

NUTRACEUTICAL VALUE OF EDIBLE FLOWERS UPON COLD STORAGE

M. LANDI^a, B. RUFFONI^b, L. COMBOURNAC^c and L. GUIDI^{a,d}

^aDepartment of Agriculture, Food and Environment (DAFE), University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

^bCREA Research Centre for Vegetable and Ornamental Crops, Corso Inglesi 508, 18038 Sanremo, Italy

^cCREAT-Chambre d'agriculture des Alpes-Maritimes, MIN fleurs 17 - box 85, Nice Cedex, France

^dInterdepartmental Research Center "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

*Corresponding author: Tel. +39 0502216620

E-mail address: marco.landi@for.unipi.it

ABSTRACT

The attraction and quality of edible flowers correlates with their high perishability. Few studies have evaluated whether edible flowers decay faster than they lose their nutraceutical value. In this experiment, ascorbic acid was negatively affected by cold storage in all the edible flowers investigated, whereas phenolic, flavonoid, and anthocyanin content were affected only in some cases. No decrease in total antioxidant activity was detected in any of the edible flowers at the end of their shelf life. Our dataset highlights that (i) the selection of edible flowers with low moisture content is key in ensuring a longer shelf life, and (ii) more effort should focus on preventing water loss in edible flowers.

Keywords: ascorbic acid, anthocyanins, brightness, edible flowers, nutraceutical value, phenolics

1. INTRODUCTION

The demand for more attractive and high quality foodstuffs is increasing in the West. The appeal of food dishes can be enhanced by edible flowers, which is why they are becoming more and more popular (AQUINO-BOLAÑOS *et al.*, 2013).

Edible flowers are mainly purchased by consumers for use in dishes as a garnish or ingredient although more often they are referred to in the literature in terms of their biologically active compounds. Some papers have extensively investigated the chemical composition of many edible flowers (LI *et al.*, 2007; GARZON *et al.*, 2009; KAISOON *et al.*, 2011; GARZON *et al.*, 2015; LOIZZO *et al.*, 2015), highlighting that they are a substantial source of chemical compounds with a high antioxidant activity (FU and MAO, 2008; GARZON *et al.*, 2015).

Phenolic acids, flavonoids, including anthocyanins, have been recognized as the most representative biologically-active compounds found in the petals of fresh edible flowers (MLCEK and ROP, 2011; NAVARRO-GONZALEZ *et al.*, 2015). Unlike freshly-marketed edible flowers, in which the profile of bioactive compounds has been extensively investigated (for a review see MLCEK and ROP, 2011), only a few papers have evaluated the stability of edible flower phytochemicals during storage (DAS *et al.*, 2010; KAZAZ *et al.*, 2010; AQUINO-BOLAÑOS *et al.*, 2013; LANDI *et al.*, 2015a).

Results are sometimes conflicting. In some cases cold storage has been found to have a negative impact on the nutraceutical value of edible flowers (DAS *et al.*, 2010; AQUINO-BOLAÑOS *et al.*, 2013), but not in other cases (FRIEDMAN *et al.*, 2007).

We investigated the effect of cold storage on various biologically-active compounds namely phenolics, flavonoids, anthocyanins and ascorbic acid in seven edible flowers belonging to five species (*Acmella oleracea* L., *Begonia semperflorens* L. with white, pink, and dark-pink, *Salvia discolor* Kunth, *Tulbaghia cominsii* Vosa, *Tropaeolum majus* L.) with different sizes, shapes and colors (the features of each edible flower are summarized in Table 1). Given that consumers are influenced by the visual appeal of edible flowers, and only high-quality produce encourages repeat purchases, we attempted to establish whether the loss of the visual appeal of edible flowers proceeds faster than the loss of their nutritional value. The overall aim was to address future research aimed at extending the shelf life of edible flowers.

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol (LC/MS grade; > 99.95 % solvent purity), and HCl (ACS reagent, 37%) were purchased from CARLO ERBA Reagents S.r.l., Milan, Italy. All the other reagents were purchased from Sigma-Aldrich S.r.l., Milan, Italy.

2.2. Flower harvest and processing

Flowers of *A. oleracea* (AO), *B. semperflorens* (with white; BS_w, pink; BS_r, and dark-pink petals; BS_{dp}), *S. discolor* (SD), *T. cominsii* (TC), *T. majus* (TM) were kindly provided by CREAT-Chambre d'agriculture des Alpes-Maritimes (Nice, France) (Table 1).

Table 1. Features of the selected edible flowers studied in this work.

| Species | Family | Abbreviation | Color | Flower size |
|---------------------------------|----------------|------------------|------------|---------------|
| <i>Acmella oleracea</i> L. | Asteraceae | AO | yellow | small |
| <i>Begonia semperflorens</i> L. | Begoniaceae | BS _{DP} | dark pink | medium |
| <i>Begonia semperflorens</i> L. | Begoniaceae | BS _P | pink | medium |
| <i>Begonia semperflorens</i> L. | Begoniaceae | BS _W | white | medium |
| <i>Salvia discolor</i> Kunth | Lamiaceae | SD | violet | small/ medium |
| <i>Tulbaghia cominsii</i> Vosa | Amaryllidaceae | TC | light pink | small |
| <i>Tropaeolum majus</i> L. | Tropaeolaceae | TM | orange | big |

Fresh flowers at maturity stage (June, 2014) were harvested early in the morning, transported in refrigerated containers (4 °C) and processed within a few hours in an aseptic laboratory in accordance with KELLEY *et al.* (2003). For each species, some flowers (about 2 g) were finely ground with liquid nitrogen and stored at -80 °C until analysis. These samples represented the first day of storage (t₀). The flowers were then randomized in air-tight hinged boxes (500 cm³, Comital Cofresco, Italy) made from polyethylene terephthalate and stored at 4 °C under light to simulate commercial shelf conditions. Each box contained about 20 g of fresh flowers. Flowers were inspected to ensure there was no visually detectable damage prior to being placed in each container. Samples were collected following the same procedure after 2, 5 and 8 d of storage for biochemical analysis. For the 8-day storage, samples were collected only for edible flowers that would still have been marketable at that time. Before being ground, some of the sample flowers were used for color determination.

2.3. Determination of moisture content

Initial (t₀) fresh weight (FW) was evaluated immediately after the preparation of the flower containers. To determine flower dry weight (DW), florets were desiccated in a ventilated oven at 80 °C until constant weight. At each sampling data (2, 5, 8 d), moisture content was evaluated as the difference between FW and DW of the flowers contained in each box, and expressed as percentage moisture content.

2.4. Color determination

For each species, color measurements were performed on five randomly selected flowers ($n=5$) at different storage times (0, 2, 5, 8 d). The value of each replicate was the mean of three independent spot measurements on each flower's surface evaluated by standard CIE $L^*a^*b^*$ color space coordinates determined by an Ocean Optic HR2000-UV-VIS-NIR spectrometer coupled with a tungsten halogen DH2000 light source (Ocean Optics, USA) as reported in LANDI *et al.* (2015b). Among all colorimetric parameters, L^* represents the lightness of colors (lightness index scale; 0 for black to 100 for white) and is a good parameter for monitoring the development of tissue darkening.

2.5. Postharvest visual quality rating

The visual appeal of flowers was scored on a 9-point scale based on visual observation of the degree of decay, as described by AQUINO-BOLAÑOS *et al.* (2013). For the sake of

simplicity, score points were grouped as follows: 9 to 7 = fresh appearance (flower with no defects or the slight beginning of decay; classified with a green triangle in Fig. 1), 6 to 4 = limit of marketability (moderately deteriorating flower with between a quarter or and half the surface area decayed; yellow triangle in Fig. 1), 3 = not suitable for sale (water-soaked, dark and wilted flower with more than half the surface area decayed; red triangle in Fig. 1). The visual quality was assessed by a panel of five people with expertise in browning phenomena and post-harvest loss of quality.

2.6. Total phenolics, flavonoids, and anthocyanins

Extraction of total phenolics and total flavonoids was based on a slight modification of the method reported by DU *et al.* (2009). An aliquot of 100 mg of flower sample was homogenized in 1 mL of ethanol:acetone (7:3, v/v) and shaken overnight at 4 °C. The extract was centrifuged at 1,000 g for 15 min at 4 °C and the supernatant was filtered using Minisart filters (pore size 0.45 µm). The filtrate was collected and stored at -20 °C until analysis. Total phenolic content was determined using the Folin-Ciocalteu assay, according to DEWANTO *et al.* (2002), using 10 µL of extract. The absorbance was read at 760 nm and the total phenolic concentration was expressed as gallic acid equivalents (mg GAE g⁻¹ DW) using a calibration curve (50-600 µg mL⁻¹).

Content of total flavonoids was determined according to DU *et al.* (2009) with a few modifications. In a 2 mL Eppendorf tube, 100 µL of flower extract were added in 1 mL ethanol 30% (v/v), 45 µL of 50 mM NaNO₂, 45 µL of AlCl₃ x 6H₂O 0.3 M. After 5 min at room temperature, 300 µL of 1 M NaOH were added and the mixture absorbance was measured at 506 nm. The content of total flavonoids was expressed as rutin equivalents (mg RE 100 g⁻¹ DW) using a calibration curve as a standard (6.25-1000 µg mL⁻¹).

Total anthocyanins were extracted as reported by LANDI *et al.* (2014). Briefly, 100 mg of ground samples were mixed with 1 mL of acidified methanol (1.5% HCl v/v) and shaken overnight at room temperature. The supernatant was filtered using Minisart filters (pore size 0.45 µm); anthocyanin-containing flower extract (50 µL) was added to 950 µL of acidified methanol (1.5% HCl v/v) and the absorbance was read from 408 to 560 nm against a blank. Total anthocyanin content was expressed as the mean value of ABS in the range 408-560 nm per 100 mg⁻¹ DW. An Ultrospec 2100 Pro spectrophotometer (GE Healthcare Ltd, Little Chalfont, England) was used for the analyses of total phenolics, flavonoids and anthocyanins, together with all the other spectrophotometric determinations.

2.7. Ascorbic acid determination

Total ascorbate (ASA_{tot}), reduced ascorbate (ASA), and dehydroascorbate (DHA) were spectrophotometrically determined as described by KAMPFENKEL *et al.* (1995). The assay is based on the reduction of Fe³⁺ to Fe²⁺ by ASA and the spectrophotometric detection of Fe²⁺ complexed with 2,2'-dipyridyl. DHA is calculated as the difference between ASA_{tot} and ASA, and data were expressed as µg g⁻¹ DW.

2.8. DPPH scavenging activity

The antioxidant activity of each sample was determined using a modified version of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay, as described by KIM *et al.* (2005). The methanolic flower extract (20 µL) was diluted to 100 µL with 80% aqueous methanol. It was then added to 0.4 mL of 0.1 M Tris-HCl buffer and 0.5 mL of 0.3 mM DPPH in methanol. The solution was mixed thoroughly and incubated in the dark for

20 min at room temperature. The absorbance of the sample mixture ($A_{\text{sample}} = A_{\text{sample } t20'} - A_{\text{sample } t0}$) was monitored at 517 nm. The absorbance of a control sample ($A_{\text{control}} = A_{\text{control } t20'} - A_{\text{control } t0}$) containing only methanol/Tris-HCl, and DPPH versus a methanol/Tris-HCl blank was also analyzed. The percentage DPPH free radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH free radical scavenging} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$$

The antioxidant activity was determined by comparing the percentage DPPH free radical scavenging of each sample to a calibration curve prepared with Trolox. Antioxidant activity was expressed as Trolox equivalents (TE; mmol of TE g⁻¹ FW) for direct comparison of the free radical scavenging capabilities between all the samples.

2.9. Statistical analysis

Visual quality was assessed by each expert in five randomly selected flowers per species at each sampling time. Reported data for flower moisture and phytochemical contents are the means (\pm SD) of five independent replicates ($n=5$), where each box was considered as a replicate. Means were compared by one-way ANOVA, following Bartlett's test to assess the homogeneity of variance among samples, considering storage as the variability factor. Percentage values were arcsine transformed prior to the analyses. Means with different letters within species are significantly different after Fisher's least-significant difference test (LSD) for $P=0.05$. For some comparisons among species (when discussed), one-way ANOVA was applied with species as the variability factor. All statistical analyses were performed using CoStat (CoHort™ Software, Berkeley, CA).

3. RESULTS AND DISCUSSIONS

3.1. Physiological weight loss and visual quality

Values of L^* are indicative of tissue darkening as browning is commonly associated with the oxidation of phenolics and their polymerization into dark brown pigments (MARTINDIANA *et al.*, 2015; LANDI *et al.*, 2015c; REMORINI *et al.*, 2015). As expected, in this experiment L^* values decreased in all the EFs under investigation, at least at the end of the storage period (8d, Fig. 1). Flowers of *A. oleracea* and *T. cominsii* were considered to be still marketable after 8 d of storage at 4 °C, whereas all the other species were classified as not suitable for sale at this storage time (Fig. 1).

Differences in the shelf life of edible flowers subjected to cold storage have already been reported (KURO *et al.*, 2012; KELLEY *et al.*, 2003). Unlike reported by KELLEY *et al.* (2003), five out of the seven edible flowers that we evaluated (including nasturtium, which was one of the edible flowers considered by KELLEY *et al.*) had a shorter shelf life at 5 °C (5 d). These differences can be attributable to the different packaging: KELLEY *et al.* (2003) used polyethylene bags, whereas we used polyethylene terephthalate boxes in order to preserve the delicate flowers. Despite the evident differences in terms of shelf life, the main determinants that contribute are less clear from the literature.

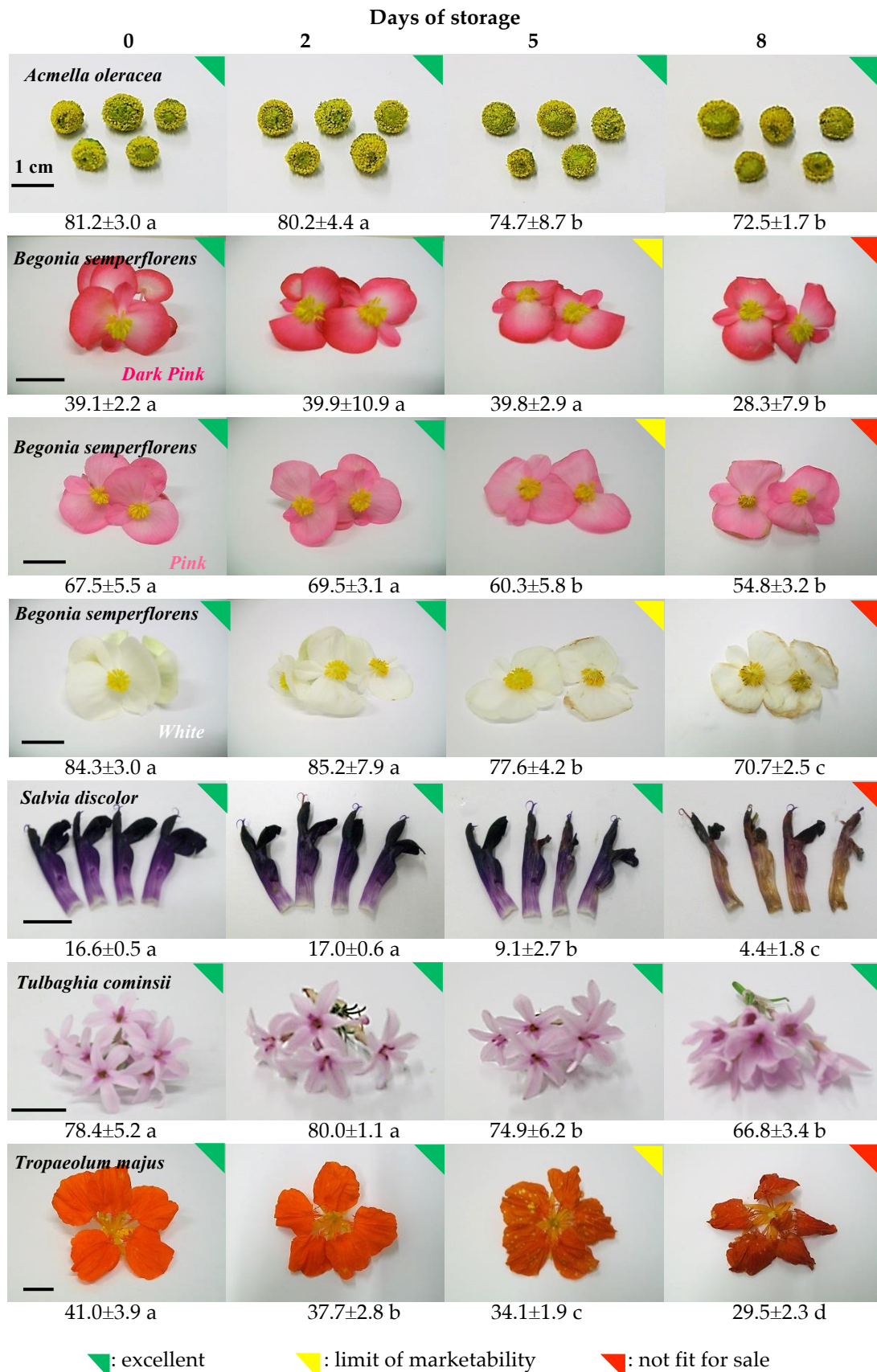


Figure 1. Visual appearance, marketability and lightness (L^*) of edible flowers. Reported results for L^* values are the mean of five replicates ($n=5;\pm SD$). Means flanked by different letters are significantly different within each flower species upon storage for $P = 0.05$ after one-way ANOVA followed by LSD test. Colored triangles at the top right of each figure represent excellent product (green), limit of marketability (yellow), or unsaleable product (red). Bars = 1 cm.

Our data suggest that the constitutive moisture content of edible flowers has the most impact on shelf life. At t0, flowers of *B. semperflorens* with dark-pink, pink and white petals and *T. majus* had a significantly higher ($p < 0.001$) moisture % (which averaged 97.34% in *B. semperflorens* with dark-pink, pink and white petals and *T. majus*) than *A. oleracea* and *T. cominsiii* (87.82 and 88.19%, respectively), and had a 5-d versus a 8-d shelf life for *A. oleracea* and *T. cominsiii* (Table 2 and Fig. 1). The hypothesis of reduced dehydration related to longer flower shelf life is also in agreement with KOU *et al.* (2012).

This hypothesis does not seem applicable to *S. discolor* flowers which had a simultaneously lower moisture content than *B. semperflorens* with dark-pink, pink and white petals and *T. majus*, but only 5 d of marketability. In this species, the reduced marketability seems mainly related to the loss of anthocyanin content (Table 3) as *S. discolor* was the only edible flower in which anthocyanin decreased during storage.

Although not measured in our experiments, the high perishability of edible flowers might be connected to their respiration rate and the production of ethylene, as is the case with other horticultural commodities (KADER and SALTVEIT, 2003). However, KOU *et al.* (2012) demonstrated that after 7 d of storage the decay index of carnation increased to a similar extent and irrespectively of the use of modified atmosphere packaging and/or 1-methylcyclopropene, which is a commonly used ethylene inhibitor. FRIEDMAN *et al.* (2007) also found that *T. majus* flower quality was not related to CO₂ or to ethylene levels inside the packaging in a short-term storage period. VILLALTA *et al.* (2004) found that the respiratory rate of yellow summer blossom remained relative constant and low during the 8-d storage period at 5 °C.

Table 2. Moisture content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_w, pink; BS_p, and dark-pink petals; BS_d), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropaeolum majus* L. (TM) during storage.

| Species | Storage (d) | | | |
|------------------|----------------------|--------------|--------------|--------------|
| | 0 | 2 | 5 | 8 |
| | Moisture content (%) | | | |
| AO | 87.82±1.74 a | 87.08±2.03 a | 79.15±5.39 b | 61.44±3.41 c |
| BS _{DP} | 97.33±0.39 a | 83.63±1.95 b | 69.15±3.22 c | 47.23±4.66 d |
| BS _P | 97.41±0.03 a | 92.89±1.08 b | 82.87±1.80 c | 61.67±3.18 d |
| BS _W | 97.38±0.35 a | 89.21±1.26 b | 74.13±3.95 c | 47.85±0.12 d |
| SD | 83.09±0.57 a | 80.11±1.48 b | 73.41±2.60 c | 57.25±1.65 d |
| TC | 88.19±0.08 a | 86.43±1.55 a | 83.22±3.40 b | 77.56±3.67 c |
| TM | 97.27±0.54 a | 91.66±4.10 b | 80.31±6.45 c | 45.03±7.24 b |

Data represent the mean±SD ($n=5$). Means flanked by different letters are significantly different within each flower species upon storage for $P = 0.05$ after one-way ANOVA followed by LSD test.

This evidence weakens the hypothesis that the flower's respiration rate is the key factor in extending an edible flower's shelf life, at least for short-term storage. On the other hand, controlling the respiration rate seems to be more important for longer storage periods (at least two weeks; KUO *et al.*, 2012).

3.2. Bioactive compounds and antioxidant activity

Phenols (including phenolic acid, flavonoids and anthocyanins) are currently the target of numerous studies since their intake has been associated with the decreased risk of cancer, cardiovascular diseases and neurodegenerative disorders (SALEM *et al.*, 2011). In our

study, constitutive levels of total phenolics ranged from 10.02 mg GAE g⁻¹ DW in *A. oleracea* to 194.33 in *T. majus* (Table 3).

Table 3. Phytochemical content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_w, pink; BS_p, and dark-pink petals; BS_{dp}), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropeolum majus* L. (TM) during storage.

| Species | Storage (d) | Total phenols (mg GAE g ⁻¹ DW) | Total flavonoids (mg RE g ⁻¹ DW) | Total anthocyanins (ABS _b 100 mg ⁻¹ DW) | DPPH (mmol TE g ⁻¹ DW) |
|------------------|-------------|----------------------------------------------|------------------------------------------------|------------------------------------------------------------------|--------------------------------------|
| AO | 0 | 10.02±2.93 c | 4.85±1.05 a | ND | 26.25±2.58 b |
| | 2 | 15.00±0.35 a | 6.17±1.39 a | ND | 32.04±1.39 a |
| | 5 | 12.62±1.59 b | 4.94±1.06 a | ND | 30.77±4.16 a |
| | 8 | 11.83±0.18 bc | 4.66±0.09 a | ND | 30.73±1.27 a |
| BS _{DP} | 0 | 64.21±1.56 b | 37.81±3.63 a | 2.20±0.37 a | 96.21±8.80 a |
| | 2 | 77.78±4.70 a | 32.77±2.23 b | 1.92±0.64 a | 90.42±12.03 a |
| | 5 | 63.85±3.86 b | 29.11±0.43 c | 1.94±0.47 a | 89.37±8.14 a |
| | 8 | - | - | - | - |
| BS _p | 0 | 51.72±4.30 c | 41.79±13.87 a | 0.84±0.16 a | 74.71±4.87 c |
| | 2 | 94.21±8.80 a | 36.50±3.10 ab | 0.76±0.21 a | 94.53±0.52 a |
| | 5 | 84.54±11.3 b | 27.43±0.97 b | 0.65±0.15 a | 83.80±5.81 b |
| | 8 | - | - | - | - |
| BS _w | 0 | 77.77±9.50 b | 32.01±6.50 a | ND | 67.94±17.34 b |
| | 2 | 95.27±1.02 a | 36.09±1.53 a | ND | 93.10±8.47 a |
| | 5 | 69.29±6.01 b | 28.37±4.60 a | ND | 63.40±9.25 b |
| | 8 | - | - | ND | - |
| SD | 0 | 26.76±0.92 a | 11.35±1.59 a | 0.27±0.03 a | 32.62±0.41 a |
| | 2 | 19.29±1.53 b | 9.60±1.45 a | 0.23±0.03 ab | 31.37±3.47 a |
| | 5 | 19.20±3.05 b | 9.76±1.22 a | 0.19±0.06 b | 29.17±0.15 a |
| | 8 | - | - | - | - |
| TC | 0 | 30.51±2.16 a | 3.02±0.24 a | 0.09±0.03 a | 44.85±0.36 a |
| | 2 | 28.55±0.47 b | 2.74±1.01 a | 0.10±0.04 a | 47.59±1.53 a |
| | 5 | 28.13±1.28 b | 3.11±0.58 a | 0.13±0.01 a | 46.79±2.10 a |
| | 8 | 25.1±1.02 c | 3.51±0.78 a | 0.13±0.01 a | 47.40±0.78 a |
| TM | 0 | 194.33±16.50 a | 28.34±3.70 a | 10.10±3.34 a | 142.13±22.16 c |
| | 2 | 140.51±9.98 c | 28.91±0.50 a | 9.81±1.70 a | 156.33±4.00 b |
| | 5 | 163.50±2.33 b | 31.55±1.41 a | 11.22±1.27 a | 181.02±1.40 a |
| | 8 | - | - | - | - |

Data represent the mean±SD (*n*=5). Means flanked by different letters are significantly different within each flower species upon storage for *P* = 0.05 after one way ANOVA followed by LSD test. ABS, absorbance; DPPH, total antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl radical; d, days; DW, dry weight; GAE, gallic acid equivalents; ND, not detectable; RE, rutin equivalent; TE, Trolox equivalents. *b*, Mean value of absorbance in the range 408-560 nm.

These values are common in flower species, as also testified by other studies (ROP *et al.*, 2012; LI *et al.*, 2014).

In many cases, the total phenol values in all the edible flowers studied here were abundantly higher than those reported for other vegetables and fruits, which are usually considered as good sources of phenols (KÄHKÖNEN *et al.*, 1999). Edible flower species could thus represent an interesting source of phenolic compounds, despite the small amount usually consumed compared to other fruits and vegetables. Interestingly, the levels of total phenols found in *T. majus* flowers are two and a half times higher than those reported for blueberry genotypes, which are usually classified as some of the richest sources of phenols, in particular due to their high level of anthocyanins (CASTREJÓN *et al.* 2008).

Overall, we found that 8 d of cold storage affected the concentration of all the phenolic bioactive compounds evaluated here only in some cases (Table 3). A reduction in total phenols was recorded only in *S. discolor*, *T. cominsii*, and *T. majus* flowers. Total flavonoids decreased only in pigmented *B. semperflorens*, whereas the loss of anthocyanins was observed only in *S. discolor*. Results regarding the effect of storage on phenolic compounds are scarce and conflicting. For example, AQUINO-BOLAÑOS *et al.* (2013) found a reduction in total phenols in yellow summer squash flowers during postharvest storage. FRIEDMAN *et al.* (2007) found no difference in anthocyanin content in *B. semperflorens* flowers and LANDI *et al.* (2015a) found no significant changes in total phenols in sage flowers during storage. We are not aware of any other work focused on the variation in phenolic content and profile in edible flowers upon storage.

Ascorbic acid is a well-known key antioxidant in plants and is an essential vitamin for humans. Unlike the polyphenols mentioned above, the concentration of ASA_{tot} decreased significantly during storage (at least at the end of the marketability stage) in almost all the edible flowers under investigation (*A. oleracea*, *B. semperflorens* with dark-pink and white petals, *T. cominsii*, and *T. majus*) (Table 4). Notably, in *B. semperflorens* with pink petals and *S. discolor*, whose level of ASA_{tot} was unchanged, we found an increased level of the oxidized form of ascorbate (decremented ratio ASA/ASA_{tot}). Thus, storage may have negatively affected the level of ASA (which is the biological active form of ascorbic acid) in all the edible flowers under investigation. A reduction in ascorbate levels in edible flowers has also been reported by DAS *et al.* (2010) and by AQUINO-BOLAÑOS *et al.* (2013) under cold storage. AQUINO-BOLAÑOS *et al.* (2013) attributed the loss of ascorbate to the loss of cell integrity and compartmentalization, which expose ascorbic acid to oxygen, which, in turn, decreases the reducing power of this key antioxidant.

The biological activities of phenolic compounds and ascorbate seem to be related to their strong antioxidant capacity *in vitro*, as also reported for many edible flower-derived compounds (KAISOON *et al.*, 2011; SALEM *et al.*, 2011; LI *et al.*, 2014; GARZON *et al.*, 2015; LOIZZO *et al.*, 2015). Interestingly, the total antioxidant activity of some edible flowers, including some of the flowers tested in our investigation (i.e., *S. discolor* and *T. cominsii*), is even higher than that of many blueberries varieties (*V. corymbosum* L.), which is one of the richest reported antioxidants (GIOVANNELLI and BURATTI, 2009).

The total antioxidant activity of edible flower extracts did not decrease in any of the edible flowers upon storage (Table 3). Conversely, in others (i.e., *A. oleracea*, *B. semperflorens* with pink petals, and *T. majus*) the total antioxidant activity was also found to increase during the storage. Our findings are in agreement with FRIEDMAN *et al.* (2007), who reported that 7-8 d of cold storage (2-5 °C) did not reduce the antioxidant activity of *B. semperflorens* flowers.

The stability of the antioxidant capacity of edible flowers seems principally related to the relative stability of the total phenol content given that many researchers have found a strong linear relation between total phenol content and the antioxidant activity of edible flowers ($R^2 > 0.93$) (LI *et al.*, 2014; NAVARRO-GONZALEZ *et al.*, 2015).

Table 4. Ascorbic acid content of edible flowers of *Acmella oleracea* L. (AO), *Begonia semperflorens* L. (with white; BS_w, pink; BS_p, and dark-pink petals; BS_{dp}), *Salvia discolor* Kunth (SD), *Tulbaghia cominsii* Vosa (TC), *Tropaeolum majus* L. (TM) during storage.

| Species | Storage (d) | | | |
|------------------|-------------------------------------------------|---------------|--------------|-------------|
| | 0 | 2 | 5 | 8 |
| | ASA_{TOT} (mg g⁻¹ DW) | | | |
| AO | 2.51±0.12 b | 3.05±0.12 a | 2.40±0.53 b | 1.04±0.09 c |
| BS _{DP} | 16.87±3.63 a | 16.06±3.12 a | 11.53±2.14 b | - |
| BS _P | 16.90±3.63 a | 17.52±2.06 a | 16.38±1.93 a | - |
| BS _W | 5.13±0.59 a | 5.76±0.51 a | 2.31±0.46 b | - |
| SD | 8.13±2.00 a | 8.18±2.66 a | 6.75±1.45 a | - |
| TC | 12.84±0.12 a | 8.07±1.29 b | 7.43±2.67 c | 8.80±1.12 b |
| TM | 89.25±15.88 a | 68.75±14.10 b | 47.01±1.33 c | - |
| | ASA/ASA_{TOT} | | | |
| AO | 0.45±0.01 a | 0.41±0.05 a | 0.47±0.04 a | 0.50±0.03 a |
| BS _{DP} | 0.74±0.01 a | 0.57±0.01 b | 0.77±0.06 a | - |
| BS _P | 0.46±0.02 a | 0.39±0.04 a | 0.39±0.02 b | - |
| BS _W | 0.53±0.08 a | 0.46±0.15 a | 0.58±0.13 a | - |
| SD | 0.73±0.10 a | 0.54±0.12 b | 0.58±0.13 b | - |
| TC | 0.86±0.03 a | 0.89±0.01 a | 0.87±0.01 a | 0.75±0.12 b |
| TM | 0.62±0.10 a | 0.58±0.18 a | 0.64±0.02 a | - |

Data represent the mean±SD ($n=5$). Means flanked by different letters are significantly different within each flower species upon storage for $P = 0.05$ after one-way ANOVA followed by LSD test. ASA, ascorbic acid (reduced form); ASA_{tot}, total ascorbate (sum of oxidized and reduced form); DW, dry weight.

Our data also highlight a good correlation between total phenol content and total antioxidant activity, although we found a lower R^2 than that mentioned above ($R^2 = 0.655$). This determination coefficient is however in agreement with that reported by LI *et al.* (2009) ($R^2 = 0.664$) and TAI *et al.* (2011) ($R^2 = 0.652$) in flowers of peony and *Sophora viciifolia*, respectively. It suggests that, despite the main role of phenolics as antioxidants, other antioxidant compounds contribute significantly to the flower antioxidant activity. This would also justify the reduction in total phenolic content found in *S. discolor*, *T. cominsii*, and *T. majus* associated with unchanged (though increased in *T. majus*) levels of their total antioxidant activity. In our experiments, the contribution of ascorbic acid to the total antioxidant activity seems less significant than total phenolics, given that ascorbic acid levels were negatively affected by the storage, while the total antioxidant activity was not.

4. CONCLUSIONS

Our data suggest that the loss of visual appeal of most edible flowers proceeds faster than the loss of their bioactive compound content. Only ascorbic acid was found to be highly susceptible to the storage process. The content of its reduced form (ASA) decreased upon storage in all the edible flowers under examination before the end of their shelf life. On the other hand, phenolic moieties were less affected by the storage. The total antioxidant activity of all the edible flowers evaluated here was stable under cold storage up to the end of their shelf life. This suggests that edible flower decay is directly related to their constitutive water content, thus (i) the selection of edible flowers with low moisture is a key factor in ensuring their longer marketability; (ii) more efforts should focus on the processes and technologies aimed at preserving (or delaying) edible flowers from water

loss, such as the use of boxes or bags made from appropriate plastic material and/or appropriately modified atmospheres, in order to extend the shelf life of edible flowers.

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