

## SCIENTIFIC ARTICLE

## Ethanol and citric acid improve longevity in *Gerbera* cv. Mistique

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### Abstract

Ethanol and citric acid can increase longevity in some cut flowers. However, its use must be extremely careful, with application methods and specific concentrations for each type of cut flower. Thus, the objective was to examine the physico-chemical and physiological changes in *Gerbera* cv. Mistique cut flowers treated with ethanol (Et) and/or citric acid (CA). Stems were selected and standardized to a length of 35 cm and maintained at  $20 \pm 2$  °C and RH  $65 \pm 2\%$ , under continuous lighting. The flowers were subjected to two application methods (pulsing for 48 h and maintenance), using different concentrations of Et (4%, 6%, and 8%) and/or CA (100 and 200 mg L<sup>-1</sup>) and distilled water (control). A visual assessment and analyses of longevity, stem bending, fresh mass, relative water content, electrolyte leakage, and peroxidase and polyphenol oxidase enzyme activities were performed at every two days. The Et (4%) + CA (100 mg L<sup>-1</sup>) solution provided the greatest longevity, regardless of the application method. These results were the basis for a third experiment, in which the stems were immersed in pulsing solutions of Et (4%) and/or CA (100 mg L<sup>-1</sup>) and distilled water (control). The *Gerbera* flowers under Et + CA solution showed lower fresh mass loss and electrolyte leakage, higher relative water content and a slower increase in polyphenol oxidase and peroxidase activities. This allowed for delayed stem bending and better appearance, resulting in greater longevity compared to the other solutions.

**Keywords:** *Gerbera jamesonii*, flowers, postharvest preservation, pulsing and maintenance.

### Resumo

#### Etanol e ácido cítrico aumentam a longevidade de gérbas cv. Mistique

Etanol e ácido cítrico podem aumentar a longevidade em algumas flores de corte. Porém, seu uso deve ser de extremo cuidado, com métodos de aplicação e concentrações específicas para cada tipo de flor de corte. Assim, o objetivo foi estudar as mudanças físico-químicas e fisiológicas na longevidade de gérbas de corte cv. Mistique, submetidas a etanol (Et) e/ou ácido cítrico (AC). As hastes foram selecionadas e padronizadas a 35 cm de comprimento, mantidas a  $20 \pm 2$  °C e UR  $65 \pm 2\%$ , sob iluminação contínua. As gérbas foram submetidas a dois métodos de aplicação (*pulsing* 48 horas e manutenção), sob diferentes concentrações de Et (4%, 6% e 8%) e/ou AC (100 e 200 mg L<sup>-1</sup>) e água destilada (controle), a cada dois dias foi realizada análise visual, longevidade, tombamento das hastes, massa fresca, conteúdo relativo de água, extravasamento de eletrólitos e atividades das enzimas peroxidase e polifenoloxidase. Observou-se que a solução de Et (4%) + AC (100 mg L<sup>-1</sup>), proporcionou maior longevidade, independente do método de aplicação. Estes resultados foram base para um terceiro experimento, no qual as hastes foram imersas por *pulsing*, em soluções de Et (4%) e/ou AC (100 mg L<sup>-1</sup>), água destilada (controle). Foi verificado que as gérbas submetidas à solução com Et + AC, apresentaram menor perda de massa fresca, maior conteúdo relativo de água, menor extravasamento de eletrólitos e aumento mais lento nas atividades da polifenoloxidase e peroxidase. Isto proporcionou retardo no tombamento das hastes e melhor aparência, resultando em maior longevidade, quando comparada as demais soluções.

**Palavras-chave:** *Gerbera jamesonii*, flores, conservação pós-colheita, *pulsing* e manutenção.

### Introduction

The floriculture sector has grown all over the globe, which has increasingly driven the economy of this segment in the last decades. In Brazil, consumer revenues amounted to BRL 6.7 billion in 2016 (IBRAFLO, 2017). *Gerbera jamesonii* is a species of great importance in the cut flower market, with hybrids that stand out for their exuberant shape and diversity of colors, which range from white to pink, orange and red (Bashandy et al., 2015). However,

lack of care during harvest, transport and stem bending may cause damage to cut flowers, compromising their quality and, consequently, increasing postharvest losses (Kumar et al., 2014).

In cut *Gerbera* flowers, the stem starts to bend in the first days postharvest. This constitutes a great difficulty to their commerce (Witte et al., 2014), because consumers demand quality, durability and freshness, which depend directly upon care since harvest and during transport (Fisher et al., 2015).

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Preservative agents with biocide properties, ethylene action inhibitors (Hossain et al., 2007) and energy sources (Norikoshi et al., 2016) are used in vase solutions to maintain the flowers viable for a longer period as they also stimulate water uptake, keeping the flowers turgid for a longer time and preventing the effects of tissue dehydration.

Sucrose application in vase solution increases the concentration of carbohydrates used for cell respiration (Rabiza-Swider et al., 2016). Sugars are normally associated with biocide agents such as 8-hydroxyquinoline citrate, silver and thiosulphate nitrates, carvacrol and thymol, increasing flower vase life. Citric acid also acts as a biocide, reducing the blockage of xylem vessels by acidifying the solution and extending the vase life of chrysanthemums (Mashhadian et al., 2012), for instance.

Studies with ethanol on carnation vase life have been one of the first works reporting its positive effect on cut flowers (Heins, 1980). However, the responses vary according to cultivar and sensitivity (Heins and Blakely, 1980), which renders studies highly specific to each situation. Low concentrations of ethanol inhibited ethylene biosynthesis and reduced respiration, ACC oxidase and synthase activities and the symptoms of senescence in chrysanthemums, increasing vase life (Bazaz et al., 2015). This response was also found in carnation (Pun et al., 2014) and lotus when combined with ascorbic acid or calcium ascorbate (Gao et al., 2017). By contrast, high ethanol concentrations cause phytotoxicity in cells, inducing plasma membrane degradation and rapid senescence (Heins and Blakely, 1980).

Gerbera cv. Good Timing showed increased lifespan when maintained in pulsing solution with benzyl adenine and gibberellic acid followed by maintenance with ethanol and sucrose (Danaee et al., 2011). Thus, ethanol was shown to be used as an auxiliary product for Gerbera flowers preservation, because they are considered little sensitive to ethylene (Nowak et al., 1990). The same was not observed in studies with ethylene-sensitive flowers like carnation, in which the effect of ethanol was evaluated separately (Wu et al., 1992). The combined use of ethanol and citric acid through pulsing and/or maintenance can minimize the physico-chemical and physiological alterations, thereby increasing longevity in Gerbera flowers. On these bases, the present study proposes to examine the physico-chemical

changes that compromise vase life in Gerbera cv. Mistique cut flowers treated with ethanol and citric acid.

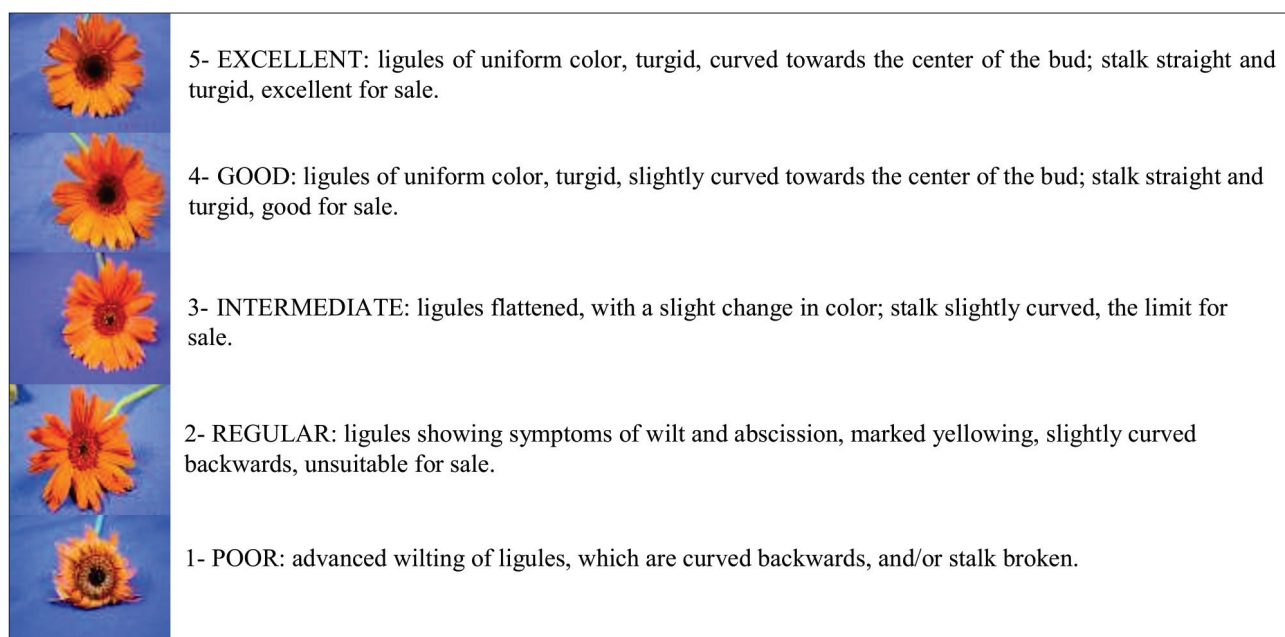
## Material and Methods

### Plant material

Orange-colored Gerbera (*Gerbera jamesonii* L.) cv. Mistique flowers were acquired at a flower shop in Gravatá - PE, Brazil (8°12'35''S and 35° 34' 10'' W; 489 m altitude). The flowers were transported at a temperature of  $20 \pm 2$  °C, for approximately 5 h, to the Laboratory of the Postgraduate Program in Plant Production (PGPV) at UFRPE/UAST, Serra Talhada, Pernambuco State. The flowers were selected, standardized to a length of 35 cm, the base of the stem cut and immersed in water. The stems were kept in an air-conditioned room at  $20 \pm 2$  °C with RH  $65\% \pm 2\%$ , under continuous lighting, as recommended by Van-Meeteren (1978).

### Pulsing and maintenance with ethanol and citric acid

The inflorescences were placed in plastic pots containing 500 mL of solution and protected with polyvinyl chloride film (PVC). In the first experiment, the flowers were immersed in 12 pulsing solutions for 48 h, namely, 1) 4% ethanol, 2) 6% ethanol, 3) 8% ethanol, 4) 100 mg L<sup>-1</sup> citric acid, 5) 200 mg L<sup>-1</sup> citric acid, 6) 4% ethanol + 100 mg L<sup>-1</sup> citric acid, 7) 6% ethanol + 100 mg L<sup>-1</sup> citric acid, 8) 8% ethanol + 100 mg L<sup>-1</sup> citric acid, 9) 4% ethanol + 200 mg L<sup>-1</sup> citric acid, 10) 6% ethanol + 200 mg L<sup>-1</sup> citric acid, 11) 8% ethanol + 200 mg L<sup>-1</sup> citric acid, and 12) distilled water (control). After this period, the solutions were replaced by distilled water, which was renewed at every two days. In the second experiment, the solutions at the same concentrations were renewed at every two days, with the concentrations maintained throughout the stem preservation period. Both experiments were set up as a completely randomized design with two application methods (pulsing or maintenance) in a 12 × 4 factorial arrangement with four days of analysis (0, 2, 4 and 6), using a volume of 500 mL of distilled water or solution. At every two days, scores were assigned individually by evaluators using a five-point Likert-type scale (Figure 1). Based on the visual analysis, we described characteristics which determine the commercial threshold of flowers, termed 'vase life'. Upon reaching this threshold, a score of 3 was assigned, representing maximum vase life.



**Figure 1.** Five-point Likert-type scale describing the preservation of cut Gerbera cv. Mistique flowers (Orange).

#### Pulsing with ethanol and citric acid in Gerbera

The experiment three was conducted as a completely randomized design with a  $4 \times 4$  factorial arrangement consisting of the pulsing solutions 1) 4% ethanol, 2) 100 mg L<sup>-1</sup> citric acid, 3) 4% ethanol + 100 mg L<sup>-1</sup> citric acid, and 4) distilled water (control) and days of analysis (0, 2, 4 and 6 days).

Forty-eight stems were separated for non-destructive analysis (longevity and fresh mass change), using 12 stems per treatment, which were sub-divided into four pots. The ligules for the physico-chemical and physiological attributes analyses were collected from a separate batch, consisting of 120 stems, 30 per treatment, sub-divided into five pots. The analyses were conducted over six days, at two-day intervals:

#### Visual assessment, longevity and stem bending

The stems were visually evaluated at every two days using a five-point Likert-type scale (Figure 1). Broken stems were considered lodged and thus unsuitable for sale and discarded. Inflorescences showing advanced symptoms of senescence such as depigmentation and wilting were also discarded.

#### Fresh mass variation

Stem fresh mass was determined as the percentage difference between the fresh mass measured on the evaluation day and the mass of the previous day (FM<sub>1</sub>), which were obtained by weighing the stems on a semi-analytical scale (ARD110 OHAUS). Fresh mass variation was calculated by the formula:

$$FMC = [(FM_1 - FM_2) / FM_1] \times 100$$

Where:

FMC: fresh mass change, %;

FM<sub>1</sub>: fresh mass of the previous day, in grams; and

FM<sub>2</sub>: fresh mass on the evaluation day, in grams.

#### Relative water content

Nine ligules were harvested per replicate (pot) at every two days (0, 2, 4 and 6), for six days. These were weighed and immersed in distilled water for 4 h. Subsequently, the ligules were dried with paper towel, weighed (turgid mass) and dried in a forced-air oven at 65 °C for 12 h. The relative water content was estimated by the equation:

$$\text{Relative Water Content} = (\text{Fresh mass} - \text{Dry mass}) / (\text{Turgid mass} - \text{Dry mass}) \times 100$$

#### Electrolyte leakage

The methodology proposed by Shanahan et al. (1990) was followed, with adaptations. A total of 0.3 g of ligules was weighed and immersed in 10 mL distilled water in closed test tubes. These were then incubated for six hours, which was the time established based on an adjustment curve, to generate the extract, which was called 'C1'. Next, electrical conductivity was measured using a conductivity meter (DDS-12DW). The same tubes were incubated at 100 °C for 1 h. After this period, they were kept at room temperature until reaching 25 °C to generate extract 'C2', in which electrical conductivity was measured.

#### Extraction and assay for peroxidase (POD, EC:1.11.1.7) and polyphenol oxidase (PPO, EC:1.10.3.1) activities

The POD activity was measured by the method of Hemeda and Kellin (1990). Using liquid nitrogen, 0.25 g of ligules were macerated in 1.3 mL 0.1 M potassium phosphate buffer (pH 7.0) previously kept at 4 °C. The extract was centrifuged at 13,000 x g for 26 min at 4 °C. The POD assay was performed by adding 100 µL of the supernatant to reaction medium containing 1.0 mL 0.01 M phosphate buffer (pH 7.0), 100 µL guaiacol (0.5%) and 100 µL hydrogen peroxide (0.08%). Readings were taken using a spectrophotometer (libra S8, Biochrom) at 470 nm, at a

temperature of 30 °C, for one minute. The POD activity was calculated based on the molar extinction coefficient of 26.6 mM cm<sup>-1</sup> for guaiacol and expressed in nmol g<sup>-1</sup> FM min<sup>-1</sup>. The PPO assay was performed by adding 100 µL of the supernatant to reaction medium containing 1.3 mL 0.01 M phosphate buffer (pH 7.0) and 1.3 mL catechol (0.2 M). Readings were taken using a spectrophotometer (libra S8, Biochrom) at 425 nm, at a temperature of 25 °C, for one minute. The PPO oxidase activity was calculated based on the molar extinction coefficient of 34 mM cm<sup>-1</sup> for catechol and expressed in nmol g<sup>-1</sup> FM min<sup>-1</sup>.

#### Statistical analysis

Means were compared by Tukey's test at the 0.05 significance level, using Sisvar 5.6 statistical software. Graphs were plotted using Sigma Plot 10.0 software.

### Results and Discussion

The use of ethanol on flowers postharvest is still a controversial topic, considering that its benefits depend on the applied concentration and that high doses may damage the integrity of membranes (Heins, 1980) that interfere with cell functioning (Heins and Blakely, 1980). Studies have shown an increase in longevity of cut flowers (carnation cv. Yellow Candy) resulting from the use of low concentrations of ethanol (Pun et al., 2014).

By contrast, high concentrations reduced the vase life of flowers such as carnation and *Bougainvillea* (Hossain et al., 2007).

#### Ethanol and/or citric acid immersion time did not influence Gerbera cv. Mistique vase life

Vase life declined too quickly, with practically all stems receiving grades below 3 on the second day (Table 1), which is considered the commercial limit. This was not the case for those kept immersed in 4% ethanol and 100 mg L<sup>-1</sup> citric acid, for which vase life was extended to four days (Table 1). The stems kept in ethanol (4%) and ethanol plus citric acid (200 mg L<sup>-1</sup>) maintained an intermediate vase life of two days (Table 1).

After application of 4% ethanol, under maintenance, the commercial quality of the Gerberas cv. Mistique inflorescences was ensured for two additional days in comparison to control stems (Table 1). The same period was obtained when 4% ethanol was applied in pulsing solution, for all concentrations (Table 1). By inhibiting the ethylene biosynthesis, ethanol reduces respiration, dehydration and chlorophyll degradation (Petridou et al., 2001), in addition to possibly acting as an energy source (Heins and Blakely, 1980), thereby extending the vase life of cut flowers. Those characteristics might have contributed to the maintenance of stem quality in the flowers treated with 4% ethanol, using either pulsing or maintenance solutions (Table 1).

**Table 1.** Mean scores for appearance in cut Gerbera cv. Mistique flowers under different separate and combined concentrations of ethanol and citric acid applied in maintenance solution. The flowers were maintained at 20 ± 2 °C and RH 65% ± 2%. \*CA: citric acid. Et: ethanol.

Solutions	Maintenance evaluation days				Evaluation days under pulsing			
	0	2	4	6	0	2	4	6
Distilled water	5,00 a	2,00 cd	2,00 b	1,00 b	5,00 a	2,00 cde	2,00 ab	1,66 ab
Ethanol, 4%	5,00 a	3,00 ab	1,66 bc	1,00 b	5,00 a	3,33 ab	1,66 b	1,33 ab
Ethanol, 6%	5,00 a	1,00 e	1,33 bc	1,00 b	5,00 a	3,00 abc	1,33 b	1,00 b
Ethanol, 8%	5,00 a	1,00 e	1,00 c	1,00 b	5,00 a	3,00 abc	1,66 b	1,00 b
CA, 100 mg L <sup>-1</sup>	5,00 a	2,33 bc	1,66 bc	1,00 b	5,00 a	2,33 bcde	1,66 b	1,00 b
CA, 200 mg L <sup>-1</sup>	5,00 a	1,66 cde	1,66 bc	1,00 b	5,00 a	1,66 de	1,66 b	1,00 b
Et, 4% + CA, 100 mg L <sup>-1</sup>	5,00 a	3,66 a	3,00 a	2,00 a	5,00 a	3,66 a	3,00 ab	2,33 a
Et, 6% + CA, 100 mg L <sup>-1</sup>	5,00 a	1,33 de	1,66 bc	1,00 b	5,00 a	2,66 abcd	1,66 b	1,33 ab
Et, 8% + CA, 100 mg L <sup>-1</sup>	5,00 a	1,33 de	1,00 c	1,00 b	5,00 a	2,33 bcde	1,33 b	1,00 b
Et, 4% + CA, 200 mg L <sup>-1</sup>	5,00 a	3,33 a	1,66 bc	1,00 b	5,00 a	2,33 bcde	2,00 ab	2,00 ab
Et, 6% + CA, 200 mg L <sup>-1</sup>	5,00 a	1,33 de	1,00 c	1,00 b	5,00 a	1,66 de	2,00 ab	1,00 b
Et, 8% + CA, 200 mg L <sup>-1</sup>	5,00 a	1,33 de	1,00 c	1,00 b	5,00 a	1,33 e	1,00 b	1,00 b

\* Means followed by the same letter in the column do not differ significantly from each other by the Tukey test (P > 0.05)

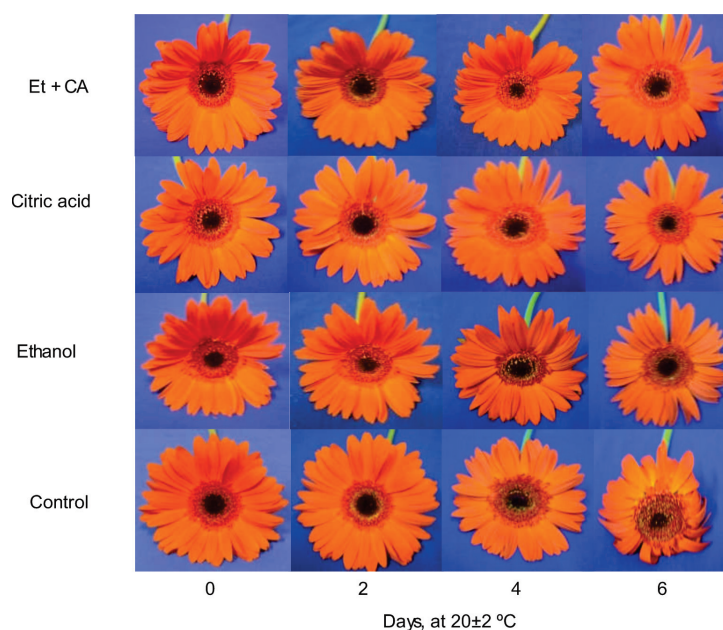
The Gerbera cv. Mistique flowers kept in pulsing solution also showed a decline in score starting on the second day. Only the treatments with ethanol and the combination of ethanol (4% or 6%) with citric acid 100 mg L<sup>-1</sup> (Table 1) maintained commercial quality in this period. At four days, only the stems treated with the combination of 4% ethanol + 100 mg L<sup>-1</sup> citric acid maintained score 3 (Table 1).

The use of only citric acid (100 and 200 mg L<sup>-1</sup>) or in combination with ethanol, maintained the flowers under a score equal to or greater than 3 for up to two days of preservation, except for the combination of 4% ethanol with 100 mg L<sup>-1</sup> citric acid (Table 1). This occurrence was not reported for *Lisianthus* in maintenance solutions with ascorbic acid and citric acid, which considerably increased its vase life (Sheikh et al., 2014). Citric acid is normally used for the adjustment of the potential of hydrogen (pH) of solutions, producing a weak acid that inhibits the growth of microorganisms and possible vascular blockage (Mashhadian et al., 2012). The concentrations tested in those experiments were likely unlikely to cause those effects, as also shown in Gerbera (Durigan et al., 2013), in which citric acid also did not increase its vase life.

In both experiments, with Gerbera cv. Mistique flowers immersed for 48 h or maintained for seven days, the solutions containing 4% ethanol + 100 mg L<sup>-1</sup> citric acid provided the greatest vase life of inflorescences (4 days) (Table 1). Depending on the consumer market, four days may be considered too little time for transporting and sale. However, the gain in vase life obtained with the applied solutions was significantly higher compared to that achieved with control treatment. These findings show that immersion for 48 h elicited a similar response from vase life in the flowers maintained in solution throughout the preservation period. Since pulsing is a more practical and economic method, and considering that the solution is replaced with water after a set time, we chose to use it as an application method in the next experiment.

#### Pulsing with ethanol and citric acid minimized physicochemical and physiological changes in Gerbera cv. Mistique cut flowers

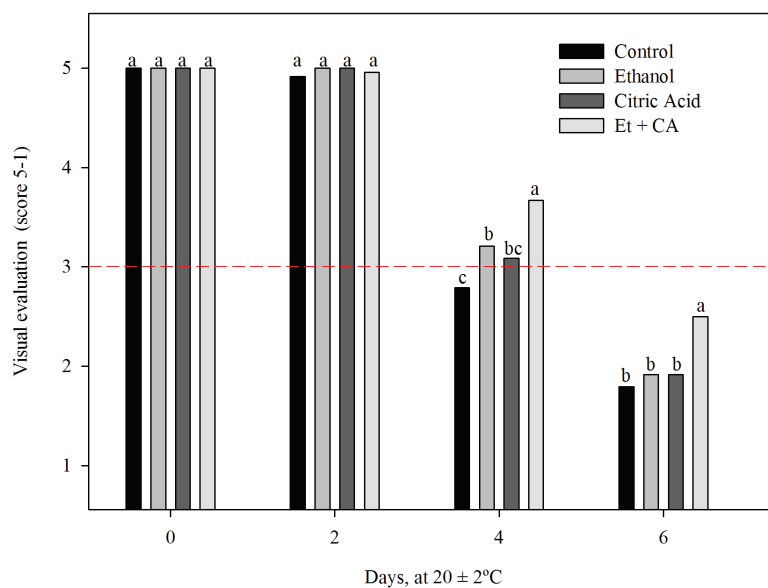
At the start of the experimentation, all Gerbera flowers were under score 5 (Figure 5), which indicates suitability for sale, with evenly colored and turgid ligules and straight stems, as described in Figure 1.



**Figure 2.** Visual aspects of cut Gerbera cv. Mistique flowers in pulsing solution with 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA); 100 mg L<sup>-1</sup> citric acid (CA); 4% ethanol; and distilled water (Control). The flowers were maintained at 20 ± 2 °C and RH 65%.

From the fourth day onwards, a slight alteration was observed in ligule color, which did not compromise the characteristics required for sale, represented by score 3 (Figures 2 and 3). The exception was the stems in the control group, which were assigned an average score of

2.8, considered below the minimum limit for sale (Figure 3). Et + CA (when applied both separately and together) maintained the quality in of Gerbera cv. Mistique cut flowers for four days, resulting in an improvement of two days in relation to control (Figures 2 and 3).

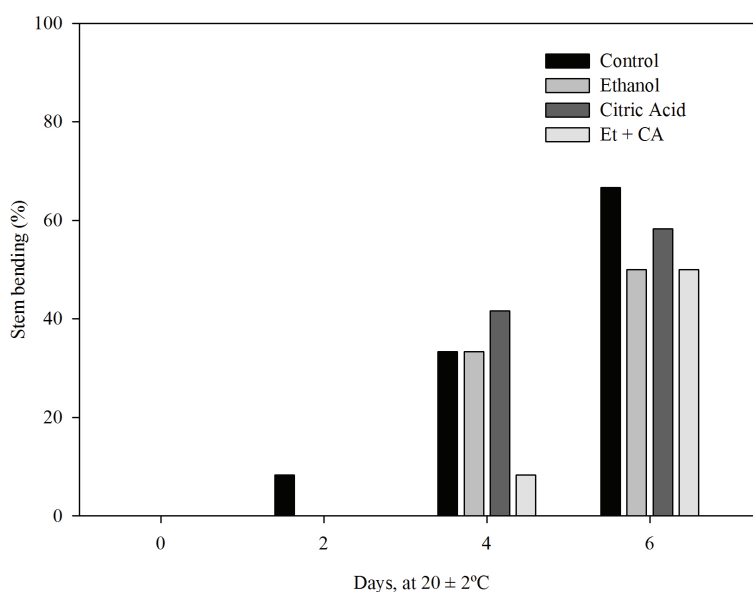


**Figure 3.** Preservation, based on a scale of scores, of cut Gerbera cv. Mistique flowers in pulsing solution with distilled water (Control); 4% ethanol (Et); 100 mg L<sup>-1</sup> citric acid (CA); and 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA). The flowers were maintained at 20 ± 2 °C and RH 65%.

Because Gerbera flowers are sensitive to handling, their vase life is highly variable, as it is influenced by factors like transport time. In the experiment of Fisher et al. (2015), Gerbera were preserved for three days when obtained from producers far from the site of preservation and sale (approximately 1,500 km) and seven days when obtained from local producers, close to the region of sale. Gerbera flowers were preserved for around nine days under the effect of 50 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) + 2.5% ethanol (Danaee et al., 2011) and 8.5 days under 64 g L<sup>-1</sup> citric acid, in pulsing solution (Durigan et al., 2013). These results

demonstrate the great variability in responses to application of ethanol and citric acid, known as anti-ethylene, biocide and antioxidant products (Pun et al., 2014; Sheikh et al., 2014).

On the fourth day, approximately 40% of the stem bending was also observed in the flowers treated with ethanol and citric acid separately. The same was found for those maintained in water (Figure 4). However, for the stems subjected to the combined application of ethanol and citric acid, stem bending did not reach 10% in the same period (Figure 4).



**Figure 4.** Stem bending of cut Gerbera cv. Mistique flowers in pulsing solution with distilled water (Control); 4% ethanol (Et); 100 mg L<sup>-1</sup> citric acid (CA); and 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA). The flowers were maintained at 20 ± 2 °C and RH 65%.

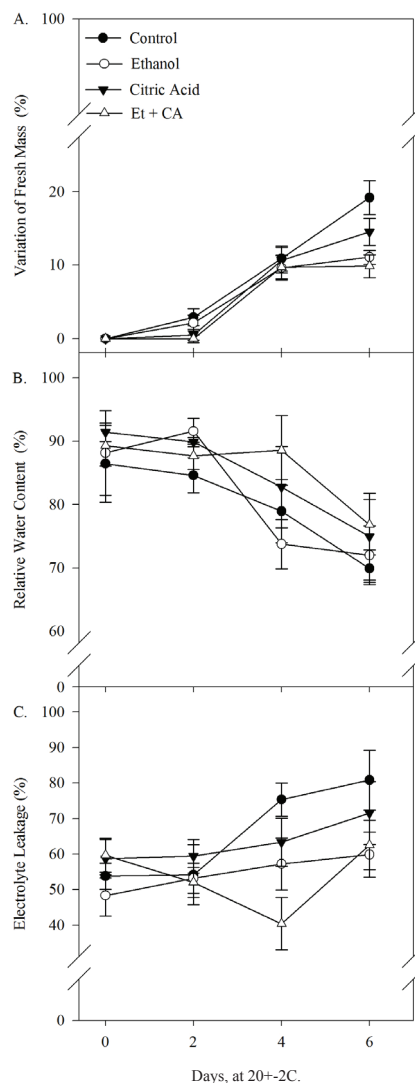
At six days, the scores dropped even further for all stems. The least impact was seen in the flowers immersed in the combination of 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA), which received score 2.5, differing statistically from the other treatment groups (Figure 3). Moreover, stem bending as equal to or higher than 50% in all stems (Figure 4).

The Gerbera cut flowers receiving ethanol application in association with citric acid always showed lower variations in the water status of their stems, from the start to the end of six days (Figures 5A and B). In this study, the water status of the stems was represented by measurements of fresh mass loss (Figure 5A), which averaged 9.9%, and relative water content, which was approximately 12.5% for the ethanol-treated stems (Figure 5B). However, the stems kept in distilled water showed a different response in the same period, with the greatest variations: 19.2% for mass loss and 16.5% for relative water uptake content (Figures 5A and B).

Those facts might have influenced stem bending (Figure 4), which was maximum (66%) under the effect of distilled water only and minimum (50%) when the

stems were treated with ethanol (4%) or ethanol combined with citric acid (100 mg L<sup>-1</sup>). Thus, Et + CA application via pulsing resulted in more-hydrated stems and possibly improved water conductivity in the vessels, which may be due to the antiseptic effect. As a result, there was a reduction of obstructions in conducting vessels caused by microorganisms (Durigan et al., 2013) or even formation of air bubbles (Pietro et al., 2012).

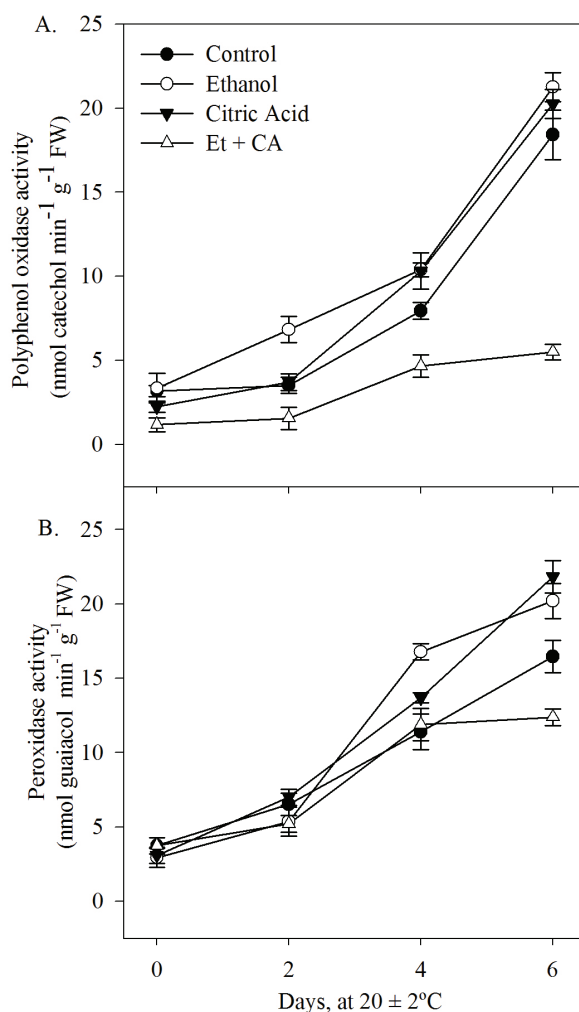
Studies have shown that increasing membrane permeability and net reactive oxygen species (ROS) production contribute to reducing energy availability in the plant (Song et al., 2014), triggering early senescence in cut flowers. In the current study, the parameter used to measure membrane permeability in the ligules was electrolyte leakage, which easily indicates their integrity. The Gerbera cut flowers treated with Et + CA showed lower electrolyte leakage on the fourth and sixth days of preservation, whereas control stems showed the highest values in the same period (Figure 5C). This might have partly contributed to the greater water preservation in the inflorescences, as can be observed in Figures 2 and 3.



**Figure 5.** Fresh mass change (A), relative water content (B), and electrolyte leakage (C) in Gerbera cv. Mistique flowers in pulsing solution with distilled water (Control); 4% ethanol (Et); 100 mg L<sup>-1</sup> citric acid (CA); and 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA). The flowers were maintained at 20 ± 2 °C and RH 65%.

Flower senescence is triggered by physiological and anatomical alterations brought about by internal (e.g. phytohormones) and environmental factors (Rabiza-Swider et al., 2016). However, the processes involving senescence in flowers not sensitive to ethylene, such as *Gladiolus grandiflora* (Kumar et al., 2014), or little sensitive, as is the case of Gerbera (Nowak et al., 1990), are not understood in depth. Dehydration, changes in membrane lipid composition (Saeed et al., 2013), oxidative enzyme activity such as peroxidases and polyphenol oxidases and growth regulators such as abscisic acid (ABA), acting on stomatal opening, are factors known to coordinate senescence. In the present study, those responses were evident, and although ABA was not quantified, it might be related to the obtained results.

The reduced activities of PPO and POD might have contributed to the physico-chemical improvement and vase life of Gerbera cut flowers in Et + CA (Figure 6), since high activity of those enzymes occurs in tissues under stress. This was observed in the stems maintained in distilled water, which exhibited greater fresh mass loss and electrolyte leakage and lower water absorption and vase life (Figures 3 and 5). The citric acid possibly reduced the pH of the PPO reaction medium, minimizing its activity (Coelho et al., 2017). Although citric acid and ethanol did not elicit the same response when applied separately in the present study, the combination of the two products provided a positive effect, reducing the PPO and POD activities during preservation (Figure 6).



**Figure 6.** Polyphenol oxidase (PPO) and peroxidase (POD) enzyme activities in ligules of cut Gerbera cv. Mistique flowers in pulsing solution with distilled water (Control); 4% ethanol (Et); 100 mg L<sup>-1</sup> citric acid (CA); and 4% ethanol + 100 mg L<sup>-1</sup> citric acid (Et + CA). The flowers were maintained at 20 ± 2 °C and RH 65%.

In practical terms, ethanol and citric acid are easily acquired, low-cost products. Additionally, ethanol is a product which requires further studies, since it can be toxic at high concentrations. Therefore, trials should be carried out aiming at methodological adequacy according to each situation. Moreover, despite the improvements obtained

with the use of ethanol in the current study, with two extra days of vase life compared to control, vase life is still limited when we consider consumers in farther locations.

The present study is thus highly relevant for the sale of Gerbera in arid and semi-arid regions, where they are often used in decoration for events, and where their cultivation



is hindered by the fact that these flowers are typical of temperate climates, which forces the farmer to purchase them from distant regions.

## Conclusions

Immersion time in ethanol (4%) and citric acid (100 mg L<sup>-1</sup>) did not interfere with vase life in Gerbera cv. Mistique cut flowers. However, considering practicality and application time, the use of pulsing solutions is recommended.

Pulsing with a mixture of ethanol (4%) and citric acid (100 mg L<sup>-1</sup>) reduced alterations in the water status of the stems and allowed for lesser membrane damage and peroxidase and polyphenol oxidase enzyme activities in the ligules after 4 days. This allowed for a delay in stem bending, improving the appearance of Gerbera cv. Mistique cut flowers.

The results suggest the potential of use of the combination between ethanol and citric acid as alternative preservatives for vase Gerbera cut flowers, not only for local, but also farther markets - in the present case, semi-arid regions.

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