

Chlorinated sanitizers preserve bioactive compounds and mitigate oxidative stress in *Begonia semperflorens* edible flowers during storage

Matheus Morais^{*}, Bruna Orsi, Ana Paula Preczenhak, Catherine Amorim, Marcos Fabian Sanabria Franco, Gabrielle Caroni Vacchi, Rafael Limão Lopes de Almeida, Giulia Belicuas Lopes, Ricardo Alfredo Kluge

Universidade de São Paulo/ESALQ – Depto. de Ciências Biológicas, Av. Pádua Dias, 11 – 13418-900 – Piracicaba, SP – Brasil.

*Corresponding author <matheusmorais@alumni.usp.br>

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ABSTRACT: *Begonia semperflorens* edible flowers are rich in bioactive compounds such as phenolics, flavonoids, and anthocyanins. This study evaluated the effects of different sanitization methods — control, distilled water (DTW), sodium hypochlorite (SH), and sodium dichlorite-s-triazinetrione (SDST) — on the retention of these compounds and oxidative stress levels during postharvest storage. Flowers were sanitized and stored at 5 °C for ten days. Analyses of bioactive compound content, ethylene (C₂H₄) and carbon dioxide (CO₂) production, and histochemical assays were conducted immediately after sanitization, and at five and ten days post-treatment. Results showed that flowers treated with SDST exhibited a 57 % increase in phenolic content and a 27 % increase in flavonoid content compared to the control and DTW treatments at the end of the storage period. Flowers that were not treated with chlorine showed increased hydrogen peroxide (H₂O₂) accumulation and a 35 % reduction in anthocyanin content after ten days of storage, compared to those treated with chlorine-based sanitizers. The absence of differences in antioxidant capacity between treatments suggests that chlorine-based sanitizers help preserve bioactive compound levels without compromising antioxidant potential. Color analysis further indicated that no sanitization was preferable to treatment with pure water. Overall, chlorine sanitizers appear to be effective options for preserving the nutraceutical quality of edible flowers, in addition to their known antimicrobial benefits.

Keywords: disinfection, food quality, non-conventional edible plants, nutraceuticals, preservation

The use of flowers in cuisine dates back to ancient times in Asia, Rome, and Greece (Mlcek and Rop, 2011). For example, marigold flowers were used in medieval France. *Antigonon leptopus* Hook. & Arn. and *Bougainvillea × buttiana* Holtum & Standl have been incorporated into Thai teas and salads (Ngoitaku et al., 2016). These flowers contain antioxidant bioactive compounds, including phenolics, flavonoids, flavones, and anthocyanins. They contribute to the prevention of chronic diseases and reduction of oxidative stress (Benvenuti and Mazzoncini, 2021; Grzeszczuk et al., 2016; Mlcek and Rop, 2011).

Begonia × semperflorens-cultorum hort., native to South America and naturalized in tropical regions (Bi et al., 2018), provides nutritional benefits due to its high content of phytochemicals, particularly anthocyanins, phenolics, and flavonoids (Grzeszczuk et al., 2016; Mlcek and Rop, 2011). However, its flowers are highly perishable, with a shelf-life of only two to five days. To extend shelf-life, several preservation techniques are employed, including low-temperature storage, drying, edible films, and sanitization (Fernandes et al., 2019).

Disinfection reduces microbial activity, enhancing both food safety and shelf-life (Mendoza et al., 2022). Chlorinated sanitizers are strong oxidizing agents. They disrupt microbial membranes, proteins, and nucleic acids (Bloomfield, 1996; Mendoza et

al., 2022). However, these treatments may degrade anthocyanins, causing undesirable color loss. While chlorinated compounds are effective for microbial control, their effects on bioactive metabolites in flower tissues remain poorly understood. Few studies have investigated the relationship between sanitization methods and the nutraceutical properties of edible flowers (Demasi et al., 2021; Friedman et al., 2007; Traversari et al., 2021).

Given the widespread industrial use of chlorinated sanitizers (Afolabi et al., 2011; Baia et al., 2020; Chinchkar et al., 2022; FDA, 2009; Kwon et al., 2011; Macnish et al., 2010; Mendoza et al., 2022), we hypothesized that different sanitizers may influence bioactive compound levels, secondary metabolism, and the overall nutraceutical value of edible flowers. This study investigated how various sanitization treatments affect bioactive compound levels, antioxidant capacity, and oxidative stress in *B. semperflorens* flowers after harvest.

A combination of pink, white, and red *B. semperflorens* flowers (including petals and sepals) at full bloom, selected based on visual quality, was harvested from a commercial supplier in Holambra, São Paulo state, Brazil (22°38'56.0" S, 47°02'21.2" W, altitude 700 m; Köppen-Geiger climate classification Aw; average temperature 21.7 °C). The treatments included: no water immersion or sanitizing solution

(control), distilled water (DTW), sodium hypochlorite (SH, 10 mL L⁻¹), and sodium dichlorite-s-triazinetriene (SDST, composition: sodium dichloro-s-triazinetriene, 40.8 % active chlorine, adjuvants *quantum satis pro* 100 mg; Clor-in®). The experiment was conducted in Piracicaba, São Paulo state, Brazil (22°42'30" S, 47°38'30" W, altitude 546 m), at a controlled temperature of 15 °C and a relative humidity (RH) of 85 ± 5 %. Flowers were immersed in 10 L of the sanitizing solution per treatment group (excluding the control).

Flowers treated with DTW, SH, and SDST were immersed for 5, 10, and 15 min, respectively, following the manufacturer's recommendations. After immersion, flowers were rinsed with DTW, blotted with tissue paper, and dried by natural evaporation at 15 °C. They were then packaged in polyethylene terephthalate containers (Galvanotek GA91, Galvanotek Embalagens Ltda; internal dimensions: 120 × 103 × 39 mm; external: 145 × 130 × 35 mm). Each package contained 5 g of flowers and was stored at 5 °C for ten days under 85 ± 5 % RH.

Fresh flowers (*in natura*) were used for analyses, including petal color, respiratory rate, ethylene production, and histochemical evaluations. Immediately after minimal processing, a portion of the flowers was frozen in liquid nitrogen at -80 °C and stored at -20 °C for subsequent lyophilization and biochemical analyses (total anthocyanins, phenolic compounds, total flavonoids, and antioxidant capacity). Due to the limited sample quantity, only pink flowers were used for analyses of color, phenolic compounds, flavonoids, anthocyanins, antioxidant capacity, and histochemical assays. The lyophilization process was conducted at -55 °C and 2 kPa pressure using a Liotop L108 freeze-dryer.

The experiment followed a completely randomized design with five replicates per treatment. Analyses were performed immediately after minimal processing (day 0), and again at five (day 5) and ten days (day 10) post-processing. Ethylene (C₂H₄) and carbon dioxide (CO₂) production were also assessed on days two, three and eight, while histochemical analyses were conducted on days zero, one, and five.

Petal and leaf surface color was evaluated with a Minolta CR-400 colorimeter. They record Commission Internationale de l'Éclairage (CIE) parameters (L*, a*, b*) and were recorded for five flowers per treatment in each replicate. Each tissue was measured twice, once on the adaxial and once on the abaxial surface.

Total phenolic content was determined using a modified Folin-Ciocalteu method (Singleton and Rossi, 1965). A 5 mg portion of previously frozen, lyophilized, and milled flower tissue (IKA®-Werke GmbH & Co. KG, model A11 Basic) was extracted in 10 mL of 80 % methanol. After 1 min of homogenization (Marconi MA-162), the extract was centrifuged at 4 °C for 10 min in a refrigerated centrifuge (Jouan BR4i) and

left to stand in the dark for 2 h. For the reaction, 400 µL of the extract was combined with 400 µL of DTW, 400 µL of Folin-Ciocalteu reagent, and 2800 µL of a 10 % sodium carbonate solution. The solution was incubated in the dark for an additional 2 h, and absorbance was measured at 765 nm using a spectrophotometer (Biospectro SP-0220). A gallic acid calibration curve (5-25 µg mL⁻¹) was used to quantify phenolic content, with results expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g⁻¹ DW).

Flavonoid content was assessed following the method described by Kramling and Singleton (1969). A 50 mg of lyophilized and milled flower tissue was extracted in 10 mL of an ethanol:HCl solution (95 % ethanol and 1.5 N HCl, 85:15 ratio) and incubated overnight at 4 °C. The mixture was centrifuged at 1.05 × 10³ rad s⁻¹ for 10 min, and the supernatant was transferred to a 50 mL volumetric flask and brought to volume with the same extraction solution. Absorbance was measured at 374 nm using a spectrophotometer. A quercetin calibration curve (Chang et al., 2002) was used to determine flavonoid content, with results expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g⁻¹ DW).

Anthocyanin content was determined following the method described by Lees and Francis (1972). A 50 mg of lyophilized, milled flower material were extracted in 10 mL of an ethanol:HCl solution (95 % ethanol and 1.5 N HCl, 85:15 ratio), stirred, and incubated overnight at 4 °C in the dark. After further stirring for 5 min, the solution was centrifuged at 1.05 × 10³ rad s⁻¹ for 10 min. The supernatant was diluted to a final volume of 50 mL with the same extraction solution. Absorbance was measured at 535 nm, and anthocyanin content was expressed in milligrams per gram of dry weight (mg g⁻¹ DW).

Antioxidant capacity was assessed using adaptations of methods by Arts et al. (2004), van den Berg et al. (1999), and Re et al. (1999), based on 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical decolorization. A 5 mg portion of lyophilized and milled sample was extracted in 10 mL of 80 % methanol, incubated in the dark for 2 h, and centrifuged at 6440 × g for 10 min at 4 °C. The supernatant's absorbance was measured at 734 nm. Antioxidant capacity was calculated using a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calibration curve (0-20 µM), with results expressed as µmol Trolox equivalent antioxidant capacity per milligram of dry weight (µmol TEAC mg⁻¹ DW).

To detect superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), *B. semperflorens* petals were immersed for 2 h at 25 ± 2 °C in nitroblue tetrazolium (NBT) and 3,3'-Diaminobenzidine (DAB) solutions, respectively. Samples were subsequently incubated in the dark for 0, 24, or 96 h, depending on the analysis time point.

The NBT solution consisted of 0.1 % NBT in 50 mM potassium phosphate buffer with 10 mM sodium azide. The DAB solution was prepared with 0.1 % DAB in DTW and adjusted to pH 3.8 with HCl. These reactions rely on the formation of insoluble colored polymers in the presence of reactive oxygen species (ROS) (Thordal-Christensen et al., 1997), yielding blue (NBT) and brown (DAB) staining. Samples were visually evaluated using a laboratory magnifying glass (Micronal).

For respiratory rate and C_2H_4 production, eight flowers were enclosed in 150 mL hermetic containers for 1 or 2 h, respectively. A 0.5 mL headspace sample was injected into a Thermo Finnigan Trace GC Ultra gas chromatograph (Thermo Fisher Scientific) equipped with a flame ionization detector, methanator, and stainless-steel column (3.175 mm and 1.8 m) packed with Porapak N 50/80. Hydrogen was used as the carrier gas at a flow rate of 25 mL min⁻¹. Column, injector, detector, and methanator temperatures were set at 110, 140, 200, and 350 °C, respectively.

Calibration curves were prepared using commercial standards of C_2H_4 and CO_2 . Results were expressed as ng C_2H_4 kg⁻¹ s⁻¹ for ethylene and mL CO_2 kg⁻¹ h⁻¹ for carbon dioxide.

Statistical analyses were performed in R Studio software (version 4.3.1). The experiment was completely randomized with five repetitions per treatment (n = 5). The results were submitted to analysis of variance and expressed as mean ± standard error. Normality and homogeneity were verified by the Shapiro-Wilk and Bartlett tests, respectively. The Tukey test was applied to test significant differences between the means at $p \leq 0.05$.

To assess the impact of sanitization on the bioactive compound content in flowers during storage, total phenolics, flavonoids, and anthocyanins were measured (Figure 1A-C). On day zero, flowers treated with SDST and SH exhibited higher phenolic content than those in the control and DTW treatments (Figure 1A). Flavonoid levels were also higher in the SDST, SH, and DTW treatments than in the control (Figure

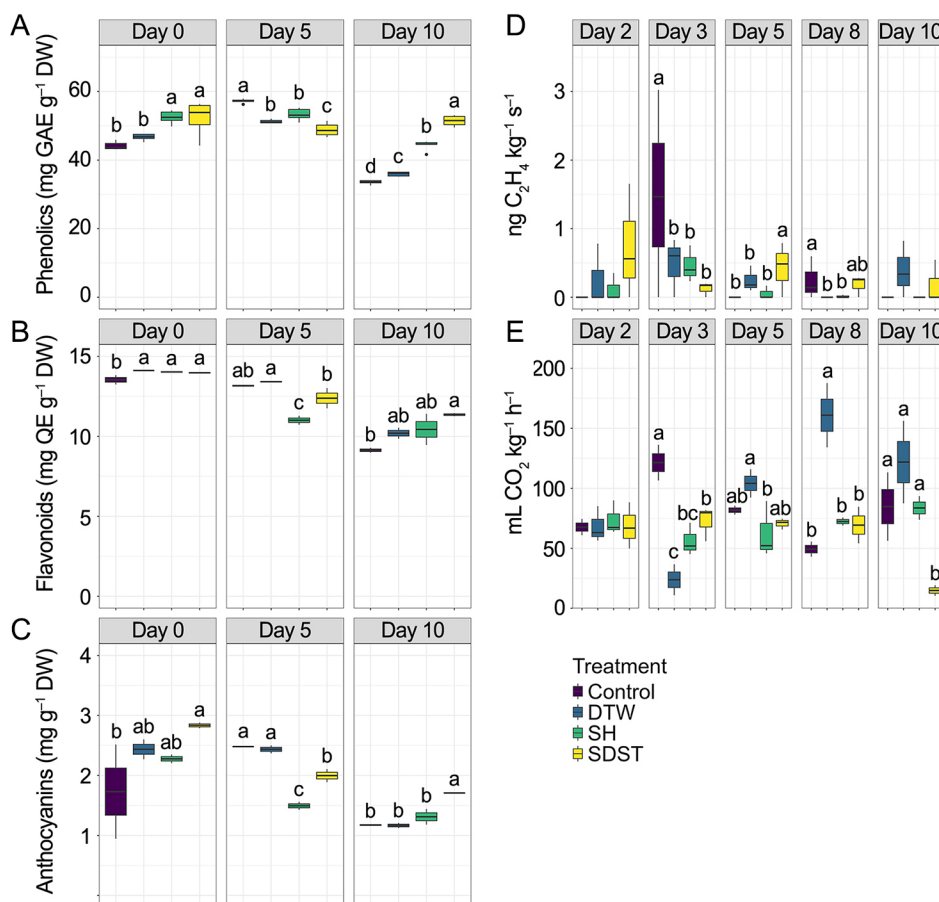


Figure 1 – A) Total phenolics content, B) total flavonoids content, C) total anthocyanins content, D) ethylene (C_2H_4) production, and E) carbon dioxide (CO_2) on the processing day (Day 0), five days after processing (Day 5), and ten days after processing (Day 10) for the control, distilled water (DTW), sodium hypochlorite (SH), and sodium dichlorite-s-triazinetriene (SDST) treatments, stored at 5 °C ± 2 and 85 % ± 5 relative humidity. Vertical bars represent the standard error of the mean. Different letters indicate significant differences at $p < 0.05$ (n = 5). GAE = gallic acid equivalents; DW = dry weight; QE = quercetin.

1B). Additionally, SDST-treated flowers showed the highest anthocyanin content on day zero (Figure 1C).

By day five, the control treatment had the highest phenolic content, while SDST-treated flowers had the lowest (Figure 1A). Flavonoid content varied from one treatment to the next, with DTW-treated flowers showing the highest levels and SH-treated flowers the lowest (Figure 1B). For anthocyanins, the control and DTW treatments had higher contents on this day (Figure 1C).

On day ten, all treatments differed in phenolic, flavonoid, and anthocyanin content. SDST treatment resulted in the highest phenolic levels, followed by SH, DTW, and control (Figure 1A). The same trend was observed for flavonoid content (Figure 1B). However, anthocyanin levels remained elevated only in the SDST treatment (Figure 1C).

Antioxidant capacity, evaluated using the ABTS assay, showed a slight increase after five days of storage for all treatments, despite no differences. Although DTW treatment showed a 1.2-fold decrease compared to the control on day ten, no difference was observed (Table 1).

To examine oxidative stress induced by the sanitizers, we conducted histochemical O_2^- and H_2O_2 using NBT and DAB staining, respectively. On day zero, DTW and SH treatments showed more intense DAB and NBT staining compared to the control and SDST (Figure 2). One day after sanitization, DTW and SH still exhibited higher DAB staining, indicating increased H_2O_2 production, whereas the control showed more intense NBT staining. On day five, both DAB and NBT staining were more intense in the control treatment. Notably, DTW-treated petals showed a broader spread of DAB staining, while SH-treated flowers showed higher and more intense NBT staining.

Ethylene production and respiratory rate (CO_2) were assessed to evaluate senescence and metabolic activity during postharvest storage. No differences in CO_2 production were observed after two days. However, after three days, all treatments showed differences, with the control producing the most CO_2 and DTW producing the least, resulting in a 5.2-fold decrease. DTW-treated flowers showed a 1.4- and 2.5-fold increase in CO_2 production on days five and eight, respectively. By day ten, DTW-treated flowers continued to show a high respiratory rate, although a difference was observed only for the SDST treatment, which showed an 8.6-fold decrease compared to DTW (Figure 1D).

Distilled water and chlorine-based treatments increased bioactive compounds levels immediately after application and at the end of storage (Figure 1A-C). Although these treatments involved more handling than the control, the findings suggest that the benefits of sanitization — such as improved bioactive compound retention — outweigh the metabolic stress caused by additional handling.

Table 1 – Total 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) right after the processing day (Day 0), five days after processing (Day 5), and ten days after processing (Day 10) for the control, distilled water (DTW), sodium hypochlorite (SH), and sodium dichlorite-s-triazinetriene (SDST) treatments, stored at $5^\circ C \pm 2$ and $85\% \pm 5$ relative humidity.

	ABTS ($\mu\text{mol TEAC mg}^{-1} \text{ DW}$)		
	Day 0	Day 5	Day 10
Control	173.57 ± 8.86^a	213.77 ± 30.47^a	186.34 ± 74.24^a
DTW	172.87 ± 30.49^a	186.83 ± 23.54^a	150.01 ± 20.60^a
SH	188.95 ± 30.49^a	191.21 ± 36.28^a	176.79 ± 41.50^a
SDST	188.5 ± 46.03^a	206.58 ± 22.6^a	206.46 ± 14.89^a

Different letters indicate significant differences at $p < 0.05$ ($n = 5$). TEAC = Trolox equivalent antioxidant capacity; DW = dry weight.

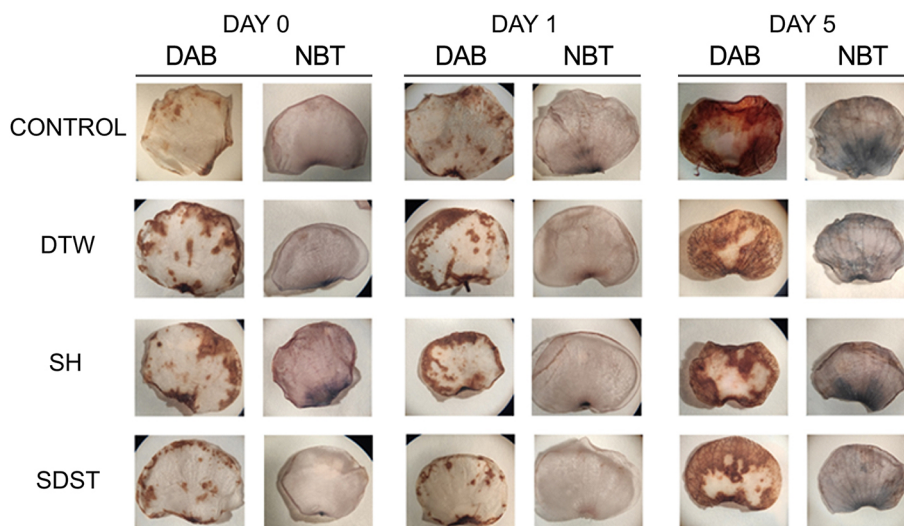


Figure 2 – Petals of *Begonia semperflorens* immersed in solutions of 3,3'-Diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) right after the processing day (Day 0), one day after processing (Day 1), and five days after processing (Day 5) for the control, distilled water (DTW), sodium hypochlorite (SH), and sodium dichlorite-s-triazinetriene (SDST) treatments, stored at $5^\circ C \pm 2$ and $85\% \pm 5$ relative humidity.

It has been well established that both biotic and abiotic stresses can influence oxidative metabolism and the production of bioactive compounds in plants. Postharvest handling is recognized as a source of abiotic stress (Meitha et al., 2020). In our study, the control treatment, which involved no sanitization, helped isolate the effects of handling-induced stress. Since both control and DTW treatments showed similar patterns in bioactive compound accumulation, the stress observed in these treatments may have originated from biotic sources. Previous studies have demonstrated the efficacy of chemical sanitizers in reducing pathogenic biotic stress (Afolabi et al., 2011; Baia et al., 2020; Chinchkar et al., 2022; FDA, 2009; Kwon et al., 2011; Macnish et al., 2010; Mendoza et al., 2022). In our results, bioactive compound accumulation peaked on day five in control and DTW treatments but declined by day ten (Figure 1A-C), suggesting a transient stress response likely triggered by biotic factors.

Interestingly, omitting washing may be more beneficial than using pure water. The control treatment, which avoided additional handling, showed reduced abiotic stress. Notably, SH treatment preserved flower color best, while DTW-treated flowers exhibited the poorest coloration. This highlights the fact that certain sanitization methods

are more effective than washing with water alone. Thus, when choosing between control and DTW, it may be preferable to skip washing altogether to avoid unnecessary handling that does not contribute to postharvest quality.

Despite differences in bioactive compound levels, antioxidant capacity remained unchanged among treatments (Table 1). The ABTS assay results support the idea that antioxidant metabolism in flowers is complex (Kucekova et al., 2013). The ABTS assay specifically measures the ability of antioxidants to quench the ABTS radical. Thus, if the additional phenolics released or formed have inherently low electron/hydrogen-donating efficiency toward this radical, due to their structure or oxidation state, the ABTS-measured antioxidant activity can remain unchanged (Csepregi et al., 2016). The lack of differences suggests that non-phenolic antioxidants, such as vitamin C or carotenoids, may play a major role in the overall antioxidant capacity. These findings indicate that chlorinated sanitizers enhance the total phenolic content without compromising antioxidant potential, even with added handling.

Histochemical DAB and NBT staining visually demonstrated the role of sanitizers in modulating oxidative metabolism, which is closely linked to stress responses. High levels of H_2O_2 and O_2^- staining

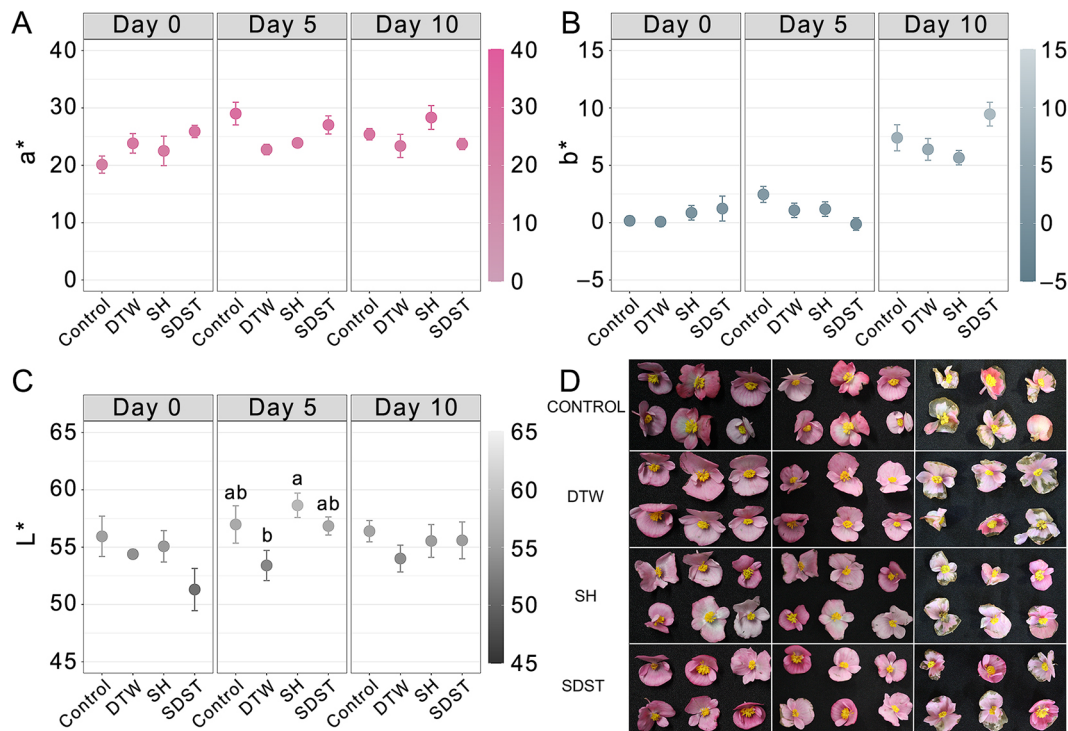


Figure 3 – Color parameters A) green-red (a^*), B) blue-yellow (b^*), C) brightness (L^*), and D) picture of the flowers right after the processing day (Day 0), five days after processing (Day 5), and ten days after processing (Day 10) for the control, distilled water (DTW), sodium hypochlorite (SH), and sodium dichlorite-s-triazinetrione (SDST) treatments, stored at $5^\circ\text{C} \pm 2$ and $85\% \pm 5$ relative humidity. Vertical bars represent the standard error of the mean. Different letters indicate significant differences at $p < 0.05$ ($n = 5$).

in the control and DTW treatments at the end of storage suggest that these flowers experienced higher stress (Figure 2). Interestingly, on day zero, the control treatment had the lowest DAB and NBT staining intensity, indicating that handling during sanitization itself could provoke abiotic stress. ROS bursts induced by stress may lead to tissue browning, commonly associated with phenolic oxidation and polymerization into dark pigments (Landi et al., 2018). Colorimetric analysis supports this, as the lowest brightness (L^*) was observed in DTW-treated flowers on day five, which also exhibited widespread DAB staining (Figure 2 and 3C). No differences were observed in the green-red (a^*) and blue-yellow (b^*) color parameters (Figure 3). ROS induction is regulated by complex signaling cascades involving plant hormones such as C_2H_4 (Zhang et al., 2016). In our study, the highest C_2H_4 production was observed in the control treatment on day three, preceding a strong ROS burst and the peak in bioactive compound accumulation on day five. These results suggest that non-sanitized flowers were under greater stress during postharvest storage.

Our findings demonstrate that chlorine-based sanitizers are more effective than non-chlorinated methods for preserving bioactive compounds in *B. semperflorens* flowers during postharvest storage. Importantly, antioxidant capacity remained stable across treatments, indicating that these sanitization methods do not compromise this key nutritional attribute. These results underscore the importance of selecting appropriate sanitization methods to maintain the nutraceutical quality of edible flowers. Future studies should investigate additional sanitization strategies and their impact on oxidative stress and antioxidant levels. Quantifying other antioxidants such as vitamin C and carotenoids, analyzing defense-related gene expression, and evaluating microbial dynamics will provide deeper insight into the biochemical changes that occur during postharvest handling and storage.

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Authors' Contributions

Conceptualization: Morais M, Preczenhak AP. **Data curation:** Morais M, Orsi B, Sanabria Franco MF. **Formal analysis:** Morais M, Orsi B. **Investigation:** Morais M, Orsi B, Preczenhak AP, Amorim C, Sanabria Franco MF, Vacchi GC, Lopes de Almeida RL, Lopes GB. **Methodology:** Morais M, Orsi B, Preczenhak AP, Amorim C. **Project administration:**

Kluge RA. **Resources:** Kluge RA. **Supervision:** Kluge RA. **Validation:** Orsi B, Preczenhak AP, Kluge RA. **Writing - original draft:** Morais M. **Writing - review and editing:** Morais M, Orsi B, Preczenhak AP, Amorim C, Sanabria Franco MF.

Conflict of interest

Authors declare no conflict of interest.

Data availability statement

Data will be made available upon request.

Declaration of use of AI technologies

No artificial intelligence technologies were used in doing this work.

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