

ARTICLE

Hot water treatments performed in the base of the floral stem reduce postharvest senescence of cutting lily

Os tratamentos com água quente realizados na base do caule floral reduzem a senescência pós-colheita do lírio de corte

Gunther Alfredo Mantilla¹ , Libertad Mascarini¹ , Hugo Daniel Chludil² , and Gustavo Adolfo Martinez^{3,*} 

¹Instituto Nacional de Tecnología Agropecuaria, Instituto de Floricultura, Hurlingham-BA, Argentina.

²Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Producción Vegetal, Cátedra de Floricultura, Buenos Aires-BA, Argentina.

³Universidad Nacional de La Plata, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Fisiología Vegetal, La Plata-BA, Argentina

Abstract: Lilies are commonly grown and marketed as cut flowers. Harvesting causes considerable stress, which triggers accelerated senescence, making this process a limiting factor of their postharvest life. Senescence control is achieved through various methodologies, including moderate-temperature thermal treatments for a short time. These treatments cause mild stress that can affect the tissues metabolism. This study aimed to determine the quality, postharvest life, and metabolism of *Lilium longiflorum* after applying thermal stress to the base of the flower stems. Heat treatments were performed by using hot water (50 °C during 5 min) in the first 2 cm above the cut on the stalk, while a similar treatment was carried out with water at 20 °C for the controls. The treated stems showed less weight loss and water consumption; but the buds opened later. Similarly, less senescence and chlorophyll loss were observed in the leaves of the treated samples. A trend towards a higher concentration of phenols was observed in the first days of storage in the leaves and tepals of the treated stems, no significant variations in flavonoid content were detected. Lower amounts of TBARS and less electrolyte loss were detected in the heat-treated samples, indicating less peroxidation and greater membrane stability. The treatment also induced higher anthocyanin accumulation in tepals. The results suggest that the hot water thermal treatment in postharvest is an appropriate method to improve stem freshness, bud opening degree, and delay senescence, prolonging vase life without negative effects during storage.

Keywords: anthocyanin, heat shock, *Lilium longiflorum*, tepal, vase life.

Resumo: Os lírios são comumente cultivados para serem comercializados como flores de corte. A colheita causa um estresse considerável, que, por sua vez, desencadeia a senescência acelerada, sendo este processo um fator limitante da vida pós-colheita. O controle da senescência é alcançado por meio de várias metodologias, incluindo tratamentos térmicos em temperaturas moderadas por um curto período de tempo. Esses tratamentos geram um estresse leve que pode afetar o metabolismo dos tecidos. O objetivo deste estudo foi determinar a qualidade, a vida pós-colheita e o metabolismo de *Lilium longiflorum* após a aplicação de estresse térmico na base dos caules das flores. Os tratamentos foram realizados durante 5 minutos a 50 °C com água nos primeiros 2 cm da área de corte, enquanto um tratamento semelhante foi realizado com água a 20 °C para o controle. Os caules tratados apresentaram menor perda de peso, menor consumo de água e os botões abriram mais tarde. Da mesma forma, foi observada menor senescência e menor perda de clorofila nas folhas das amostras tratadas. Uma tendência a uma maior concentração de fenóis foi observada nos primeiros dias de armazenamento nas folhas e tépalas dos caules tratados, enquanto não foram detectadas variações significativas no teor de flavonoides. Menores quantidades de TBARS e menor perda de eletrólitos foram detectadas nas amostras tratadas com calor, indicando menor peroxidação e maior estabilidade da membrana. O tratamento também induziu maior acúmulo de antocianinas nas tépalas. Os resultados sugerem que o tratamento térmico com água quente no pós-colheita é um método adequado para melhorar a qualidade das hastes, o grau de abertura dos botões e atrasar a senescência, prolongando a vida em vaso sem efeitos negativos durante o armazenamento.

Palavras-chave: antocianina, choque térmico, *Lilium longiflorum*, tépalas, vida útil em vaso.

Introduction

The lily is a highly valued ornamental species and one of the most economically important within the floriculture sector. With a wide range of colors, shapes, and sizes, lilies are prominent in the floral industry, adorning bouquets, arrangements, and gardens alike. However, despite their aesthetic appeal and popularity, lilies face notable challenges related to postharvest senescence, which can negatively affect their marketability and overall quality. Senescence is a physiological deterioration that can begin prematurely in response to a stressful situation, such as harvesting (Wang et al., 2020). Senescence is accompanied by numerous physiological, biochemical, and molecular changes that lead to degradation of cellular structures, and in the early stages involves nutrient remobilization from senescing organs to other parts of the plant (Zhang et al., 2021).

In cut flowers, senescence is manifested as leaf yellowing and wilting in sepals or tepals, processes regulated by external and internal factors integrated into the senescence program (Sun et al., 2021). Leaf yellowing, a common occurrence that often precedes flower senescence, can drastically reduce the ornamental value of the plant.

Various strategies have been implemented to reduce postharvest senescence in lilies. Among these, hormone-based sprays (combinations of gibberellins and benzyladenine) effectively reduce leaf yellowing in various lily cultivars. However, these treatments may not be effective

when leaf senescence occurs without ethylene, such as during storage under low-temperature conditions (Van Doorn and Han, 2011).

In addition to hormone-based solutions, the potential of thermal treatments has been explored as a non-chemical alternative to prevent leaf yellowing and tepal wilting. Thermal treatments, which have been effective in disinfection and disease reduction in other flower crops, could provide a promising solution for extending the vase life of lilies. The use of thermal treatments with moderate temperatures does not cause irreversible damage but triggers changes and mechanisms that allow plants to cope with new stress. These changes include maintaining membrane stability, removing reactive oxygen species (ROS), producing antioxidants, and accumulating compatible solutes. Therefore, hydrothermal exposure induces momentary stress in the plant, reducing the expression of genes related to these processes and thereby delaying normal senescence. Immersion treatment in hot water has successfully extended postharvest life of cut ornamentals, such as red ginger flowers (Chantrachit and Paull, 1998) or *Banksia* spp (Seaton and Joyce, 1993).

In vegetables, the use of thermal treatments to delay senescence has been extensively studied (Šola et al., 2023). For example, in broccoli, thermal treatments have been applied to the entire head as well as to the cut area of the stem (Perini et al., 2017), the section of tissue with the higher rate of ethylene biosynthesis (Pattyn et al., 2020). In both types of

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treatment: heating of the entire head or eating only in the stem cut area, a lower rate of senescence was detected during broccoli postharvest storage.

Based on the foregoing, in this study we applied a hot water thermal treatment to the first 2 cm of the stem cutting zone of 'Forza Red' lily. The objective of this research was to determine the effect of such type of treatment on flower senescence and quality during post-harvest storage, aiming to extend its shelf life and enhance its commercial appearance.

Materials and methods

Plant material, treatments and samples

Plant materials were obtained from a crop of *Lilium longiflorum* cv. 'Forza red'. Planting was carried out in a greenhouse with a 150 µm thermal polyethylene cover, equipped with passive lateral and zenithal ventilation and an automated irrigation system, in beds of 0.4 m width x 0.3 m depth x 20 m length, with perlite substrate.

Bulbs of 16-18 cm caliber were obtained from a local representative and placed at a density of 20 bulbs m⁻². The average ambient temperature during cultivation was 26.6 °C. Irrigation was applied daily, using drip tapes with a nominal flow rate of 1 L h⁻¹ per emitter, with 20 emitters per linear meter of bed. The irrigation rate was established according to the estimated evapotranspiration using the Penman-Monteith method modified by FAO (Allen et al., 2000) and fertilization began to be applied after stem emergence, approximately 20 days after planting. When the crop reached commercial maturity (first sprout showing color) at 23 weeks of age, harvesting was carried out and samples were immediately transported to the laboratory.

A total of 80 lily stems (*Lilium spp.*), homogeneous in physiological development and number of buds, were used. Two treatments were applied: (i) immersion of the stem base (2 cm) in hot water at 50 °C for 5 minutes and (ii) immersion in water at 20 °C for the control group. Each treatment included 40 stems. After treatment, the stems were placed in previously disinfected 250 mL test tubes containing distilled water and sealed with plastic film to prevent evaporation. The stems were stored at 16 °C, with 54% relative humidity (RH), and under a 12-hour photoperiod provided by 36 W white fluorescent lamps (irradiance: 6 W m⁻²). The distilled water used for maintenance had a pH of 5.8, total dissolved solids (TDS) of 5 ppm, and electrical conductivity (EC) of 0.6 µS cm⁻¹. Water consumption was replenished daily based on stem uptake.

Samples were stored for 12 days, during which water consumption, weight loss, and the degree of flower opening were recorded. Additionally, leaf 3 was used for SPAD index measurement, while leaves from the first (1 - 3) and second third (4 - 6) of the stem were analyzed for chlorophyll content, total sugars, phenols, and flavonoids. To make the method clearer, the working procedure is made in schematic form (Fig. 1).

On the other hand, the internal tepals from the 3 terminal blossoms, where blossom 1 (main axis terminal tepal), blossom 2 (second order axis

terminal tepal) and blossom 3 (third order axis terminal tepal) were used for anthocyanins, sugars, phenols, flavonoids, TBARS and electrolyte leakage determinations. In all cases (except for electrolyte loss measurements), samples were frozen with liquid nitrogen and stored at -80 °C until use.

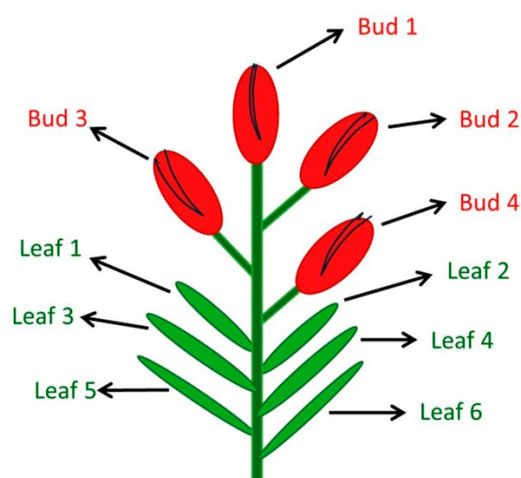


Fig. 1. Diagram representing the positions of buds and leaves used for the different determinations.

Analytical determinations

Water consumption

Daily measurements of the volume of water in the test tube containing the flowering stem were made at the same hour of each day of storage. Water consumption was evaluated by the differences in volume detected in 24 h.

Weight Loss

Daily variations in the fresh weight of lily stems were recorded during the trial. Weight loss was evaluated as a percentage of the initial weight and labeled as weight loss (%).

Degree of opening of the blossoms

Morphological changes in the blossoms were determined descriptively, using a scale of opening grades from grade one (I) in ascending order up to grade six (VI): grade I (closed bud showing color), grade II (initial bud opening), grade III (anthesis), grade IV (turgid tepals), grade V (dehydrated tepals, color changes), grade VI (senescent tepals) (Adapted from Arrom and Munné-Bosch, 2012) (Fig. 2).



Fig. 2. Degree of opening of lily blossoms

SPAD measurements

Leaf senescence was monitored by estimating chlorophyll from the green index measurement (SPAD) according to Mantilla et al. (2021). A Konica-Minolta SPAD-502, Japan, was used. Three determinations were made at the same leaf position three of each cane. The data were expressed in SPAD (Soil Plant Analysis Development) units which indicate a specific

dimensionless measure of the relative amount of chlorophyll present in the leaves and photosynthetic activity.

Chlorophyll content measurement

Approximately 0.1 g of frozen leaves were taken and vigorously mixed with 1 ml of acetone and allowed to stand for 4 h at 4 °C in the

dark. The resulting suspension was centrifuged at $10,000 \times g$ for 10 min at 4°C . The chlorophyll content in the supernatant was determined spectrophotometrically according to Barriga Lourenco et al. (2023). All measurements were performed in triplicate and expressed as grams of chlorophyll per kg of fresh tissue.

Ethanol extracts preparation

Approximately 0.5 g of frozen tepals or leaves were ground in liquid nitrogen and mixed with 3 mL of methanol 80% for 24 hours. The mixture was centrifuged for 10 min at $13,000 \times g$ and 4°C . The supernatant was recovered and utilized to measure flavonoids, and phenolic compounds. Three extracts from each condition were obtained.

Total sugar content

The determination of sugar content was carried out from frozen tissue by using the phenol-sulfuric acid method (Barriga Lourenco et al., 2023). Approximately 5 mg of tissue sample were weighed and homogenized with 1 mL of distilled water and 1 mL of H_2SO_4 98% w w⁻¹, the volume was adjusted to 5 mL with water and shaken for 30 minutes. Homogenate was centrifuged for 10 min at $10000 \times g$ and 4°C . Then, 200 μL were taken from the supernatant, and the volume was adjusted to 500 μL with water and added with 0.5 mL of phenol 10% w v⁻¹ and 2.5 mL of H_2SO_4 98% w w⁻¹. Samples were left to stand for 20 min at room temperature and absorbance at 490 nm was measured in a spectrophotometer (T70 UV-VIS, PG Instruments, United Kingdom). Three extracts from each condition were obtained and three replicates per extract were determined. Glucose was used as standard and results were expressed as gram of glucose per kilogram of fresh tissue.

Determination of total phenolic compounds

Total phenol content was determined using the Folin-Ciocalteu reagent (Barriga Lourenco et al., 2023). 100 μL of extracts in methanol were mixed with 1.0 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (1:1 v v⁻¹). After mixing, 1.5 mL of 2% w v⁻¹ Na_2CO_3 was added and incubated at room temperature for 30 min with intermittent shaking. Absorbance was measured at 765 nm. three replicates per extract were determined. Calibration curve was performed with gallic acid as standard and total phenolic content was expressed as grams gallic acid equivalent (GAE) per kilogram of fresh tissue.

Total flavonoids content

Total flavonoids content was determined by using a protocol according to Barriga Lourenco et al. (2023). Briefly, 400 μL (tepals) or 200 μL (leaves) of methanolic extract, 100 μL of 5% w v⁻¹ NaNO_2 , 100 μL of 10% w v⁻¹ AlCl_3 , 3 mL of distilled water and 1.7 mL of 95% methanol were mixed. The mixture was homogenized and incubated for 45 minutes. Absorbance was read at 415 nm. Measurements were done in triplicate for each extract. The calibration curve was constructed using catechin as a standard. The results were expressed as g of catechin equivalent per kilogram of fresh tissue.

Determination of TBARS

Thiobarbituric acid reactive substances (TBARS) were measured according to Page et al. (2001). Frozen plant material 0.5 g was homogenized with 1 mL trichloroacetic acid (10% w v⁻¹), 10 mL acetone was added and centrifuged at $1300 \times g$ for 15 min. The precipitate was washed with 5 mL of acetone, vortexed and then centrifuged at $1300 \times g$ for 10 min (washing was repeated 4 times). The precipitate was dried under N_2 and incubated at 100°C for 30 min with 3 mL of 1% w v⁻¹ H_3PO_4 and 1 mL of 0.6% w v⁻¹ thiobarbituric acid. The reaction was terminated by cooling the tubes on ice and 3 mL of 1-butanol was added. The samples were shaken vigorously to achieve an emulsion and then centrifuged to separate the phases. Absorbance was measured at 530 nm in the aqueous phase. Three extracts were prepared for each storage time and measurements were performed in duplicate on each extract. The results were expressed as Abs 530 nm per g of tissue.

Electrolyte leakage

Electrolyte leakage was determined according to Aliniaiefard et al. (2020) with some modifications. Approximately five grams of fresh tepals were weighed and placed in 30 mL of an iso-osmotic mannitol-water solution, then incubated at 20°C for 1 h. The conductivity of the solution was measured at the beginning and at the end of the incubation period using a Hanna Instruments 9813-6 conductivity meter (Woonsocket, USA). The total electrical conductivity was obtained by boiling the sample for 10 min. The relative electrolyte leakage was expressed as a percentage of the total conductivity. Measurements were performed in triplicate.

Anthocyanins determination

Anthocyanin content of the tepal extract was measured using the pH differential method described by Giusti and Wrolstad (2001). Approximately 0.2 of frozen petals were homogenized with 1 mL of methanol and left to stand at 4°C overnight. After that, homogenates were centrifuged at $10,000 \times g$ during 10 min at 4°C . The total anthocyanin content was determined using the differential pH method and employing two solutions systems: potassium chloride (pH 1.0; 0.025 M) and sodium acetate (pH 4.5; 0.4 M). The extract (400 μL) was mixed with 3.6 mL of the respective solutions, and absorbance was read at 510 and 700 nm. The total anthocyanin content was measured via spectrophotometry using the formula $A = [(A_{510} - A_{700}) \text{pH } 1.0 - (A_{510} - A_{700}) \text{pH } 4.5]$ with a molar extinction coefficient of 29600 (cyanidin-3-glucoside). Results are expressed as milligrams of cyanidin-3-glucoside equivalents per gram of plant material.

Statistical analysis

A complete randomized design was used for the experiments, consisting of two treatments: a thermal treatment and a control. Each treatment included 40 experimental units, totaling 80 plants. Four replicates were assigned per treatment. Data were analyzed using ANOVA in InfoStat v.2020 software, and mean comparisons were performed using Dunnett's test ($p < 0.05$). Analysis of variance (ANOVA) is the most used and appropriate methodology for conducting statistical analysis in this type of trial. Dunnett's test is a post hoc statistical test used in ANOVA when comparing an experimental group with a single reference control group. In the case of bud opening grade, an analysis was performed using the Wilcoxon signed rank test. The Wilcoxon Signed-Rank test is used when dealing with ordinal scales or ranks instead of precise numerical values. The whole experiment was repeat twice.

Results and Discussion

Lilium longiflorum is one of the most important cut flowers employed as ornamentals. However, its short postharvest life limits its uses and reduces the possibility of commercialization for extended periods. One way to prolong the postharvest life of flowers and horticultural products is through heat treatments (Malakar et al., 2023). These treatments consist of brief heat pulses that induce non-lethal stress, allowing the tissue to recover and potentially gain some resistance to the progression of senescence. In this study, it was applied a heat treatment with hot water only to the base of the stem, where mechanical damage and cutting stress occurred.

Table 1 shows the water consumption and weight loss recorded during the postharvest storage of the stems. Overall, a trend towards lower water consumption was detected in the treated samples on all storage days. Similarly, the stems subjected to the treatment showed less weight loss, particularly towards the end of the storage period. Cut flowers maintain their metabolism active even after being harvested, including water transpiration. Likewise, harvesting triggers senescence, which leads to the degradation of macromolecules, causing weight loss, and to the dismantling of membranes, further increasing water loss. Heat treatment, as shown in the results below, delays the progression of senescence by reducing metabolism and consequently decreasing weight loss while maintaining membrane integrity, thereby slowing down water consumption.

Table 1. Water consumption (mL day⁻¹) and weight loss (%) recorded during postharvest storage at 16 °C of *L. longiflorum* sticks which has been applied water at 50 °C for 5 min at the base of the stem (HT). Control samples were treated at 20 °C for 5 min.

Days	Water Consumption		Weight loss	
	Control	HT	Control	HT
3	44.6 ± 1.5	42.5 ± 1.9	0.1 ± 0.1	0.2 ± 0.1
6	54.2 ± 1.3	49.0 ± 2.1 *	0.8 ± 0.5	0.4 ± 0.1
9	58.6 ± 1.1	45.4 ± 2.5 *	6.1 ± 0.3	5.2 ± 0,4
12	45.8 ± 1.9	25.2 ± 2.0 *	30.3 ± 1.5	14.5 ± 1,8 *

Asterisks indicate significant differences at the same storage time ($p < 0.05$).

In the following determinations, analyses were performed on the four main buds and on groups of leaves separately, as their different stages of ontological development may be affecting the chemical composition to be analyzed.

The opening of buds and the development as well as progression of flower senescence are central characteristics in determining the quality of lily stems. Figure 3 shows the opening grade of each of the first

four flowers on the stem. A delay in the opening grade was observed in thermally treated stems. Significant differences were detected using the Wilcoxon signed-rank test during storage days 4 to 10.

The time required for each bud to reach opening grade 5 was also analysed. It was found that the time required to reach this stage was longer in treated stems for all the buds analyzed (Table 2).

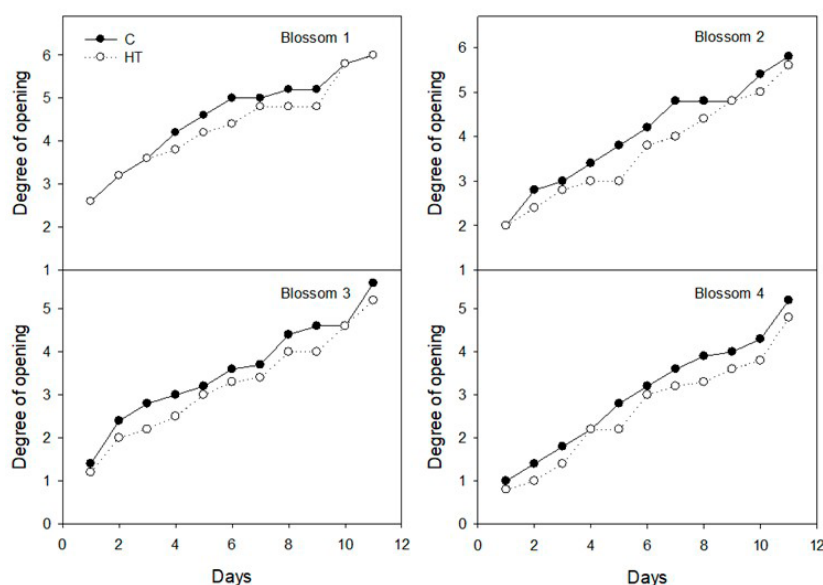


Fig. 3. Degree of flower opening in different stem positions of *Lilium longiflorum* during postharvest storage. C: Control. HT: Heat Treated

Table 2. Postharvest storage days required to reach stage 5 in the first four blossoms of *L. longiflorum* stems stored at 16 °C.

	Control	HT
Blossom 1	5.4 ± 0.3	7.0 ± 0.8*
Blossom 2	6.4 ± 0.4	9.0 ± 0.5*
Blossom 3	8.6 ± 0.4	10,4 ± 0.6*
Blossom 4	9.6 ± 0.5	10,6 ± 0.2*

Asterisks indicate significant differences ($p < 0.05$).

As previously described, the tissues of cut flowers remain active after harvest, and one of the processes that continues to develop is bud opening. Considering that heat treatment likely causes a decrease in metabolism, this may lead to a delay in bud opening in the treated samples.

One of the most notable factors in the progression of senescence is leaf yellowing, which can be evaluated through chlorophyll content. This parameter was estimated using SPAD readings and chemical determination (Table 3).

Table 3. SPAD values (Leaf 3) and chlorophyll content (expressed as g kg⁻¹ fresh weight) in leaves of groups A (positions 1 and 2) and B (positions 4; 5, and 6) measured in *L. longiflorum* sticks.

Days	Leaves A (1 and 2)						Leaves B (4; 5 and 6)			
	SPAD (leaf 3)		Chlorophyll a		Chlorophyll b		Chlorophyll a		Chlorophyll b	
	Control	HT	Control	HT	Control	HT	Control	HT	Control	HT
0	69.8 ± 0.8	70.2 ± 0.6	0.49 ± 0.09	0.51 ± 0.10	0.22 ± 0.07	0.23 ± 0.59	0.44 ± 0.07	0.46 ± 0.10	0.20 ± 0.08	0.21 ± 0.04
3	65.4 ± 0.5	66.6 ± 0.8	0.45 ± 0.08	0.53 ± 0.09	0.21 ± 0.09	0.24 ± 0.07	0.46 ± 0.08	0.49 ± 0.06	0.19 ± 0.04	0.22 ± 0.10
6	60.7 ± 0.8	64.3 ± 0.7*	0.46 ± 0.07	0.56 ± 0.08*	0.19 ± 0.05	0.24 ± 0.10*	0.47 ± 0.04	0.53 ± 0.05	0.20 ± 0.04	0.23 ± 0.03
9	54.0 ± 0.3	59.8 ± 0.1*	0.35 ± 0.10	0.44 ± 0.02*	0.12 ± 0.04	0.20 ± 0.06*	0.29 ± 0.09	0.40 ± 0.08*	0.11 ± 0.01	0.17 ± 0.02*
12	43.2 ± 0.4	53.9 ± 0.6*	0.29 ± 0.03	0.37 ± 0.06*	0.09 ± 0.01	0.14 ± 0.02*	0.22 ± 0.02	0.31 ± 0.02*	0.07 ± 0.02	0.11 ± 0.02

Asterisks indicate significant differences at the same storage time ($p < 0.05$).

During storage, SPAD values and chlorophyll a and b content decreased. However, this decline was more pronounced in the control samples than in the treated ones. In particular, on days 9 and 12, a marked difference in chlorophyll content was observed, with significantly higher values in the stems treated with hot water (HT).

In many cases, postharvest senescence of cut flowers is accelerated by ethylene. In *Lilium*, the effects of ethylene are controversial; some varieties appear to be ethylene-sensitive, while others seemingly do not respond to this hormone (Al-Ajlouni et al., 2023). In some cases, 1-MCP (1-methylcyclopropene, an inhibitor of ethylene action) treatments have proven effective in reducing senescence in *Lilium* (Costa et al., 2021). Ethylene is continuously synthesized under normal conditions, but its production is also induced by mechanical damage (Ferrante, 2023). In

cut flowers and horticultural products, this damage occurs in the area where the cut is made during harvest. In broccoli, it has been shown that the tissue just a few centimeters near the cut is the area with the highest prevalence of ethylene synthesis (Kato et al., 2002). Heat treatments applied to the stem base effectively prevent the degreening of broccoli heads, similar to the delay in leaf yellowing observed in *Lilium* (Perini et al., 2017)

The concentration of sugars is strongly linked to the progression of senescence. It has been reported that the sugar content at harvest influences the postharvest life of *Lilium* flowers (Sun et al., 2022). Likewise, preserving flowers with stems submerged in sugar-containing solutions is a common postharvest practice (Zhang et al., 2022). Table 4 shows the results of total sugar content in the tepals and leaves of *Lilium* stems.

Table 4. Total sugars content (expressed as gram of glucose per kg of fresh weight) measured in tepals and in leaves of groups A (positions 1 and 2) and B (positions 4; 5 and 6) of *L. longiflorum* sticks.

Days	Tepal 1		Tepal 2		Tepal 3		Leaves A (1 and 2)		Leaves B (4; 5 and 6)	
	Control	HT	Control	HT	Control	HT	Control	HT	Control	HT
0	0.38 ± 0.04	0.37 ± 0.08	0.36 ± 0.03	0.38 ± 0.10	0.39 ± 0.02	0.34 ± 0.04	0.40 ± 0.04	0.38 ± 0.04	0.39 ± 0.01	0.37 ± 0.02
3	0.36 ± 0.02	0.32 ± 0.03	0.33 ± 0.04	0.35 ± 0.09	0.34 ± 0.05	0.31 ± 0.10	0.35 ± 0.07	0.36 ± 0.07	0.31 ± 0.10	0.30 ± 0.03
6	0.32 ± 0.01	0.34 ± 0.06	0.29 ± 0.04	0.35 ± 0.03*	0.32 ± 0.06	0.31 ± 0.03	0.36 ± 0.04	0.34 ± 0.08	0.30 ± 0.06	0.29 ± 0.08
9	0.28 ± 0.05	0.36 ± 0.05*	0.29 ± 0.05	0.31 ± 0.05	0.30 ± 0.07	0.28 ± 0.02	0.30 ± 0.01	0.35 ± 0.03*	0.29 ± 0.02	0.31 ± 0.04
12	0.32 ± 0.08	0.32 ± 0.03	0.29 ± 0.01	0.33 ± 0.06	0.27 ± 0.06	0.37 ± 0.05*	0.24 ± 0.03	0.28 ± 0.04	0.26 ± 0.02	0.31 ± 0.05*

Asterisks indicate significant differences at the same storage time ($p < 0.05$).

In control samples, a general decrease in sugar content was detected in both tepals and leaves, although the decrease was more pronounced in leaves. In tepal 1, which typically exhibits more advanced senescence than the others, there is an increase in sugar concentration towards the last day of storage, suggesting a probable degradation of the cell wall. The stems treated with hot water maintained a slightly higher sugar concentration on certain days of storage, which varied depending on the tepal position, indicating lower sugar consumption that correlates with slower senescence progression.

One of the metabolic objectives of senescence is nutrient recycling, for which energy is required, usually obtained from sugar consumption.

Simple sugars are generated in the first stage by the hydrolysis of starch and gluconeogenesis of amino acids and lipids, and in the second stage by the degradation of cell walls. Therefore, the content of simple sugars may decrease in the first few days and then increase due to the contribution of cell wall breakdown (Barriga Lourenco et al., 2023).

Phenolic compounds have numerous and varied functions in plants. One of them is to act as protective agents against oxidative stress (Zagoskina et al., 2023). During senescence, a significant accumulation of ROS contributes to tissue deterioration (Zhao et al., 2022; Hajam et al., 2023). Table 5 shows the content of phenols in leaves and tepals.

Table 5. Content of total phenolics (expressed as gram of gallic acid per kg of fresh weight) measured in tepals and in leaves of groups A (positions 1 and 2) and B (positions 4; 5 and 6) of *L. longiflorum* sticks

Days	Tepal 1		Tepal 2		Tepal 3		Leaves A (1 and 2)		Leaves B (4; 5 and 6)	
	Control	HT	Control	HT	Control	HT	Control	HT	Control	HT
0	0.90 ± 0.05	0.94 ± 0.07	0.92 ± 0.03	0.89 ± 0.09	0.90 ± 0.10	0.92 ± 0.06	1.02 ± 0.05	0.99 ± 0.07	1.16 ± 0.07	1.13 ± 0.10
3	0.92 ± 0.03	1.49 ± 0.4	1.01 ± 0.05	1.37 ± 0.06*	1.03 ± 0.09	0.95 ± 0.07	0.92 ± 0.10	0.94 ± 0.05	1.13 ± 0.10	1.08 ± 0.08
6	0.88 ± 0.02	1.46 ± 0.05*	0.95 ± 0.12	1.31 ± 0.07*	1.02 ± 0.02	1.10 ± 0.05	0.91 ± 0.11	0.95 ± 0.08	0.80 ± 0.09	1.15 ± 0.05*
9	1.33 ± 0.06	1.48 ± 0.03*	0.95 ± 0.09	1.26 ± 0.06*	1.21 ± 0.13	1.44 ± 0.07*	0.93 ± 0.13	0.95 ± 0.05	0.83 ± 0.04	1.14 ± 0.12*
12	1.88 ± 0.10	0.91 ± 0.04*	1.59 ± 0.11	1.08 ± 0.09*	1.22 ± 0.10	1.32 ± 0.09	1.09 ± 0.08	0.96 ± 0.09	0.62 ± 0.09	0.84 ± 0.09*

Asterisks indicate significant differences at the same storage time ($p < 0.05$).

An increase in total phenol values was detected in the tepals of control rods during storage. This increase was much more marked in the tepals of the treated rods on days 3, 6, and 9. By day 12, the increase stopped, so that lower values were obtained in the treated rods with respect to the controls. In the leaves of group A (positions 1 and 2), the total phenol content did not show significant changes during storage. On the other hand, in the leaves of group B, the total phenol content decreased with storage time in the controls and remained higher in the treated leaves on days 6, 9, and 12.

One of the common plant responses to stress conditions is an increase in the synthesis of phenols, which act as protectors against the action of free radicals. Several studies have shown that moderate postharvest heat treatment can act as an activator of the biosynthesis of phenolic compounds through the activation of the transcription of genes such as phenylalanine ammonia-lyase (PAL) (Perini et al., 2017, and the references therein).

Regarding flavonoid content, an increase in the concentration of these compounds was detected in untreated tepals during storage (Table 6).

Table 6. Content of flavonoids (expressed as gram of catechin per kg of fresh weight) measured in tepals and in leaves of groups A (positions 1 and 2) and B (positions 4; 5 and 6) of *L. longiflorum* sticks.

Days	Tepal 1		Tepal 2		Tepal 3		Leaves A (1 and 2)		Leaves B (4; 5 and 6)	
	Control	HT	Control	HT	Control	HT	Control	HT	Control	HT
0	10.90 ± 0.09	11.05 ± 0.07	12.10 ± 0.02	12.86 ± 0.10	10.99 ± 0.03	10.92 ± 0.03	36.97 ± 0.11	35.95 ± 0.05	31.16 ± 0.08	30.14 ± 0.09
3	11.60 ± 0.10	15.40 ± 0.05*	12.24 ± 0.04	14.25 ± 0.04*	11.27 ± 0.09	10.19 ± 0.11	36.48 ± 0.08	29.93 ± 0.09	32.88 ± 0.04	31.94 ± 0.12
6	13.57 ± 0.08	13.93 ± 0.08	11.52 ± 0.07	12.44 ± 0.05	11.67 ± 0.06	13.41 ± 0.07*	35.82 ± 0.09	22.24 ± 0.07*	31.98 ± 0.06	39.23 ± 0.09
9	15.12 ± 0.02	12.97 ± 0.07*	13.02 ± 0.04	11.65 ± 0.04*	13.91 ± 0.03	14.84 ± 0.06	34.66 ± 0.06	25.20 ± 0.05*	33.43 ± 0.05	40.13 ± 0.07*
12	16.70 ± 0.04	12.41 ± 0.06*	16.54 ± 0.10	12.17 ± 0.08*	15.48 ± 0.02	13.07 ± 0.03*	39.32 ± 0.06	25.67 ± 0.04*	37.76 ± 0.05	37.66 ± 0.06

Asterisks indicate significant differences at the same storage time ($p < 0.05$).

Heat treatment generated a different pattern of change in flavonoid content, with a significant increase at short days of storage (day 3 in tepals 1 and 2; day 6 in tepal 3) and a decrease towards the end of storage (day 12). In the case of leaves, decrease in phenol content was detected in the A group of the treated samples, and no differences were observed between controls and treated samples in the B group. B. These results suggest that hot water treatment may generate some stress that favors the accumulation of phenols and flavonoids, in this case at short times, and particularly in tepals, that in turn could contribute to protection against oxidative stress during postharvest storage, in agreement with the findings of Lu et al.

(2020) on the use of selenium to regulate postharvest physiological characteristics in *Lilium*. Similarly, Al Fayad and Othman (2024) found that pre-harvest GA3 treatments determine greater postharvest longevity of *Lilium* flowers, which correlates with greater accumulation of phenolic compounds.

The increase in ROS and the marked catabolism that occurs during senescence leads to lipid oxidation, which in turn leads to the deterioration of cell membranes (Rogers and Munné-Bosch, 2016). A good indicator of this deterioration is the measurement of thiobarbituric acid reactive substances (TBARS) (Fig. 4).

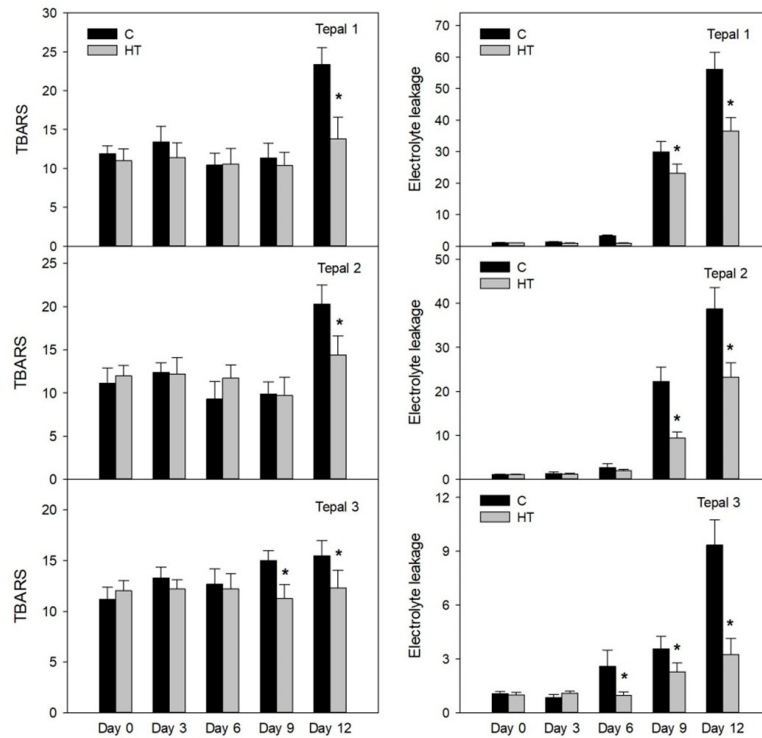


Fig. 4. TBARS and electrolyte leakage (expressed as percentage of the total conductivity) measured in tepals of *Lilium longiflorum* sticks. Measurements were done during postharvest storage at 16 °C of sticks treated 5 min with water at 50 °C at the base of the stem (HT) and control sticks (treated at 20 °C for 5 min).

We detected a marked increase in TBARS values in tepals 1 and 2 around day 12 in the control samples. This increase was significantly lower in treated samples. Differently, in tepal 3 the increase in TBARS in the controls was more gradual and of smaller magnitude, while no increase was detected in the treated ones.

Damage to cell membranes can be further assessed by measuring of electrolyte leakage. An increase in these values was detected during storage of *Lilium* (Fig. 4). The increases were greater in tepal 1 relative to 2, and in the latter relative to tepal 3, suggesting a greater deterioration of cell membranes in tepal 1. Also, electrolyte leakage was generally lower in the heat-treated samples, particularly towards the storage end.

Taken together, results indicate that hot water treatment may have a protective effect against lipid oxidation and membrane deterioration in *Lilium* tepals. Similarly, postharvest treatments such as H₂ fumigation (Wu et al., 2024) or storage in solutions containing gibberellic acid, benzyl amino purine and sucrose (Singh et al., 2008) improve postharvest life by reducing lipid peroxidation (measured as TBARS) and membrane permeability.

Anthocyanin content is an indicator of the quality of *Lilium* flowers. In most plants, anthocyanin synthesis in the flowers is under developmental regulation and its accumulation coincides with petal growth and in the later stages of petal development (Muhammad et al., 2022). In this case an increase in anthocyanin content was detected up to day 9 in untreated tepals coinciding with development even after harvest (Table 7).

Table 7. Content of anthocyanins (expressed as mg cyanidin-3-glucoside g⁻¹) measured in tepals of *L. longiflorum* sticks.

Days	Tepal 1		Tepal 2		Tepal 3	
	Control	HT	Control	HT	Control	HT
0	9.76 ± 0.19	9.66 ± 0.32	10.02 ± 0.13	9.87 ± 0.19	10.52 ± 0.25	10.33 ± 0.22
3	11.06 ± 0.43	10.94 ± 0.29	11.06 ± 0.22	10.40 ± 0.44	11.42 ± 0.31	10.88 ± 0.40
6	17.72 ± 0.13	16.69 ± 0.27	16.45 ± 0.36	12.78 ± 0.54*	18.65 ± 0.78	13.04 ± 0.29*
9	23.44 ± 0.33	24.94 ± 1.19	23.23 ± 0.99	22.97 ± 1.09	20.82 ± 0.84	21.47 ± 0.59
12	16.99 ± 1.78	29.87 ± 0.98*	14.78 ± 0.46	26.56 ± 1.68*	13.82 ± 0.49	23.62 ± 0.84*

Different letters indicate significant differences at the same storage time ($p < 0.05$).

However, anthocyanin levels decreased by day 12. In contrast, a continuous increase in anthocyanin content was observed in the heat-treated samples, including day 12. Consequently, the treated samples showed higher anthocyanin content by the end of storage than the controls in all three tepals. These results suggest that in the controls the accumulation of anthocyanins was halted by the advance of senescence, while in the treated ones, considering that senescence is delayed,

a continuous synthesis could be detected, thus allowing a greater accumulation of pigments, which could contribute to their coloration and better visual attractiveness. It has been described that the use of simple sugars in flower preservation solutions can cause an increase in anthocyanin concentration (Zhang et al., 2022 and the references therein). These authors demonstrated that simple sugars activate the transcription of MYB2, a transcription factor that induces anthocyanin synthesis. In the

present work, treated samples presented a higher concentration of sugars, probably due to a lower consumption. This higher level of sugars could be, in turn, a stimulus for anthocyanin accumulation in heat treated samples.

Conclusions

In this study, brief hot water treatments applied to the stem cutting area delayed postharvest senescence in *Lilium longiflorum*. The treated stems showed reduced water consumption, weight loss, and bud opening, with a clear delay in the time required to reach stage 5 (dehydrated tepals). The treatment resulted in less yellowing and chlorophyll loss in the leaves, while increasing the levels of phenols and flavonoids in both leaves and tepals. Additionally, a lower concentration of TBARS and greater membrane integrity was observed in the petals of the treated stems, which could be associated with higher antioxidant levels (phenols and flavonoids). The stems treated with hot water tended to maintain a slightly higher sugar concentration, with variations depending on the tepal position. This also suggested lower sugar consumption, which was associated with a slower progression of senescence. Probably, this higher sugar content and mild stress are the cause of the increased anthocyanin levels in the petals of the thermally treated samples. These findings could be valuable for the commercial floriculture industry, as they provide a potential means to extend the vase life of cut lily flowers

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Author Contribution

GM: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing – Review & Editing. **LM:** Conceptualization, Writing – Review & Editing. **HC:** Supervision, Conceptualization, Writing – Review & Editing. **GAM:** Conceptualization, Supervision, Data Curation, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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