Effects of Ascorbic Acids on Post-Harvest Longevity of Chrysanthemum Cut Flowers

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Kurniawan Budiarto
Indonesian Ornamental Crops Research Institute (IOCRI), Jl. Raya Pacet-Ciherang, Pacet Cianjur, West Java (43253), Indonesia
Corresponding author, email:  bud1arto@yahoo.com

ABSTRACT
Longer vase life of chrysanthemum cut flower is one of preferable quality traits in marketing for growers, retailers and consumers. Several compounds, like ascorbic acid have been reported to be able to prolong the post-harvest quality and longevity of cut flowers. Thus, the purpose of the study was to assess the ascorbic acid effects in several concentrations (0, 100, 200, and 300 ppm) in extending the fresh life of two chrysanthemum cultivars, i.e. cv. Remix (spray) and Yellow Fiji (standard). The results showed that the termination of flower freshness was visually characterized by wilting of leaves and florets and the change in floret color (paler). The supplementation of ascorbic acid solution at the concentrations of 200 and 300 ppm prevented and lengthened leaves and florets turgidity and postponed the wilting up to 2 – 3 days compared to control. At the same concentrations, the solution also slowed down the degradation rates of chlorophyll content on leaves during vase life periods.

Keywords: Ascorbic acid, chlorophyll content, chrysanthemum (Dendranthema grandiflora), freshness, vase life, wilting

INTRODUCTION
Chrysanthemum (Dendranthema grandiflora [Ramat.] Kitam.) is one of famous ornamental cut flowers in almost all parts of the world, including Indonesia. Longer vase life of chrysanthemum cut flower is one of preferable quality traits in marketing for growers, retailers and consumers (Bayat, Aminifard, 2017). Various preservative solutions containing antibiotics (bactericide and/or fungicide), growth regulators, inhibitors like ethylene or abscisic acid, carbohydrates (Clark et al., 2010; Dole, Carlson, Crawford, & McCall, 2013; Elbimabi, 2011), and mineral compounds (Banjaw, 2017) are commonly employed to prevent the wilting, thus prolong the cut flower fresh life.

The termination of fresh life is indicated by wilting (Azizi, Onsinejad, & Kaviani, 2015). Wilting of leaves and florets are mostly generated by imbalance between water and mineral supply and loss due to the resistance of water flow in the stem (Ahmad, Dole, & Blazich, 2014; Satoh, Nukui, & Inokuma, 2005; In, Seo, & Lim, 2016). Water facilitates the chemical reaction within the cell to keep biological activities and maintain cell turgor and flower freshness (Dung, Seaton, & Singh, 2016; Soleiman-Fard, Hemmati, & Khalighi, 2013). The inhibition of water flow within the stem is caused by several factors, i.e. water trapped within membrane cells generally filled with air (embolism) (Van Ieperen, Van Meeteren, & Nijsse, 2002), physical injuries leading to tissue decay(Wang, Zheng, & Xu, 2014)and water blockage due to microorganism activities (Kazemi & Ameri, 2012; Nemati, Tehranifar, Esfandiari, & Rezaei, 2013). Among these factors, microorganism activities
resulting the tissue decay was the most common causes of water blockage (Hashemabadi, Kaviani, Shirinpour, & Zahiri, 2013; Hashemabadi, 2014; Liu, Ratnayake, Joyce, He, & Zhang, 2012; Jowkar, Kafi, Khalighi, & Hasanzadeh, 2012). The activities of microorganism produce ethylene and toxic compounds that induce the acceleration of senescence (Basiri, Zarei, Mashayekhy, & Pahlavany, 2011; Rahman, Ahmad, & Lgu, 2012; Bhaskar, Rao, & Reddy, 2017). These processes result in the loss of cell turgor and tissue wilting (Sudaria, Uthairatanakij, & Nguyen, 2017).

Ascorbic acid (vitamin C) has been reported to have impact on the increase of water uptake in cut flower and prolong vase life of cut flower (Abdulrahman, Ali, & Faizi, 2012). Several authors also reported that ascorbic acid has played important roles in some biochemical activities as an enzyme cofactor (Szarka, Bánhegyi, & Asard, 2013), electron transport (Ivanov, 2014) and antioxidant on chloroplast cell membrane (Gallie, 2013). Endogenous ascorbic acid also prevents and maintains issues from oxidative damages due to tissue decay and microorganism activities (Ghadimian & Danae, 2015). The supplementation of ascorbic acid solution has been observed to induce the increase of endogenous ascorbic acid content within the stem, thus lengthen the vase life of several cut flowers, i.e. lisianthus (Azizi et al., 2015), snapdragon (Abdulrahman et al., 2012), gerbera (Mehdikhah, Onsinejad, & Hashemabadi, 2016), and gladiolus (Ravanbakhsh, Mobasser, & Hasandokht, 2016). The purpose of the study was to evaluate the effects of ascorbic acid at several concentrations on extending the fresh life of spray and standard chrysanthemum cut flowers.

MATERIALS AND METHODS
Sample Preparation

The study was conducted in the Indonesian Ornamental Crops Research Institute (IOCRI), located at 1100 masl, from January to May 2017. A factorial experiment was arranged in completely randomized design with three replications. The first factor was chrysanthemum cultivars, namely cv. Yellow Fiji (standard type) and cv. Remix (spray type) and the second factor was the ascorbic acid concentrations, i.e. 0 (control), 100, 200, and 300 ppm.

Treatment application

The ascorbic acid (L-ascorbic acid, PubChem – 54670067) was weighed at 100, 200, and 300 mg and each was put into 100 ml aquadest (distilled water) and stirred for 10 minutes. The solutions were made just before the experimental was set up (freshly mixed). The solutions were then separately put into vase flasks based on the experimental set up with the volume of 300 ml per flask.

Sample collection and observation

The chrysanthemum flowers were harvested in the morning. The stem was cut and leaved 40 cm stalk from the terminal flowers. The basal stalks were then diped into flasks containing 300 ml/flask of ascorbic acids solutions according to the experimental set up. The flasks were arranged in certain distance to facilitate the flowers not in contact with each others. The experimental set up was conditioned in an ambient temperature room with proper aeration and protected from direct sunlight. During the night, artifical lights were provided using 22 watt LED lamps that were put 3 m above the treated flower stalks.

The variables observed included: (1) duration of leaves freshness, counted as number of days from the first day of treatment application until the leaves were defined wilting, (2) duration of floret freshness, referred to number of days from the first day of treatment application until the outer most florets were defined wilting, (3) floret color, observed using RHS color chart at the first
day of treatment application and the day when the florets were defined wilting and (4) chlorophyll content, measured using portable chlorophyll meter SPAD (Konica Minolta). The observations were conducted every two days starting from the first day of treatment application. Two healthy, fully expanded, vegetative leaves were selected for chlorophyll content measurement. The reading values were recorded and converted into predicted values of chlorophyll content using the equation from Davies, He, Chau, Heinz, & Cartmill (2004):

\[ y = 0.001 x^3 + 0.0104 x^2 - 1,730 x + 11.702 \] (r = 0.98),

where \( y \) = predicted chlorophyll content (µg/cm\(^2\)) and \( x \) = SPAD reading value.

Data analysis
The collected data were analyzed using ANOVA and subjected to Least Significant Difference (LSD, \( \alpha = 5\% \)) test.

RESULTS AND DISCUSSION
Characteristic of wilting and floret color change
The wilting of leaf and flower was determined by visual observation on leaf and floret turgor. Fresh leaves and florets have an optimal cell turgor. With optimum cell turgor, the position of leaf and floret blade is seated straight or has an upward angle from the base (Figure 1a, c and e). The termination of leaf and floret freshness were characterized by the irreversible loss of cell turgor resulted in the wilting of leaves and florets (Figure 1b, d and f).

In both chrysanthemum cultivars, the cessation of flower freshness affected the floret colors. The color of wilted florets in both cultivars displayed the same pattern when wilting in all ascorbic acid concentrations (Table 1). These indicated that the ascorbic acid did not direct the color change into certain color type. The changes of flower color when wilting during vase life were also reported on orchid (Khan, Mehraj, Taufique, Ahsan, & Jamaluddin, 2015), gerbera (Heidarnezhadian, Eghbali, & Kazemi, 2017), rose (Zamani, Kazemi, & Aran, 2011) and carnation (Kazemi, Gholami, & Bahmanipour, 2012). The change of floret color was associated with the decreased carbohydrate content used for respiration and supply due to low photosynthetic rates during vase life period.

Figure 1. (a) Initial leaf freshness, (b) wilted leaves, (c) Initial and (d) wilted flower of cv. Remix, (e) initial and (f) wilted flower of cv. Yellow Fiji
Carbohydrate was one of the main compounds in pigment biosynthesis process and other secondary metabolites, including anthocyanins (Khan et al., 2015; Heidarnezhadian et al., 2017; Zamani et al., 2011). However, ascorbic acid was predicted to have relation in slowing down the process of color change along with the induction of prolonged flower freshness during vase life.

Table 2. Leaf and flower freshness of two chrysanthemum cut flower cultivars supplemented with ascorbic acid solution at different concentrations

| Treatments | Duration of freshness* (days) | Leaves | Flowers |
|------------|------------------------------|--------|---------|
| Chrysanthemum cultivars | | | |
| cv. Remix | 11.11 a | 13.76 a |
| cv. Yellow Fiji | 11.44 a | 13.72 a |
| Concentration of ascorbic acid (ppm) | | | |
| 0 (control) | 10 a | 11.67 a |
| 100 | 10.55 ab | 13.84 b |
| 200 | 12.5 b | 14.57 b |
| 300 | 12.05 b | 14.89 |
| Treatment combinations | | | |
| cv. Remix in 0 ppm ascorbic acid (control) | 10 a | 11.67 a |
| cv. Remix in 100 ppm ascorbic acid | 10.55 a | 13.57 ab |
| cv. Remix in 200 ppm ascorbic acid | 12.11 b | 14.8 b |
| cv. Remix in 300 ppm ascorbic acid | 11.77 ab | 15 b |
| cv. Yellow Fiji in 0 ppm ascorbic acid (control) | 10 a | 11.67 a |
| cv. Yellow Fiji in 100 ppm ascorbic acid | 10.55 a | 14.1 b |
| cv. Yellow Fiji in 200 ppm ascorbic acid | 12.89 b | 14.33 b |
| cv. Yellow Fiji in 300 ppm ascorbic acid | 12.33 b | 14.77 b |

Remarks: *) Values in the same column under each criterion of cultivars, ascorbic acid concentrations and treatment combinations followed by different letters differ significantly based on Least Significant Different test (LSD, α ≤ 5%).

Table 1. Initial and wilted floret color of chrysanthemum cv. Yellow Fiji and cv. Remix during vase life

| Chrysanthemum cultivar | Floret color (RHS color chart) |
|------------------------|--------------------------------|
| Yellow Fiji            | Yellow 12 B, Yellow 6 B        |
| Remix                  | Red Purple 59 B, Greyed Purple 187 C |

Leaf and floret fresh life period

Analysis of variance on the effects of ascorbic acid solution at several concentrations as holding solution for cut flowers of two chrysanthemum cultivars revealed that there was no significant interaction effect between ascorbic acid concentrations and chrysanthemum cultivars on lifespan of leaf and floret longevity. The duration of flower freshness of both tested cultivars was not significantly different (Table 2). This condition inferred that both chrysanthemum cultivars that have different genetic backgrounds performed similar retention on the duration of flower freshness. Similar findings were also reported by Baskaran, Jayanthi, Janakiram, & Abirami (2010) and Sharma & Srivastava (2014) on the observation of vase life of several chrysanthemum cultivars with or without chemical supplements.

Table 2 showed that the lifespan of leaf and flower freshness were also affected by the application of ascorbic acid. The cut flowers supplemented with ascorbic acid solution had prolonged leaf and flower freshness. The longest leaf freshness was observed on the cut flower dipped in 200 ppm ascorbic acid solution with the average of 2.5 days longer than control. At higher concentration (300 ppm), however, the effects were descended. Different phenomena were observed in petal freshness. The prolonged petal freshness was induced in lower ascorbic acid concentration. Longer period of petal freshness, in average of 2 days, was observed in chrysanthemum cut flower supplemented with 100 ppm. At higher concentrations (200 and 300 ppm), the induction of prolonged petal freshness was not significant.

After the flowers were harvested (the stem was cut) and during vase life period, the photosynthetic activities continued with diminished rates. The flower stalks use carbohydrate storage within the organ tissues for respiration to support cells’ life.
(Hossain, Boyce, & Majid, 2008). During vase life, ascorbic acid is needed to prevent leaf wilting, thus maintain the water balance and photosynthetic activities (Forti & Elli, 1995; Kobayakawa & Imai, 2012) although in lower rates. This mechanism is predicted to have relation with higher concentration of ascorbic acid needed to prolong leaves and petals freshness. These phenomena were detected on both tested cultivars cv. Yellow Fiji and cv. Remix (Table 2).

Several studies reported that ascorbic acid prolonged the flower longevity through several mechanisms. Ascorbic acid might be lowering the respiration rate and ethylene synthesis (Abri, Ghasemmezhad, Hasansajedi, & Bakhshi, 2013). The low respiration infers the ability of flower stalk to minimize water loss and maintain water content through active metabolic process (Abdulrahman et al., 2012). Ascorbic acid plays important role in improving water uptake by depleting the stem resistance. The antimicrobial characteristics of the substance delay the tissue decay and maintain tissue-water conductivity within the stem, thus increase water uptake by the basal stem (Bhaskar et al., 2017).

Chlorophyll content

The chlorophyll content gradually decreased based on every two day-SPAD measurements in all treated cut flowers during the vase life period (Figure 2). Sharper chlorophyll decrease was detected on the cut flower leaves of control treatment. The decrease of chlorophyll content indicates chlorophyll degradation (Basiri et al., 2011) which is generally caused by mineral translocation and microorganism activities (Elhindi, 2012). Without ascorbic acid supplement, the chlorophyll degradation is predicted to be faster. The faster chlorophyll degradation on control treatment was detected on both tested cultivars. The measurement of chlorophyll content on control treatment was terminated after 14 days since the leaves had wilted and dried.

The decrease of chlorophyll content on the leaves of cut flowers treated by ascorbic acid solution were slower especially after 4 (cv. Yellow Fiji) and 8 (cv. Remix) days during vase life period (Figure 2). On both tested cultivars, the highest chlorophyll content (slowest chlorophyll degradation) was detected on cut flower treated with 300 ppm, followed by 200 and 100 ppm ascorbic acids treatments. The slow chlorophyll degradation is related with lower respiration rate and the increase of water uptake of cut flower (Balouchi, Peyvast, Ghasemnezhad, & Dadi, 2012). This condition is predicted due to the preservative effect of ascorbic acid in preventing water blockage in the transport tissues (Banaee, Hadavi, & Moradi, 2013), thus prolong the flower freshness during vase life period.

![Figure 2. Chlorophyll content in the leaves of chrysanthemum cut flower stem cv. Remix (left) and Yellow Fiji (right) treated by ascorbic acid solution in various concentrations during vase life period.](image-url)
Based on the SPAD chlorophyll measurements, the increase of chlorophyll content was not detected in any ascorbic acid concentrations during vase life period. The application of ascorbic acid solution affected only chlorophyll retention thus slowed down the chlorophyll degradation. These phenomena are not in line with the findings of Ghadimian & Danaee (2015) on roses and Asrar (2012) on snapdragon, in which ascorbic acid treatments increased the chlorophyll content of the cut flowers during vase life. Further evaluations on the effect of ascorbic acid at higher concentrations with and/or without other synergistic preservatives supplements are needed to improve chlorophyll content of cut flower during vase life, thus prolong the flower longevity.

CONCLUSIONS

Termination of chrysanthemum cut flower freshness was characterized by the wilting of leaves and florets and the change of floret color (less colored) on both tested chrysanthemum cultivars, cv. Remix and Yellow Fiji. Supplementation of ascorbic acid at concentration of 200 to 300 ppm delayed the leaf and petal wilting up to 2 to 3 days compared to control (without ascorbic acid) treatment. At the same concentrations, ascorbic acid treatments also showed down chlorophyll degradation during vase life period.

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