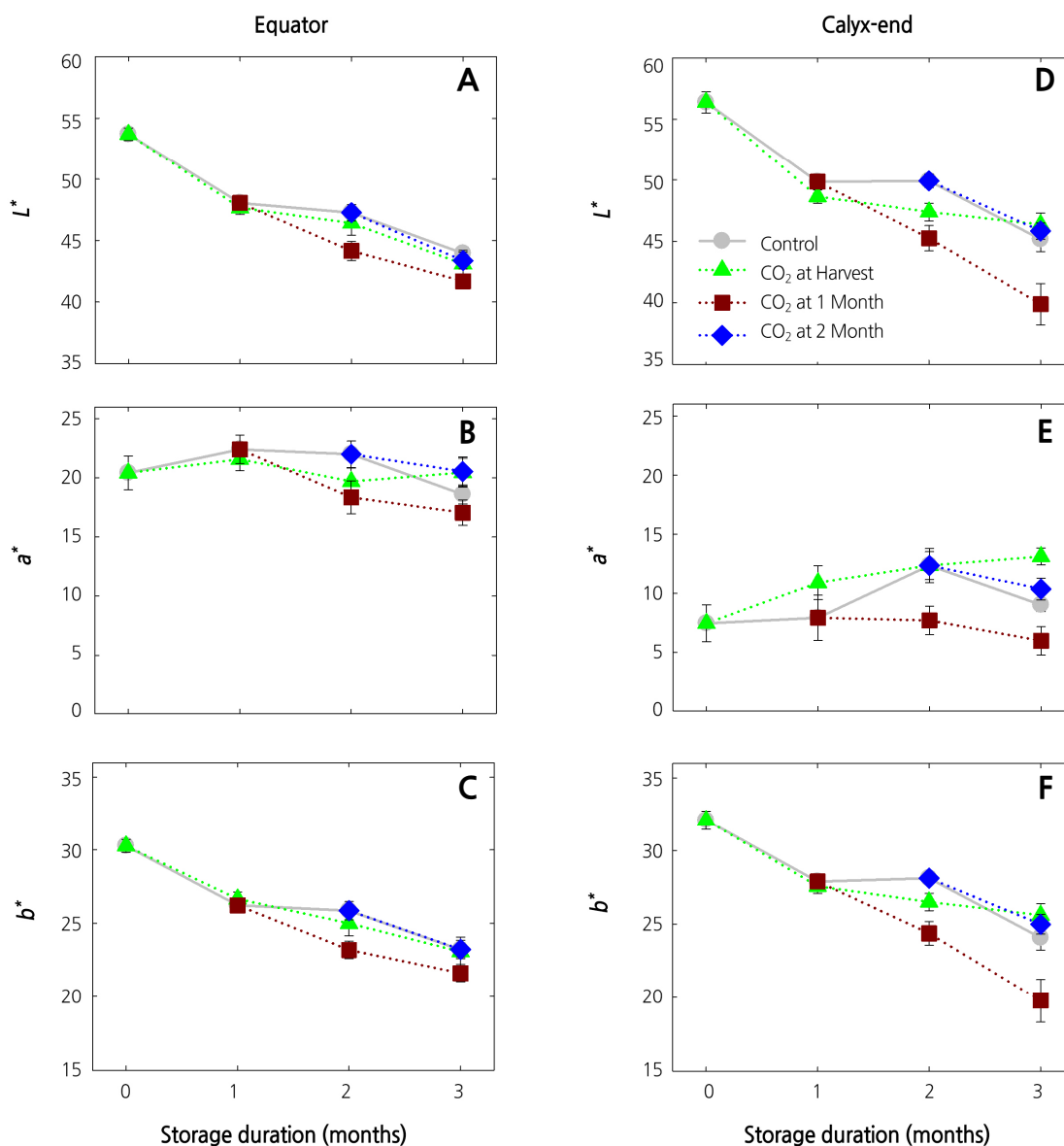


Fig. 2. Changes in peel color variables (L^* , a^* , and b^*) at equator (A, B, and C) and calyx end (D, E, and F) regions of ‘Sangjudungs’ persimmon fruit after different application timing of CO₂ destringency removal at harvest, one or two month during cold storage, and then stored for up to 3 months at -1°C . All values are expressed as means \pm standard error ($n = 15$).

However, the respiration rate of CO₂-treated fruit gradually increased throughout cold storage, and was significantly higher than that of the untreated fruit. Fruit treated with CO₂ after two months of cold storage exhibited higher respiration rates than that of the other treatments (Fig. 1F). After CO₂ application, the soluble TI of fruit exposed to deastringency treatment rapidly decreased. Untreated fruit retained higher soluble tannin content throughout the cold storage period ($p < 0.05$, Fig. 1C).

The peel color variables were obviously affected at the calyx-ends, compared with the equator regions. Fruit treated with CO₂ after one month of cold storage showed significantly decreased L^* and b^* values, while a^* was increased ($p < 0.05$, Fig. 2A-C). Similar results were also found in the equator regions of the fruit, especially at the end of cold storage (Fig. 2D-F).

The CO₂ deastringency treatment also resulted in an increase in flesh browning during cold storage. Fruit softening, wilting, peel blackening, and flesh browning are physiological disorders that are visually apparent and are commonly detected during cold storage. In this study, fruit treated with CO₂ at harvest had high levels of physiological disorders, especially at the end of cold storage (Fig. 3). Fruit softening was slightly affected by CO₂ application at one or two months of cold storage (Fig. 3A). However, none of these treatments exhibited fruit peel blackening at early cold storage, but fruit treated with CO₂ at harvest showed a high peel blackening at the end of storage, compared with others ($p < 0.05$, Fig. 3C). The storage temperature of

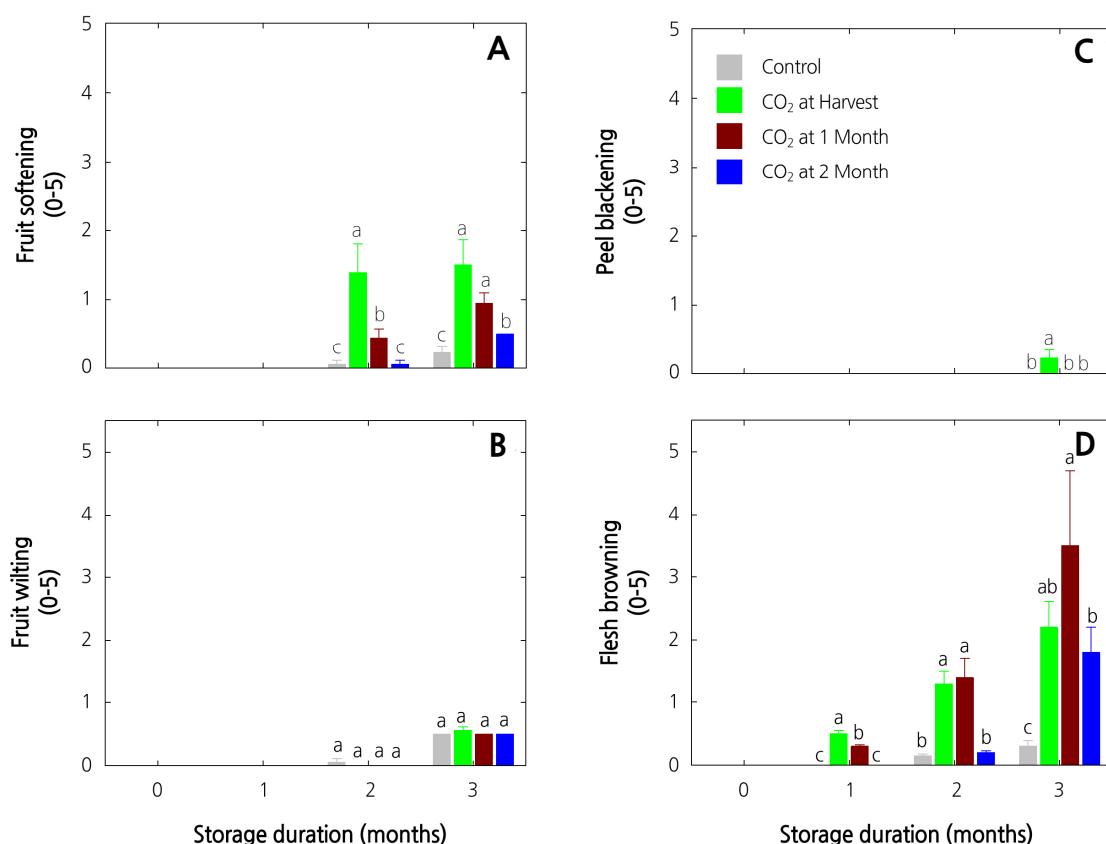


Fig. 3. Severity rate of fruit softening (A), fruit wilting (B), peel blackening (C), and flesh browning (D) in ‘Sangjudungs’ persimmon fruit with different application timing of CO₂ deastringency removal at harvest, one or two month during cold storage, and then stored for up to 3 months at -1°C. All values are expressed as means ± standard error ($n = 15$). The severity rate of physiological disorders was subjectively scored as 0 = 0%, 1 = 1 to 10%, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% area of the peel, referencing the largest area with the corresponding symptoms. The different letters in this figure represent different significant levels at $p < 0.05$.

-1°C was not found to impact the physiological disorders of untreated fruit during cold storage. At the end of the storage period, fruit wilting was detected to have occurred in all treated fruit during cold storage (Fig. 3B). All deastringency treated fruit scored highly on the flesh browning index, compared with untreated fruit ($p < 0.05$, Fig. 3D). When the fruit were cut in longitudinal and horizontal sections, strong internal flesh browning of the treated fruit was observed at the end of the cold storage period, especially for fruit treated with CO₂ after one month of cold storage. Early CO₂ applications at harvest and after one month of cold storage exhibited stronger flesh browning than other treatments at the end of cold storage. Compared with CO₂ treatments, the untreated fruit remained unchanged, and cold storage did not affect the internal flesh color (Fig. 3).

Discussion

For consumption of fresh fruit of astringent persimmon, the removal of astringency may be necessary at harvest and during storage. Exposure of fruit to CO₂ is the most commercially useful treatment to ensure the removal of astringency (Cheng et al., 2015). The optimum duration of CO₂ treatment depends on the fruit maturity stage and differences among cultivars (Besada et al., 2010). However, the time of application also plays a key role in astringency removal, and excessive application leads to losses in fruit quality (Novillo et al., 2014a).

Deastringency treatment using CO₂ reduced flesh firmness immediately after application, and similar results were found throughout the course of cold storage. Early CO₂ application resulted in higher losses of flesh firmness, compared with fruit treated later. CO₂ treatment caused a loss in firmness for all maturity stages of persimmon (Novillo et al., 2014b; Harima et al., 2003). However, Salvador et al. (2008) reported that CO₂-treated fruit had similar firmness values to that of untreated fruit after 30 days of storage, when stored at 15°C followed by 5 days of shelf life at 20°C. In this study, CO₂-treated fruit exhibited lower firmness than did untreated fruit at the end of cold storage. However, the firmness value for all treated fruit remained above 10 N after three months of cold storage. This result indicated that fruit subjected to deastringency treatment still retained fair marketability after cold storage. In this study, SSC decreased after CO₂ application throughout the cold storage period - a result that could be associated with the loss of astringency - indicating a decrease in the content of soluble tannin in fruit due to deastringency treatment (Kim and Ko, 1995; Chung et al., 2015; Arnal and Del Rio, 2003; Salvador et al., 2007).

A high rate of respiration was found for all CO₂ deastringency treatments in this study. CO₂ deastringency treatment increased the fresh weight loss after the application of CO₂, and during cold storage. The respiration rate increased immediately after CO₂ treatment at three of the maturity stages, but ethylene was not detected in later maturity stages (Novillo et al., 2014b). In this study, CO₂ treatment did not significantly affect ethylene production. Persimmon is one of the climacteric fruit; although the fruit produce low levels of ethylene (Besada et al., 2010), high CO₂ concentration could act as a stress to induce the production of ethylene (Harima et al., 2003). Also, ethylene was not produced until 4 months of storage in cold-stored 'Sangjudungsi' persimmon fruit (Win et al., 2017b). Peel color variables (measured using CIELab) are an important fruit quality attribute and affect consumer preference. Fruit ripening, as indicated by peel color variables, was more apparent at the calyx-ends than at the equator regions; a similar result was previously been reported for 'Sangjudungsi' persimmon (Win et al., 2017b; Yoo et al., 2016). In this study, the CIELab color values decreased with longer storage time. Chung et al. (2015) also observed that L^* and b^* values decreased after CO₂ application and cold storage. The changes in L^* value can be used to determine the browning index in fruit and vegetables (Caster et al., 1999). A decreased L^* value may be due to biochemical oxidation by polyphenol oxidase and peroxidase, which produce a brown pigment by an enzymatic

browning reaction (Tomas-Barberan and Espin, 2001).

Flesh browning is one of the main disorders that causes fruit quality loss during persimmon storage. Deastringency treatment itself results in a stress to the fruit that provokes numerous physiological disorders during cold storage and shelf life. Excessive application of CO₂ deastringency can also lead to flesh browning (Besada et al., 2018). The enhancement of browning index in astringent 'Niuxin' persimmon during cold storage is related to a decrease in soluble tannin content by CO₂ deastringency treatment (Min et al., 2018). Therefore, in this study, the earlier application of deastringency treatment completely removed the soluble tannin content, which might have led to the increase in flesh browning observed during storage. Furthermore, fruit maturity stage should be considered when determining the duration of treatment to avoid flesh browning by CO₂ overexposure, and the extent of flesh browning also appeared to increase when the fruit were stored at lower temperature rather than at moderate temperature (Besada et al., 2010; Novillo et al., 2014a). Early CO₂ application might contribute to fruit ripening by causing other physiological disorders, such as fruit softening, decay, and peel blackening at the end of cold storage. These symptoms were also reported for 'Sangjudungsi' and 'Tonewase' persimmon fruit stored at -1°C (Win et al., 2017a, 2017b).

In conclusion, the results of this study suggest that following harvest, earlier CO₂ treatment could reduce fruit quality attributes and increase the incidence of physiological disorders during cold storage. The application of deastringency treatments at harvest was determined to have a greater effect on fruit than did later treatments. Therefore, it is suggested that CO₂ deastringency treatment should be applied close to the end of the storage period to minimize loss of fruit quality in 'Sangjudungsi' astringent persimmon fruit.

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