

Different growing conditions can modulate metabolites content during post-harvest of *Viola cornuta* L. edible flowers

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Abstract: Edible flowers are inflorescences traditionally used in various part of the world to enrich sweet and savoury recipes. The flowers of *Viola* spp. were appreciated since the Romans, and today the fresh products are now incorporated as ingredients in different culinary preparations. In this work, cultivation of potted *Viola cornuta* L. cv. Penny Lane was performed in greenhouse with different environmental conditions (basal heating, additional LED lighting and moisture management) and therefore the biomass production (number of flowers per square meter and plant dimension per pot) was assessed. The plants are characterised by flowers with dark purple and orange petals in the same corolla. The shelf-life of detached flowers was studied in post-harvest conditions at 0 and 4 days of cold storage at 4°C (polyethylene boxes, 12/12 h light/dark condition) to simulate the condition of I gamma products. Sugars and secondary metabolites were analysed. Basal heating seems not to increase flower number but could contribute to reach a well-balanced simultaneous presence of different antioxidant molecules (polyphenols, anthocyanins, carotenoids). Our data highlight that the short cold storage under light condition lead to an increase in the content of total polyphenols and antioxidant activity, although a general reduction in pigments and sugars is observed.

1. Introduction

Edible flowers are currently part of a niche market and perceived as a culinary novelty, even if their consumption is known for thousands of years. In fact, there are several historical evidences that highlight the use of the inflorescences to prepare and garnish dishes, from some ancient

civilisation as Greeks and Romans, to more recent times, e.g. the Victorian period in England (Mlcek and Rop, 2011; Cunningham 2015). Most appreciated species were roses (*Rosa* spp.), calendula (*Calendula officinalis* L.), saffron (*Crocus sativus* L.), dandelion (*Taraxacum officinale* L.), and elder inflorescences (*Sambucus nigra* L.) (Mlcek and Rop, 2011). In Indian and Chinese cultures, edible flowers are used as components of medicines based on herbs, in addition to culinary purposes (Wongwattanasathien *et al.*, 2010). Several edible flowers are beneficial to human health showing anti-inflammatory effects and antioxidant and ROS scavenging activities (Mlcek and Rop, 2011).

Today, around 180 species are known to produce edible flowers (Lu *et al.*, 2016), and *Viola* spp. are among the most common and currently consumed. These flowers are characterized by a sweet and refreshing taste, in addition to a pleasant velvety texture (Neumann and O'Connor, 2009; Koike *et al.*, 2015). Edible *Viola*s belong to 3 different species, namely *Viola cornuta* L. (horned pansy), *Viola tricolor* L. (Johnny Jumpup), and *Viola × wittrockiana* Gams (garden pansy) (Neumann and O'Connor, 2009). The plants are similar to each other except for flower size, which its diameter is in garden pansies (up to around 11.5 cm) > horned pansies (up to around 2.5 cm) > Johnny Jump ups (less than 2.5 cm in diameter) (Bailey, 1998; Kessler *et al.*, 1998). Over the years, intensive breeding programs selected new varieties with unique flower colours (pure-colour or multi-coloured flowers), greater flowers number, and plant temperature tolerance (Bailey, 1998). The cultivation of *V. cornuta* is similar to the one of *V. × wittrockiana*. These species are grown as autumn and spring bedding plants, although they are also raised for the summer and winter markets (Pearson *et al.*, 1995). In order to produce edible flowers safe for human consumption, chemical products, such as synthetic fertilizers and pesticides, has to be avoided during plants production; for this reason, only organic cultivation is allowed (Fernandes *et al.*, 2017). No special needs for cultivation are required, indeed well drained commercial potting soil can be used. *Viola* flowers are often cultivated in greenhouse, and properly defined environmental factors, such as temperature, photoperiod and irradiance are fundamental for the quality of the flowers (Gandolfo *et al.*, 2016). Pansies should be grown between 4 and 13°C, in order to reduce plant growth rate, internode elongation and to ensure high quality flowers (Cavins *et al.*, 2000). In fact, flower size (mm²) decreased linearly with increasing temperature between 9 and 31°C (Pearson

et al., 1995). The ideal temperature for growth and flowering ranges from about 14°C to 21°C (Kessler *et al.*, 1998). Moreover, pansies are obligate FR (far red)-dependent long-day plants and, for this reason, FR radiation are required to promote the flowering process, in addition to red (R) radiation (Kozai *et al.*, 2016). Blue light is able to reduce the time required to produce flower buds in *V. × wittrockiana* (Rashidi *et al.*, 2018).

Full-bloomed, edible flowers can be sold in pots or, mainly, in small and medium rigid plastic packages to avoid their rapid drying and to preserve their fragile texture (Whitman, 1991; Kelley *et al.*, 2001). However, flowers are high perishable so that different approaches were performed to prolong their shelf-life. Cold storage is documented for *V. tricolor* and *V. × wittrockiana*, using sealed low-density polyethylene film bags. These two species were able to preserve their commercial attractiveness up to 2 weeks of storage, when kept between 0 and 2.5 °C (Kelley *et al.*, 2003). More recently, different new post-harvest technologies were applied on *Viola* spp. Edible coatings (e.g. alginate), crystallization and osmotic dehydration improved *Viola*s shelf-life, as shown by a good visual quality for prolonged period (Fernandes *et al.*, 2018 a, b, 2019 a, b). Coated pansies contained higher level of polyphenols and antioxidant activity than uncoated ones, on all assayed storage times (up to 14 days). Gamma irradiation are also tested, and this methodology increased polyphenols content and antioxidant activity in *V. tricolor* flowers, compared to no irradiated controls (Koike *et al.*, 2015). Edible flowers are selected and perceived by their fragrance, appearance, size and colour. Consumers prefer yellow and orange flowers rather than blue (Kelley *et al.*, 2001, 2002). Within this regard, *V. cornuta* cv. 'Penny Lane' with orange-violet flowers have been selected for this work.

The aim of this work was to cultivate *V. cornuta* L. 'Penny Lane' and test the effect of different cultivation strategies for a higher production of flowers with good quantities of nutritional compounds. Moreover, the post-harvest treatment has been performed to analyze the change in bioactive compounds. Storage temperature was maintained around 4–6°C (cold storage) and flowers were also exposed to artificial light to simulate the refrigerated sector of the grocery market. Metabolites (polyphenols, anthocyanins, carotenoids, sugars) were analysed to determine the shelf-life of packaged flowers as I gamma products. To the best of our knowledge, any investigation of metabolites during post-harvest cold storage studies

were performed on *V. cornuta*.

2. Materials and Methods

Plant cultivation, greenhouse condition and flower blooming

Plants of *Viola cornuta* L. ‘Penny Lane’ with orange-violet flowers (Fig. 1) were purchased by Gruppo Padana - Ortifloricoltura dei Fratelli Gazzola S.S. Società Agricola (Paese, TV, Italy) and planted in 420 pots with a diameter of 14 cm (1 L volume). They were placed on 4 benches of an iron-glass greenhouse (called SAM-LAB) equipped with a climatic control system at the “Centro di Sperimentazione e Assistenza Agricola” (CeRSAA) in Albenga (SV) 43° 3’ 14” North and 8° 13’ 1” East). At the beginning of the experiment, plants were 3 cm height with a diameter of 2.5 cm. The substrate used was “TS4” soil from “Turco Silvestro” company (Albenga, SV, Italy), char-



Fig. 1 - Flowers of *Viola cornuta* cv. Penny lane grown in pot in SAMLAB greenhouse.

acterized by pH 6.5, electrical conductivity 0.56 dS/m, dry bulk density 250 kg/m³ total porosity 90% v/v. The experimental design foresees 7 treatments (60 plants each) in the SAMLAB and reported in Table 1. The presence or absence of basal heating was guaranteed by either electric mat WARMSET at 50°C or water at 35°C (by hydraulic coil) and the addition of 1 or 2 hours of light after the astronomical sunset to extend the photoperiod, as reported in Table 1. In consideration of the number and the layout of the benches of the greenhouse and the possibility to subdivide the lighting of led lamps used for the experimentation, only the selected treatments were tested (Table 1). Each bench was equipped with 4 LED lamps placed at a distance of 1.50 meters from the surface of the pallet depending on the type of lamp and culture. For the tests, specifically, VALOYA B200 LED lamps with AP673L spectrum were used (blue 12% - green 19% - red 61% - far red 8% - PAR 92%). Each lamp has a total power consumption of 192 W, a photon flux in the range 400-700 nm of 284 μmol s⁻¹ and a photon flux in the range 300-900 nm of 311 μmol s⁻¹). During the trial in the greenhouse the registered average temperature was 18°C and the average humidity was 64%. From 01/14/2019 to 02/28/2019 evaluations were carried out to observe whether different kind of basal heating and supplementary light could affect plant growth. At the end of the trials, the investigations included the measurement of plant diameter (cm) per each pot and the number of flowers per meter square. Thus, flowers were picked by hand in the morning for further analyses and cold treated.

Flowers storage conditions

Fresh picked flowers were stored in polyethylene boxes at 4 °C with a 12 hours photoperiod in order to simulate better the condition of supermarket fridge

Table 1 - Greenhouse treatments (basal heating of benches and/or additional light) of *Viola cornuta* cv. Penny lane. Treatments are carried out for 3 months, from transplantation through flowering period until the end of trials

Treatment	Basal heating			Additional light		
	Electric mat 50°C	Hot water 35°C	Absent	1 hour	2 hours	Absent
1	X			X		
2	X					X
3			X		X	
4		X		X		
5		X				X
6		X			X	
7			X			X

counter. The refrigerated cells were equipped with LED lamps (Valoya, Finland) having the following spectrum: blue 21% - green 38% - red 35% - far red 6% - PAR 94%. Flower storage was evaluated after 4 days post-harvest (time 4) performing the following biochemical analyses: total phenolics content, antioxidant activity (DPPH assay), total anthocyanins content, total carotenoids content and total soluble sugars content. For each biochemical analysis three homogeneous biological replica were used. Each replica was stored at -20°C until further analyses. Fresh flowers (time 0) are used as control.

Polyphenol content

Polyphenols were extracted as reported by Bretzel *et al.* (2013). Fresh flowers (200 mg) were homogenized in 2 mL of methanol 70 %, and of total phenolic content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). The absorbance was read at 765 nm in a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan) and the total phenolic concentration was expressed as catechin equivalents per gram of fresh weight.

DPPH scavenging activity

The antioxidant activity of each sample was determined through the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay, as described by Brand-Williams *et al.* (1995). The absorbance was read at 517 nm. Antioxidant activity was expressed in IC_{50} , which represent the concentration of the sample able to reduce the initial amount of radical DPPH by 50%. Consequently, lower IC_{50} value of sample corresponds to greater antioxidant activity.

Anthocyanin content

Total anthocyanins were extracted as reported by Bretzel *et al.* (2013). 200 mg of sample were homogenized in 750 μ l of acidified methanol (MeOH/HCl 10:0.1). The absorbance was read at 535 nm and the total anthocyanin concentration was expressed as malvidin equivalents per gram of fresh weight.

Carotenoids content

Determination of total carotenoids content was determined using Lichtenthaler's formula (1987). Fresh flowers (100 mg) were added to 5 ml of methanol 99 % and it was incubated for 24 hours at 4°C. The absorbance was read at 665.2 nm, 652.4 nm and 470 nm.

Total soluble carbohydrates content

Total soluble carbohydrates content was estimated from dried flowers (20 mg) using anthrone proto-

col according to Yemm and Willis (1954). The absorbance was read at 630 nm, using glucose as external standard.

Statistical analysis

The normal distribution of the residuals and the homogeneity of variance was determined and then data were statistically analysed. The results of biomass (number of flowers and growth in pot) were expressed as mean values and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSSD Test with $p=0,05$. The results of post-harvest treatments have been performed using ANOVA Student's t-test to determine the significant difference of each treatment between fresh samples (time 0) and samples after 4 days (time 4), with $p < 0.05$. Biochemical results were analysed by one-way ANOVA followed by Fisher's probable least-squares difference test with cut-off significance at $p \leq 0.05$ (StatView®, Version 5.0, SAS® Institute Corporation). The dependent variables were analysed using two-way ANOVA, with the factors "Treatment" and "Post harvest days" (PHD).

3. Results and Discussion

Horned pansy (*Viola cornuta*) is a biannual plant with long flowering period through different seasons. It is also considered as cold-tolerant plant, since the minimum temperature for flowering is around 4°C, while the optimal temperature is around 26°C (Blanchard and Runkle, 2011). Winter temperature and light are very important factors to determine a good production of flowers (Boldt and Altland, 2019), and heating and supplemental lighting are often provided in greenhouse cultivation to improve the quantity and quality of flowers (Dieleman and Meinen, 2007; Oh *et al.*, 2010). For this reason, horned pansy plants were subjected to different treatments (Table 1) to evaluate their effect on the growth and flowers number (Table 2) and thus the yield of edible flowers.

The results indicated that the treatment n. 7 (control, no basal heating, no additional light hours) and treatment n. 3 (no basal heating, 2 h of supplementary light) determined the larger diameter of plants (Table 2). However, there is no statistically significant difference (T student analysis) between treatment n. 3 and the control n. 7 (18.0 and 17.1 cm/plant respectively), probably due to the effect of light towards vegetative growth. The addition of basal temperature of the benches by hot water (treatment

5) corresponded to a decrease of the growth (15,6 cm/plant), while when also additional lighting was performed the decrease was not significant (treatments n. 1, 4, 6). The growth of the plants seems to be affected when the additional light is added, in the absence or with higher temperature of the benches (Table 2). The effect of supplemental LED lighting is known to affect positively many plant growth parameters of several plants, including pansy (Koksai et al., 2015), so these results are in agreement with previous reports. The treatment 2 (electric mat 50°C) highlighted the lowest value of number of flowers (604.44 flowers/m²) followed by treatment n.6 (636.94 flowers/m²), while the other trials showed similar higher amounts (Table 2). Taken together, the biometric parameters suggest that the single elongation of photoperiod (2h) plays a positive role to increase the biomass and to produce more flowers, and the temperature is a secondary effect. The quality of plants in relation to light and temperature is debated since long time (Liu and Heins, 1997; Adams et al., 1998), and the lower temperatures and higher irradiance seems to produce higher quality of flowers, including pansy (Pearson et al., 1995; Boldt and Altland, 2019). The results presented here are in agreement of the observed influence of the exposure duration, intensity and combinations of light to the growth and flowering of *V. × wittrockiana* (Oh et al., 2010). The lengthening of the photoperiod has been confirmed as important factor in *V. × wittrockiana* 'Rose', during experiments aimed to determine the influence of photoperiod and phytochrome (Rashidi et al., 2018). In that research, the night interruption decreased the plant dimension. The quality of flowers, especially the edible ones, are related to

the visible characteristics and nutraceutical components (Benvenuti et al., 2016). Thus, the influence of light and temperature for the production of different metabolites was also determined.

Edible flowers are considered a good source of antioxidant molecules (Rop et al., 2012; Loizzo et al., 2015) and polyphenols (including phenolic acids and anthocyanins) are considered the main antioxidant compounds. A first detail of phenolic composition and properties of *V. cornuta* edible flowers highlighted that their polyphenols content is lower than *V. × wittrockiana* (Moliner et al., 2019). The metabolites were analyzed at the time of harvest (time 0), and after short period of post-harvest in a chamber at lower temperature and in the presence of light (12h). The post-harvest treatment was chosen to mimic the condition of the benches of grocery stores. Pigments, as carotenoids and anthocyanins are the important compounds for evaluating the visual quality of flowers. At time of harvest (time 0), the worst treatment resulted the n. 3 (addition of 2h light), since the recorded amount of both pigments were the lowest, 0.14 and 5.63 mg/g FW for carotenoids and anthocyanins, respectively (Table 3). Instead, the highest values were determined with the treatment n.1 (temperature 50°C by electric mat and light 1h (0.32 and 10.42 mg/g FW for carotenoids and anthocyanins). The increased temperature, either by electric mat (n. 2) or by hot water (n. 5), did not support any increase in flower pigmentation, both for carotenoid and anthocyanins. Control flowers showed good carotenoid values (0.30 mg/g FW), while anthocyanins suffered without addition of light or temperature (6.93 mg/g FW). Of our knowledge only few papers have been published so far on the

Table 2 - Effect of different cultivation (basal heating and/or supplementary light) on biomass production of *Viola cornuta* cv. Penny lane

Treatment	Diameter (cm)	Number of flower per m ²
1 Electric mat 50°C + light 1 h	15.8±0.40 ab	838.42±42.9 a
2 Electric mat 50°C	16.2±0.46 ab	604.44±48.75 b
3 Light 2 h	18.0±0.40 a	864.42±40 a
4 Hot water 35°C + light 1 h	16.6±0.33 ab	851.42±33.8 a
5 Hot water 35°C	15.6±0.38 b	812.43±44.85 a
6 Hot water 35°C + light 2 h	16.1±0.24 ab	636.94±43.55 ab
7 Control	17.1±0.50 a	793.93±38.35 a

Plant diameter (cm) and the number of flowers (per meter square) were detected at the end of flowering period.

Data are expressed as mean value (n=60) and analyzed using one-way analysis of variance (ANOVA) followed by Fisher's probable least-square difference test with p=0.05.

Table 3 - Determination of carotenoids, anthocyanins, polyphenols, radical scavenging activity (DPPH assay), and soluble sugars of *Viola cornuta* flowers grown under different greenhouse conditions (AV1-7, see Table 1) and cold stored for 0 (time 0) or 4 (Time 4) days postharvest

Treatment	Carotenoids (mg/g FW)	Anthocyanins (mg/g FW)	Polyphenols (mg/g FW)	DPPH assay (IC50 mg/ml)	Soluble sugars (mg/g FW)
<i>Time 0</i>					
1 Electric mat 50°C+ light 1 h	0.32 ± 0.00 a A	10.42 ± 0.32 a A	12.42 ± 0.11 a A	0.82 ± 0.00 a A	209.69 ± 3.91 a A
2 Electric mat 50°C	0.27 ± 0.00 c B	8.05 ± 0.20 c A	9.93 ± 0.68 b B	0.80 ± 0.01 a A	183.84 ± 6.97 b A
3 Light 2 h	0.14 ± 0.01 e B	5.63 ± 0.14 e B	11.00 ± 0.09 ab A	0.84 ± 0.02 ab A	179.57 ± 3.98 b A
4 (Hot water 35°C + light 1 h)	0.29 ± 0.01 bc A	9.28 ± 0.49 bc A	11.85 ± 0.40 ab A	0.86 ± 0.02 ab A	207.45 ± 4.85 a A
5 Hot water 35°C)	0.28 ± 0.01 c A	10.29 ± 0.25 ab A	9.35 ± 1.06 b A	0.89 ± 0.01 b B	181.29 ± 3.84 b A
6 Hot water 35°C + light 2 h	0.26 ± 0.00 d A	6.93 ± 0.42 d A	7.36 ± 0.16 c B	1.19 ± 0.05 d B	183.90 ± 10.96 b A
7 Control	0.30 ± 0.00 b A	8.71 ± 0.57 cd A	7.66 ± 0.25 c B	1.09 ± 0.01 c B	179.90 ± 1.44 b A
<i>Time 4</i>					
1 Electric mat 50°C+ light 1 h	0.33 ± 0.01 b A	4.81 ± 0.19 d B	12.46 ± 0.25 a A	0.78 ± 0.01 a A	203.61 ± 2.03 a A
2 Electric mat 50°C	0.36 ± 0.01 a A	7.63 ± 0.18 c A	11.62 ± 0.16 b A	0.82 ± 0.02 a A	158.91 ± 2.90 d A
3 Light 2 h	0.21 ± 0.00 e A	9.22 ± 0.19 b A	10.88 ± 0.21 bc A	0.84 ± 0.01 b A	161.09 ± 0.36 d B
4 Hot water 35°C + light 1 h	0.28 ± 0.01 c A	10.73 ± 0.56 a A	11.65 ± 0.32 ab A	0.90 ± 0.02 c A	175.50 ± 1.30 c B
5 Hot water 35°C	0.23 ± 0.00 d A	6.65 ± 0.44 c B	10.43 ± 0.45 c A	0.77 ± 0.02 a A	164.42 ± 3.41 d A
6 Hot water 35°C + light 2 h	0.25 ± 0.01 d A	4.75 ± 0.49 d B	9.32 ± 0.04 d A	0.94 ± 0.02 c A	159.48 ± 2.99 d B
7 Control	0.27 ± 0.01 c B	7.49 ± 0.79 c A	10.37 ± 0.27 c A	0.78 ± 0.01 a A	192.31 ± 2.56 b A
<i>ANOVA p-value</i>					
Treatment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PHD	0.0014	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment × PHD	< 0.0001	< 0.0001	0.0041	< 0.0001	0.0060

Data are expressed as means (n=3, ± SE.) ANOVA followed by Fisher's probable least-square difference test was used, with a cut-off significance at p=0.05. Small letters indicate comparisons between treatments at the same postharvest day (PHD); capital letters indicate comparisons between the two PHD for the same treatment. Interaction between treatments and PHD were analysed by two-way ANOVA.

influence of light and temperature on the content of pigments in *Viola* spp. (Rashidi *et al.*, 2018), so these results will contribute to define the effect of these factors and their contribution to the pigmentation. Other metabolites of horned pansy were determined at time of harvest, such as polyphenols and sugars, that are expected as fundamental nutraceutical components, as well as the scavenger reducing power (by DPPH assay). With regards to total soluble carbohydrates (TSS) statistically significant differences were observed among the various treatments: higher sugars amounts were observed in the treatments n. 1 and 4, characterized by the addition of light (1 h) and higher temperature. Although the other treatments showed the similar less quantities of sugars, the lowest amount is observed in treatment n. 3 (TSS 179.57 mg/g FW). The highest amount of total polyphenols (12.42 mg/g FW) was measured in treatment n. 1 (basal electric heating at 50 °C with 1 hour of additional light), and the lowest value in the control and n. 6 (7.66 and 7.36 mg/g FW, respectively). The antioxidant activity is higher in the treatments of

addition of temperature by electric mat (n. 1 and 2) with IC₅₀ (DPPH assay) values of 0.80 and 0.82 mg/ml respectively, followed by the trials n.3 and 4. Flowers of the control and treatment n.6 showed the lower scavenger reducing activity. In the present work the concentration of total polyphenols ranged 7.36 and 12.42 mg GAE/g fresh weight. These values agree with those found in *V. × wittrockiana*, reported by other authors (Rop *et al.*, 2012). However, the polyphenol values could be underestimated by the method of extraction, as already shown in González-Barrio *et al.* (2018). In fact, they reported different polyphenol amounts in *V. × wittrockiana* by using either acidic hydrolysis or maceration instead of the method adopted in this work (Bretzel *et al.*, 2014).

Other reports showed the influence of storage at different temperature in different flowers (Kelley *et al.*, 2003). Moreover, different packages used for the storage conditions can affect the quality of flowers (Landi *et al.*, 2018). Changes of appearance, and aesthetic value were performed on *V. × wittrockiana* (Kelley *et al.*, 2003). The results obtained at the time

of cold storage (time 4) were compared to those at the time of harvest (time 0). The data reported here indicated that the purple-pink flowers maintained the carotenoids content after 4 days of cold storage in the treatments n. 1, 4 and 6, whereas in the treatments n. 2 and 3 values of carotenoids increased. Meanwhile, in flowers of treatment n. 5 and 7 (control) the amount of carotenoids decreased (Table 3). After 4 days of postharvest treatment, anthocyanins are the most affected metabolites by cold storage. In fact, the amount of anthocyanins decreased in the treatments n. 1, 5 and 6, whereas in the treatment n. 3 values increased (9.22 mg/g FW). The treatment n. 1 showed the largest decrease, 10.42 mg/g FW at time 0 and 4.81 mg/g FW at time 4. However, the loss of pigmentation is not always documented, but it is peculiar of each species and variety, as already demonstrated in other species as *Acmella oleracea*, *Salvia discolor*, *Begonia semperflorens*, *Tropaeolum majus* (Landi et al., 2018). The total polyphenols content after cold storage maintained the same values of that detected at Time 0, with the exception of treatments n. 2, 6 and control, where polyphenols increased, 11.62, 9.32 and 10.37 mg/g FW at time 4, respectively (Table 3). The antioxidant activity increased in the treatments with hot water (n.4, 5, 6). Different susceptibility to the storage process was observed in other edible flowers, with different changes (increase or decrease) on nutraceutical values up to 8 days of postharvest (Landi et al., 2018). The soluble sugars dropped significantly in the treatment n. 4, since the values was the highest at time 0 but reduced at 80% at time 4 (207.45 and 175.5 mg/g FW). Other decrease in the content of sugar is observed for the treatments n.3 and 6. Soluble sugars are important nutritional components of the flowers and represent a good characteristic for the choice of edible flowers (Mlcek and Rop, 2011). However, there are few works on the sugar profile of edible flowers, e.g. *Rosa micrantha* (Guimarães et al., 2010). In experiment done with cut lily flowers was discussed the role of reducing sugars, as a typical reaction of plants that defend themselves against injury due to chilling or frost (Van doorn and Han, 2011).

4. Conclusions

The different cultivation treatments used in this work are differently correlated with the analyzed metabolites. In order to obtain flowers with high

quality of brilliant color, one hour of supplementary lighting and a basal heating of 50°C seem to be the right combination of factors. The cold storage imposed to the flowers as the post-harvest treatment indicated that the flowers treated with additional 2 h of light (treatment n. 3) retained values of the metabolites during the post-harvest, with the exception of sugars. However, even if the additional lighting seems to preserve the flowers from depigmentation and to maintain the nutraceutical compounds, the decreased content of the observed sugars could be a consequence of the phenomenon of senescence. Further studies on the influence of illumination on plastic bags and the evaluation of ethylene production can be useful for the definition of the post-harvest process in *V. cornuta*. In addition, the investigation of the other minor nutritional components can be crucial to define a more detailed condition of storage.

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References

- ADAMS S.R., PEARSON S., HADLEY P., 1997 - *The effects of temperature, photoperiod and light integral on the time to flowering of Pansy cv. Universal Violet (Viola x wittrockiana Gams.)*. - Ann Bot. London, 80(1): 107-112.
- BAILEY D.A., 1998. - *Commercial pansy production*. - Hortic. Information Leaflet, 521: 1-8.
- BENVENUTI S., BORTOLOTTI E., MAGGINI R., 2016 - *Antioxidant power, anthocyanin content and organoleptic performance of edible flowers*. - Sci. Hortic., 199: 170-177.
- BLANCHARD M.G., RUNKLE E.S., 2011 - *Quantifying the thermal flowering rates of eighteen species of annual bedding plants*. - Sci. Hortic., 128: 30-37.
- BOLDT J.K., ALTLAND J.E., 2019 - *Timing of a short-term reduction in temperature and irradiance affects growth and flowering of four annual bedding plants*. - Horticulturae, 5(1): 15.
- BRAND-WILLIAMS W., CUVELIER M.E., BERSET C., 1995 - *Use of a free radical method to evaluate antioxidant activity*. - LWT - Food Sci. Technol., 28(1): 25-30.
- BRETZEL F., BENVENUTI S., PISTELLI L., 2014 - *Metal conta-*

- mination in urban street sediment in Pisa (Italy) can affect the production of antioxidant metabolites in *Taraxacum officinale* Weber. - Environ. Sci. Pollut. Res., 21(3): 2325-2333.
- CAVINS T.J., DOLE J.M., STAMBACK V., 2000 - *Unheated and minimally heated winter greenhouse production of specialty cut flowers*. - HortTechnology, 10(4): 793-799.
- CUNNINGHAM E., 2015 - *What nutritional contribution do edible flowers make?* - Journal of the Academy of Nutrition and Dietetics, 115(5): 856.
- DIELEMAN J.A., MEINEN E., 2007 - *Interacting effects of temperature integration and light intensity on growth and development of single-stemmed cut rose plants*. - Sci. Hortic., 113: 182-187.
- FERNANDES L., CASAL S., MAGALHAES A., BAPTISTA P., PEREIRA J.A., SARAIVA J.A., RAMALHOSA E. 2019 a - *Effect of osmotic drying on physicochemical properties of pansies (Viola × wittrockiana)*. - Intern. J. Food Studies, 8(2): 23-33.
- FERNANDES L., CASAL S., PEREIRA J.A., SARAIVA J.A., RAMALHOSA E., 2017 - *Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health*. - J. Food Compost. Anal., 60: 38-50.
- FERNANDES L., CASAL S., PEREIRA J.A., SARAIVA J.A., RAMALHOSA E., 2018 a - *Effect of alginate coating on the physical-chemical and microbial quality of pansies (Viola × wittrockiana) during storage*. - Food Sci. Biotechnol., 27(4): 987-996.
- FERNANDES L., CASAL S., PERERA J.A., PEREIRA E.L., SARAIVA J.A., RAMALHOSA E., 2019 b - *Physicochemical, antioxidant and microbial properties of crystallized pansies (Viola × wittrockiana) during storage*. - Food Sci. Technol. International, 25(6): 472-479.
- FERNANDES L., PEREIRA J.A., BAPTISTA P., SARAIVA J.A., RAMALHOSA E., CASAL S., 2018 b - *Effect of application of edible coating and packaging on the quality of pansies (Viola × wittrockiana) of different color and sizes*. - Food Sci. Technol. International, 24(4): 321-329.
- GANDOLFO E., HAKIM G., GERACI J., FEURING V., GIARDINA E., DI BENEDETTO A., 2016 - *Responses of pansy (Viola × wittrockiana Gams.) to the quality of the growing media*. - JEAJ, 1-10.
- GONZÁLES-BARRIO R., PERIAGO M.J., LUNA-RECIO C., JAVIER G.A.F., NAVARRO-GONZÁLEZ I., 2018 - *Chemical composition of the edible flowers, pansy (Viola wittrockiana) and snapdragon (Antirrhinum majus) as new sources of bioactive compounds*. - Food Chem., 252: 373-380.
- GUIMARÃES R., BARROS L., CARVALHO A.M., FERREIRA I.C., 2010 - *Studies on chemical constituents and bioactivity of Rosa micrantha: An alternative antioxidants source for food, pharmaceutical, or cosmetic applications*. - J. Agr. Food Chem., 58(10): 6277-6284.
- KELLEY K.M., BEHE B.K., BIERNBAUM J.A., POFF K.L., 2001 - *Consumer ratings of edible flower quality, mix and color*. - HortTechnology, 11: 644-647.
- KELLEY K.M., BEHE B.K., BIERNBAUM J.A., POFF K.L., 2002 - *Combinations of colours and species of containerized edible flowers: effect on consumer preferences*. - HortSci., 37(1): 218-221.
- KELLEY K.M., CAMERON A.C., BIERNBAUM J.A., POFF K.L., 2003 - *Effect of storage temperature on the quality of edible flowers*. - Postharvest Biol. Technol., 27: 341-344.
- KESSLER J.R., HAGAN J.A., COBB P., 1998 - *Pansy production and marketing*. - Alabama A&M and Auburn Universities, ANR-596.
- KOIKE A., BARREIRA J.C.M., BARROS L., BUELGA C.S., VILLAVINECIO A.L.C.H., FERREIRA, I.C.F.R., 2015 - *Edible flowers of Viola Tricolor L. as a new functional food: Antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation*. - Food Chem., 179: 6-14.
- KOKSAL N., INCESU M., TEKE A., 2015 - *Supplemental LED lighting increases pansy growth*. - Hortic. Bras., 33(4): 428-433.
- KOZAI T., FUJIWARA K., RUNKLE E.S., 2016 - *LED lighting for urban agriculture*. - Springer Science+Business Media Singapore.
- LANDI M., RUFFONI B., COMBOURNAC L., GUIDI L., 2018 - *Nutraceutical value of edible flowers upon cold storage*. - Ital. J. Food Sci., 30(2): 336-346.
- LICHTENTHALER H.K., 1987 - *Chlorophylls and carotenoids: pigments of photosynthetic biomembranes*. - Methods Enzymol., 148: 350-382.
- LIU B., HEINS R.D., 1997 - *Is plant quality related to the ratio of radiant energy to thermal energy?* - Acta Horticulturae, 435: 171-182.
- LOIZZO R.M., PUGLIESE A., BONESI M., TENUTA M.C., MENICHINI F., XIAO J., TUNDIS R., 2015 - *Edible flowers: a rich source of phytochemicals with antioxidant and hypoglycemic properties*. - J. Agric. Food Chem., 64(12): 2467-2474.
- LU B., LI M., YIN R., 2016 - *Phytochemical content, health benefits, and toxicology of common edible flowers, a review (2000–2015)*. - Crit. Rev. Food Sci. Nutr., 56 (Suppl 1): 130-148.
- MLCEK J., ROP O., 2011 - *Fresh edible flowers of ornamental plants - A new source of nutraceutical foods*. - Food Sci. Technol., 22: 561-569.
- MOLINER C., BARROS L., DIAS M.I., REIGADA I., FERREIRA I.C., LOPEZ V., RINCON C.G., 2019 - *Viola cornuta and Viola × wittrockiana: Phenolic compounds, antioxidant and neuroprotective activities on Caenorhabditis elegans*. - J. Food Drug Analysis, 27(4): 849-859.
- NEWMAN S.E., O'CONNOR A.S., 2009 - *Edible flowers*. - Colorado State University Extension, 12/96, Fact Sheet no. 7.237.
- OH W., RUNKLE E.S., WARNER R.M., 2010 - *Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy*. - HortScience, 45(9): 1332-1337.
- PEARSON S., PARKER A., ADAMS S.R., HADLEY P., MAY DR.,

- 1995 - *The effects of temperature on the flower size of pansy (Viola x wittrockiana Gams)*. - J. Hortic. Sci., 70: 183-190.
- RASHIDI A., TEHRANIFAR A., NEMATI H., 2018 - *Effect of light combination and timing of supplemental lighting on growth characteristics and flowering of pansy (Viola xwittrockiana Rose)*. - J. Ornamental Plants, 8(4): 227-240.
- ROP O., MLCEK J., JURIKOVA T., NEUGEBAUEROVA J., VABKOVA J., 2012 - *Edible flowers a new promising source of mineral elements in human nutrition*. - Molecules, 17: 6672-6683.
- SINGLETON V.L., ROSSI J.A., 1965 - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents*. - Am. J. Enol. Vitic., 16: 144-158.
- VAN DOORN W.G., HAN S.S., 2011 - *Postharvest quality of cut lily flowers*. - Postharvest Biol. Tec., 62(1): 1-6.
- WHITMAN A.T., 1991 - *Edible flowers and culinary herbs: New uses for traditional crops, new crops for traditional growers*. - GrowerTalks, 54(13): 22-33.
- WONGWATTANASATHIEN O., KANGSADALAMPAI K., TONGYONK L., 2010 - *Antimutagenicity of some flowers grown in Thailand*. - Food Chem. Toxicol., 48: 1045.
- YEMM E.W., WILLIS A., 1954 - *The estimation of carbohydrates in plant extracts by anthrone*. - Biochem. J., 57(3): 508.

