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Effect of Internal Anthocyanin of Petals and External Sucrose on Some Postharvest Traits of Four Carnation Cut Flower Cultivars

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The amount of internal anthocyanin in cut carnation flowers may be one of the factors affecting the vase life of colored carnations. Two experiments were conducted to examine the effect of changing the internal anthocyanin concentration in petals and using external sucrose on the vase life of four cultivars of carnation cut flowers, including white (White Liberty), yellow (Yellow Liberty), pink (Tabor), and red carnations (Grand Slam). The first experiment investigated the variation in internal anthocyanin of petals and its effect on the vase life of the four cultivars. The second experiment evaluated the effect of external sucrose concentration $(0, 1)$ 2, and 4%) on postharvest traits of the studied cultivars. The results of the first experiment revealed the possible effectiveness of internal anthocyanin in the vase life of carnation. The vase life of the cultivars that contained no internal anthocyanin (white and yellow) was twice (14 days) as long as that of the red and pink cultivars containing anthocyanin (7 days). The results of the second experiment indicated that external sucrose improved the vase life-related traits. The shortest vase life was observed in the red cultivar (13.7 days) without the application of external sucrose, and the longest was related to the pink cultivar (18 days) treated with 4% external sucrose. Increasing internal anthocyanin in the absence of external sucrose generally reduced vase life. The application of external sucrose extended the vase life of the cultivars containing more anthocyanin.

Keywords: Anthocyanin, Carnation, Concentration, Sucrose, Vase life.

Abstract

Abstract

INTRODUCTION

Today, maintaining the physical quality of cut flowers is the most important objective in local and international markets, for which it is crucial to consider the factors affecting the vase life of cut flowers, including internal and environmental factors. Researchers have studied factors like vascular occlusion by bacterial activity and how to avoid or control it (Kazem Alvandi et al., 2010; Chamani et al., 2012; Angioni et al., 2016).

Carnation *(Dianthus caryophyllus)* is one of the world's most popular cut flowers. It ranks sixth in the international trade of cut flowers (Blankenship and Dole, 2003). Exploration into the methods of extending the postharvest life of flowers is of great importance for flower producers and consumers (Kofranek and Halevy, 1972). Cut flower color is one of the valuable traits of ornamental plants. Flower color results from the accumulation of pigments, such as flavonoids, carotenoids, and betacyanins, in the epidermal cells (Mol *et al.*, 1998). Anthocyanins are the chief group of natural pigments in plants after chlorophyll. They are non-toxic water-soluble *eta* compounds commonly found in plants' intracellular fluid (Chandrasekharm *et al.*, 2012; Lee *et al.*, 2005). Phenolic compounds, especially anthocyanins occurring in cells, protect plants from ultraviolet damage. When anthocyanidin combines with glycoside, the new compound is known as anthocyanin (Castaneda-Ovando et al., 2009).

Sucrose is an important carbon compound synthesized by and then transported in plants (Lolonde *et al.*, 1999). It is also a primary resource for petal growth (Woodson and Wang, 1987). External application of sugars significantly delays the aging process in flowers whose aging process is influenced by ethylene (van Doorn *et al.*, 1995). It has been reported that the application of sugars to carnation flowers reduces the effect of external ethylene application (Mayak and Dilley, 1976). These effects are probably due to the inhibition of ethylene synthesis in petals. It has been reported that almost all mRNA genes delay senescence in the presence of high concentrations of sucrose in cut carnation flowers (Hoeberichts *et al.*, 2007). Studies have also shown that high amounts of sugars can accelerate the degradation of *ein*³ gene transcription factors in proteasomes (Yanagisawa *et al.*, 2003). Other sugars, such as trehalose, mannitol, and inositol, also delay the senescence process in some plant species. Studies on tulips (Iwaya-Inoue and Nonami, 2003) and gladiolus (Otsubo and Iwaya-Inoue, 2000; Yamada et al., 2003) have demonstrated the influence of these sugars on delaying the senescence process. The application of external sugars to the aging process in ethylene-insensitive flowers is associated with a delay in the expression of genes involved in mobilizing fatty acids and proteins (Eason *et al.*, 2002). Adding sucrose to preservative solutions positively influences the postharvest life of most flowers, including carnations. Senescence delaying in the petals of the carnation cultivar 'White Sim' has been described with the use of sucrose. Since the use of sucrose alone in preservative solutions promotes microbial growth, it is necessary to apply antimicrobial agents (Solgi et al., 2009; Kazemi and Amiri, 2012).

To date, no study has reported the effects of internal anthocyanin concentration of petals and sucrose application on the vase life of cut carnation flowers. Therefore, this study aimed to investigate the impact of different commercial cultivars of cut carnation flowers and external sucrose on variations in internal anthocyanin pigments and postharvest traits of cut carnation flowers.

MATERIALS AND METHODS

Plant materials

Cut carnation flowers, including four commercial cultivars of white (White Liberty),

yellow (Yellow Liberty), pink (Tabor), and red (Grand Slam), were collected at the budding stage (half-open) from a commercial greenhouse in Mahalat, Iran and transferred to the laboratory $(Fig. 1)$.

Fig. 1. Four cut carnation flower cultivars used in this research: yellow (Liberty), white (White Liberty), red (Grand Slam) and pink (Tabor).

Treatments

In the laboratory, all flowers with a length of 35 cm were re-cut under water and transferred to vase solutions. The study consisted of two experiments. In the first experiment, the vase solution contained distilled water plus silver nanoparticles (10 mg L^{-1}) as an antimicrobial agent. The first experiment aimed to examine the effect of variations in internal anthocyanin concentration of two colored cultivars compared to two colorless ones. For this purpose, four cultivars of cut carnation flowers (white, yellow, pink, and red) were selected. The vase solution was applied permanently. The experiment was conducted in a completely randomized design with three replications and five observations per replication. The evaluated traits were vase life, relative fresh weight $(2, 4, 6,$ and 8 days), solution uptake rate $(2, 4, 6,$ and 8 days), and anthocyanin content $(2, 4, 6,$ and 8 days).

The second experiment was conducted to investigate the effect of external sucrose levels $(0, 2, \text{ and } 4\%$ sucrose) on some traits of cut flowers of the four cultivars mentioned in the previous experiment (white, yellow, pink, and red). The vase solution of all treatments contained distilled water plus silver nanoparticles $(1:10;$ silver nitrate: plant extract) and was kept there until the end of the experiment (permanent). The lids of the containers containing cut flowers were covered with cellophane to prevent preservative solution evaporation. The studied traits were vase life, relative fresh weight (measured every other day from day 2 to day 18), solution uptake rate (measured every other day from day 2 to day 18), anthocyanin concentration $(4, 10, 10)$ and 16 days), and amount of soluble solids $(4, 10, \text{and } 16 \text{ days})$.

Measurement of traits

Vase life is the most important trait in the carnation postharvest stage. Vase life is defined as the number of days from the first day after applying the treatments until the flowers lose their commercial value. This trait was measured by observation every day. The vase life of cut carnation flowers was evaluated according to the petal wilting rate, in which 50% of petal wilting marked the end of vase life.

To measure the relative fresh weight, the weight of the flowers was measured every other day $(2, 4, 6, \text{ and } 8 \text{ days})$ using a digital scale (with a precision of 0.01 g). The weight of each flower on the target days was divided by the weight of the same flower on day zero and multiplied by 100. The relative fresh weight was calculated in grams per gram of initial fresh weight per day (Solgi *et al.*, 2009; van Meeteren, 1978).

The solution uptake was determined by subtracting the weight of the container with vase solution from the container with vase solution on the previous day. Solution uptake was calculated every other day. The solution uptake was calculated in ml using the following formula (Solgi et al., 2009; van Meeteren, 1978):

Solution uptake (m) = Vase solution weight of respective day - Vase solution weight of the previous day

Anthocyanin concentration was measured by the method of Wagner (1979). Initially, 0.1 g of the petals were crushed with acidified methanol and kept in the dark for 24 hours. The supernatant containing anthocyanin was then removed in a centrifuge at 4000 rpm. Finally, anthocyanin concentration was calculated using a spectrophotometer at 550 nm according to the following formula:

 $A = \varepsilon BC$

In which A is the absorbance read, $\varepsilon = 33000$ is the extinction coefficient. B is the cuvette path length, and C is the anthocyanin concentration.

The phenol-sulfuric acid method was used to measure soluble sugar. A portion of the petals was cut and placed in an oven at 70° C for 72 hours. 0.05 g of the resulting dry matter was added to 3 ml of distilled water and then crushed. It was next passed through a filter paper, and the extract was mixed with 5 ml of distilled water. 2 ml of the supernatant was taken and mixed with 50 μ l of phenol and 5 ml of sulfuric acid. The solution was left still for 10 minutes, and then, the absorbance was measured at 490 nm (DuBois *et al.*, 1956).

Statistical analysis

The first experiment was based on a completely randomized design with three replications and five observations per replication. Also, the second experiment was laid upon a factorial arrangement based on a completely randomized design with three replications and five observations per replication. Data were analyzed by ANOVA using SAS software. Duncan's multiple range test (DMRT) was performed to determine significance at the 1% and 5% probability levels.

RESULTS

First experiment

Based on the analysis of variance, the statistical evaluation revealed that the cultivars significantly differed in internal anthocyanin and solution uptake $(P<0.01)$ and relative fresh weight on the evaluated days (Table 1).

Table 1. The analysis of variance of internal anthocyanin, relative fresh weight, and solution uptake in four cut carnation flowers during postharvest in the first experiment.

According to table 2, the red cultivar had the highest petal anthocyanin content (0.04146) μ g g⁻¹ fresh weight). The pink cultivar had the second-highest petal anthocyanin. In contrast, no anthocyanin was found in the white and yellow cultivars. Similar to the results for petal anthocyanin, the highest solution uptake rate was found in the red cultivar $(29.080 \text{ ml g}^{-1}$ fresh weight) and the pink cultivar. The minimum solution uptake was observed in the white and yellow cultivars, respectively (Table 1).

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using Duncan's multiple range test.

The comparison of means revealed that solution uptake and relative fresh weight reduced over time. The highest solution uptake (43.692 mL) and relative fresh weight (109 g g^{-1} initial fresh weight) were achieved on day 2, and the lowest values on day 8 (11.110 mL and 99.5 g g^{-1} initial fresh weight, respectively) (Figs. 2 and 3).

Fig 2. The comparison of means for the solution uptake of four cultivars of cut carnation flower during postharvest days. Data is expressed as means of three replicates. Different letters indicate significant differences ($P \le 0.05$) by Duncan's multiple range test.

Fig 3. The comparison of means for the relative fresh weight of four cultivars of cut carnation flower during postharvest days. Data is expressed as means of three replicates. Different letters indicate significant differences $(P < 0.05)$ by Duncan's multiple range test.

The analysis of variance for vase life indicates significant differences among the four cultivars in this trait (Table 3). As shown in Fig. 3, the vase life of white and yellow cultivars (14 days) was the longest, twice as long as that of the pink (6 days) and red cultivars (7.33 days) (Fig. 4). These results are in contrast with those of solution uptake and anthocyanin content of petals. The vase life of colored cultivars decreases with the increase in anthocyanin content and solution uptake.

Table 3. Analysis of variance for the postharvest vase life of four cut carnation flowers in the first experiment.

**: Significant differences at $P < 0.01$ based on Duncan's multiple range test.

Fig. 4. The comparison of means for the vase life of the four cultivars of cut carnation flower. Data are expressed as means of the three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test.

Second experiment

According to the analysis of variance, there was no significant interaction between the cultivars and sucrose concentrations, except for vase life and relative fresh weight on day 12 $(P < 0.01)$. Furthermore, the effect of different cultivars on anthocyanin content was significant $(P < 0.01)$ on all estimated days and also was significant on the amount of soluble solids on day 10. Soluble solids on days 4 ($P<0.05$) and 10 ($P<0.01$) and anthocyanin on day 16 ($P<0.01$) were also affected by sucrose levels significantly (Table 4). The comparison of means revealed that the anthocyanin content of the red cultivar was the highest, but that of the white and yellow cultivars was the lowest (Table 5). These results confirm our findings in the first experiment regarding the presence of more anthocyanin in the colored cultivars. Moreover, as shown in Table 2, extending the postharvest period significantly increased the anthocyanin content of the colored cultivars, especially in the red cultivar from day 4 to day 16.

Table 4. The analysis of variance for the effects of two concentrations of sucrose and four carnation cut flowers on internal anthocyanin and total soluble solids on days 4, 10, and 16 in the postharvest period in the second experiment.

		MS						
S.o.V	df	Internal anthocyanin (Day 4)	TSS (Day 4)	Internal anthocyanin (Day 10)	TSS (Day 10)	Internal anthocyanin (Day 16)	TSS (Day 16)	
Cultivars (C)	3	$1.040e^{9**}$	0.0102^{ns}	$2.1292e^{9**}$	$0.0934*$	$1.8734e^{-8**}$	0.1080 ^{ns}	
Sucrose (S)	2	$2.9352e^{-12ns}$	$0.0363*$	$4.9604e^{-12ns}$	$0.2339**$	4.3999e- $10**$	0.0425 ^{ns}	
$C \times S$	6	5.6175 ^{-12ns}	0.0087 ^{ns}	3.9625e ^{-11ns}	0.0294 ^{ns}	1.8186e ^{-10ns}	0.0221 ^{ns}	
Error	24	$2.9034e^{-11}$	0.0105	$4.4118e^{-11}$	0.0292	7.3837e ⁻¹¹	0.0410	
CV(%)		64.91	3.12	11.55	4.88	24.13	599	

*, ** and s : Significant differences at $P < 0.05$, $P < 0.01$ and insignificant differences based on Duncan's multiple range test, respectively.

Table 5. The comparison of means for internal anthocyanin synthesis of four cultivars of cut carnation flower during postharvest.

Cultivar	Internal anthocyanin (μ g g ⁻¹ petal fresh weight)		
	Day 4	Day 10	Day 16
White	0.00013c	0.00006c	0.00155c
Yellow	0.00016c	0.00029c	0.0191c
Pink	0.01011 ^b	0.01540 ^b	0.04073 ^b
Red	0.02281 ^a	0.03246°	0.09824 ^a

In each column, means with similar letter(s) are not significantly different $(P < 0.05)$ based on Duncan's multiple range test.

According to the results of means comparison (Fig. 5), the pink cultivar exhibited the highest amount of soluble solids, although it was not significantly different from the red and yellow cultivars. The lowest amount of this trait was found for the white cultivar. Results specified that increasing the concentrations of sucrose $(2 \text{ and } 4\%)$ was related to an elevated level of soluble solids (Table 6).

Cultivars of carnation cut flower

Fig. 5. The comparison of means for the total soluble solids of the four cultivars of cut carnation flower on day 14. Data are expressed as means of three replicates. Different letters indicate significant differences $(P < 0.05)$ by Duncan's multiple range test.

Sucrose	TSS			Solution uptake	
$(\%)$	Day 4	Day 10	Day 4	Day 6	Day 12
$\overline{0}$	3.23 ^b	3.36 ^b	33.43^b	24.06 ^b	17.71 ^b
2	3.32 ^a	3.51 ^a	34.34 ^b	24.01 ^b	17.76 ^b
4	3.33a	3.64°	36.32 ^a	$28\,71a$	20.67 ^a

Table 6. The comparison of means for the effects of sucrose level on total soluble solids on days 4 and 10 and solution uptake on days 4, 6, and 12 of the four cultivars of cut carnation flower.

In each column, means with similar letter(s) are not significantly different $(P < 0.05)$ based on Duncan's multiple range test.

The application of sucrose had a significant effect on the anthocyanin content of petals on day 16. According to Fig. 5, the anthocyanin content of petals was reduced by increasing the external sucrose, and the minimum amount was obtained in 4% sucrose treatment (Fig. 6).

Fig. 6. The effect of different levels of sucrose on petal anthocyanin content on day 16. Data are expressed as means of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test.

According to the analysis of variance, the interaction between different cultivars and sucrose levels was significant on relative fresh weight only on day 12 ($P<0.01$). Different cultivars affected relative fresh weight significantly on days 6 and 10 ($P<0.05$) and also on days 8, 12, 14, 16, and 18 ($P<0.01$). The application of external sucrose also displayed a significant effect on relative fresh weight on days 6 and 8 ($P<0.01$) and on day 16 ($P<0.05$). As well, sucrose levels significantly affected the solution uptake on days 4 and 6 ($P \le 0.05$) and 12 $(P<0.01)$ (Table 7).

As presented in Fig. 7, the highest relative fresh weight was found in the yellow cultivar in combination with 4% sucrose treatment (115.3 $g g^{-1}$ initial fresh weight), and the lowest was in the white cultivar (90.5 g g^{-1} initial fresh weight) without sucrose and in pink cultivar (88.9 g g^{-1} initial fresh weight) together with 2% sucrose. The comparison of means (Table 8) revealed that the maximum relative fresh weight was associated with the yellow cultivar and the lowest one with the other cultivars on all days.

Table 7. Analysis of variance for the effect of two concentrations of sucrose and four cut carnation flowers on internal relative fresh weight and solution uptake (on days $2, 4, 6, 8$, and 10) during postharvest in the second experiment.

 $\frac{\text{CV } (\%)}{\text{Cov } \text{V}}$ 5.53 37.66 4.11 26.28 4.07 16.60 4.32 22.93 5.99 22.00
 $\frac{1}{\text{V}}$ and ^{ns}: Significant differences at P < 0.05 and P < 0.01 and insignificant differences based on Duncan's multiple range test, respectively.

Table 7. Continued.

*, ** and n_i : Significant differences at $P < 0.05$ and $P < 0.01$ and insignificant differences based on Duncan's multiple range test, respectively.

Fig. 7. The comparison of means for the effect of different cultivars of cut carnation flower and different levels of sucrose on relative fresh weight on day 12. Data are expressed as means of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test.

Table 8. The comparison of means for the effect of different cultivars of cut carnation flower on relative fresh weight during several days of postharvest.

In each column, means with similar letter(s) are not significantly different $(P < 0.05)$ based on Duncan's multiple range test.

Based on data in table 9, the highest rate of solution uptake was exhibited by the yellow and pink cultivars on day 12 and the pink and red cultivars on days 16.

Table 9. The comparison of means for the effect of different cultivars of cut carnation flower on solution uptake during several days of postharvest.

Cultivars		Solution uptake (mL)	
	Day 12	Day 16	Day 18
White	16.88c	14.89c	13.28 ^b
Yellow	21.75^a	15.46°	14.03 ^b
Pink	20.73^{ab}	18.18^{b}	18.26^a
Red	18.57 ^{bc}	20.16 ^a	17.90°

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) based on Duncan's multiple range test.

According to the analysis of variance, the effect of different cultivars and sucrose levels and their interactions were significant on vase life. The comparison of means for the effect of cultivars showed that the vellow cultivar achieved the highest vase life (16.3 days), whereas the pink (15.8 days) and white (15.7 days) cultivars did not show a significant difference $(Fig. 7)$. The minimum longevity was found in the red cultivar (14.8 days) . On the other hand, the application of external sucrose extended the vase life, whose highest value was observed in the 4% sucrose level (16.5 days). The results of the interaction showed that the longest vase life was related to the pink cultivar treated with 4% sucrose (18 days). In contrast, the lowest vase life was related to the white and red cultivars treated with no sucrose (13.6 days) (Fig. 8). Based on the results, the application of external sucrose had a positive effect on the vase life of the different cultivars.

Different cultivars of carnation cut flower

Fig. 8. The effect of different cultivars of cut carnation flower and different sucrose levels on vase life. Data are expressed as means of three replicates. Different letters indicate significant differences $(P < 0.05)$ by Duncan's multiple range test.

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DISCUSSION

Generally, major causes of cut flower loss include vascular occlusion, the decline in carbohydrates and water uptake, and ethylene production, which induce the emergence of senescence symptoms, such as leaf and petal wilting, neck bending, and tissue dryness (Put 1990; de Witte and van Doorn, 1988). Therefore, cutting tools, flower containers, and preservative solutions should be bacteria-free (Armitage *et al.*, 2003). Anthocyanins in petals display many physical changes in the postharvest period. Some reports suggest that decreasing anthocyanins in cut rose petals directly correlates with reduced vase life and marketability (Hatami *et al.*, 2012). Similarly, the color of lisianthus flowers decreased after separation from the mother plant (Griesbach, 1992). Moreover, color reduction impaired the quality of peony (P. suffruticosa 'Luoyang Hong') (Zhang et al., 2014). Similarly, Schmitzer et al. (2009) revealed noticeable changes in flower color during senescence in red rose 'KORcrisett'.

The present study found that the amount of anthocyanin in the petals of colored cultivars increased at the postharvest stage. This observation is not consistent with the findings of previous researchers mentioned above. This research also showed that the internal anthocyanin in the petals had a negative effect on the vase life of cut carnation flowers. Definitely, the cultivars with out anthocyanin (white and yellow cultivars) exhibited longer vase lives than those with anthocyanin (red and pink cultivars).

Anthocyanins are very unstable compounds. So, they are highly susceptible to degradation at high temperatures and pH changes. The stability of anthocyanin is influenced by the presence of enzymes, light, oxygen, ascorbic acid, sugars, sulfur dioxide, metal ions, co-
pigments, and other compounds, such as flavonoids and minerals (Francis *et al.*, 1989).

Based on the results of the second experiment, the highest level of external sucrose $(4%)$ increased the relative fresh weight, soluble solids, and vase life in all cultivars, particularly in the yellow and pink cultivars. Nevertheless, anthocyanin content was decreased. The longest vase life was observed in two colorless cultivars.

The petal's color changes through anthocyanin formation from the budding to the senescence stage of cut flowers could be attributed to such factors as changes in the content of phenolic compounds, increase in pH of cell sap, light, temperature, plant growth regulators, and sugars (Sood and Nagar, 2003; Sood et al., 2006). Glucose, the most important source of energy production and regulator of osmotic potential, plays an effective role in plant growth and development (Smeekens, 2000). It was shown that the germination and growth of *Arabidopsis* increased in the medium containing a high sugar level (Solfanelli *et al.*, 2006). Studies have revealed that sugar-derived signals act in the same way as major plant hormones (Das *et al.*, 2012). Furthermore, sugar acts as an internal stimulus and increases the gene expression of anthocyanin synthesis in *Arabidopsis*, petunia, grape, and radish (Smeekens, 2000; Solfanelli *et al.*, 2006; Zheng *et al.*, 2009).

According to previous studies, some sugar solutions improve the vase life of cut flowers (Han, 2003; Ichimura and Korenaga, 1998). Sucrose increases water uptake by increasing osmotic concentration in flowers and leaves. Subsequently, it maintains water balance in stems and flowers (Ichimura et al., 2000). Verlinden and Garcia (2004) showed that sucrose reduced ethylene production, decreased senescence, and extended the vase life of cut carnation flowers, which confirms our results. Using a vase solution containing sucrose is very effective for flower blooming compared to pure water in flowers like roses, carnations, chrysanthemums, and gladiolus (Nair et al., 2003; Chamani et al., 2012; Solgi et al., 2009; Put, 1990). Likewise,

the presence of sucrose induces stomatal closure and, subsequently, decreases water loss in cut flowers. Sucrose improves water balance, and the treatments containing sucrose have more fresh weight (Jen *et al.*, 2000). These findings are consistent with the results of the present study.

The increasing respiratory substrates due to the presence of sugar compounds in preservative solutions enhance the quality and increase vase life (Knee, 2000). The effect of sucrose in delaying the senescence process is due to the delay in the degradation of proteins, ribonucleic acids, preservation of cell membranes, and mitochondria. Fundamentally, sucrose provides the energy required for the development and expansion of flower buds and contributes to their longevity (Mayak and Dilley, 1976).

CONCLUSION

This study showed that the internal anthocyanin of petals influenced the vase life of cut carnation flowers with different colors. The highest amount of petal's internal anthocyanin and the shortest vase life were found in the red and pink cultivars. In contrast, the white and yellow cultivars exhibited the lowest internal anthocyanin and the maximum vase life. In addition, the application of external sucrose (especially at higher concentrations) improved the soluble solids, relative fresh weight, and vase life of the colored cultivars (more anthocyanin) compared to the colorless cultivars (less anthocyanin). This study demonstrated the different effects of internal anthocyanin of petals and the application of external sucrose on the postharvest traits of four cultivars of cut carnation flowers.

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