

Acid influences postharvest quality and oxidative activity of gerbera cut flowers

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Abstract

Gerbera is a very popular ornamental plant and used in gardens as well as for cut flower. However, as a cut flower its postharvest quality is minimal, efforts are required to increase longevity and one of the possible way is to use acidic solutions. The study was conducted to understand if the action of maintenance solutions with acids influence the postharvest physiology of gerbera cv. 'Piang'. The experimental design was factorial completely randomized consisting of two factors: four postharvest treatments and seven evaluation times. The flowers were kept in 200 mg L⁻¹ of citric acid solution (ACS reagent, ≥ 99.5 % - Sigma-Aldrich), 200 mg L⁻¹ of boric acid solution (ACS reagent, ≥ 99.5 %) and 200 mg L⁻¹ of salicylic acid solution (ACS reagent, ≥ 99.0 %) and potable water as a control. During the postharvest period, fresh mass loss, water absorption, petal luminosity, total longevity, peroxidase (POD) enzyme activity, superoxide dismutase (SOD), total protein and total carbohydrate content were studied. Treatments with boric and salicylic acids recorded higher percentage of fresh weight loss. Citric acid showed higher water absorption rate and better appearance indicated by luminosity of the petals. The total protein and carbohydrate content decreased during the evaluation period but in the citric acid treatment, the reduction was not so significant. On the other hand, citric acid induced higher peroxidase and superoxide dismutase activity on the second day of evaluation and lower activity until the tenth day.

Key words: *Gerbera jamesonii* Bolus, ethylene, senescence, oxygen reactive species

Introduction

An essential condition in postharvest quality of plant products, such as cut flowers, is the balance between free radical production and antioxidant defenses. Free radicals are produced during cellular functioning of aerobic organisms, especially under reactive oxygen species (ROS). Hydrogen peroxide (H₂O₂) and superoxide anion (O₂^{•-}) are relatively in low amount, but can form harmful species to essential cell components such as hydroxyl radical (•OH), which can initiate lipid peroxidation and also damage to DNA, proteins and many small molecules (Arora *et al.*, 2002). The ROS content depends on the production systems, which are catalyzed reactions and defense mechanisms in the endogenous system, which may be non-enzymatic and enzymatic (Apel and Hirt, 2004). Non-enzymatic defenses include compounds with antioxidant properties such as ascorbic acid, alkaloids, carotenoids, glutathione and tocopherol, while enzymatic mechanisms primarily involve superoxide dismutase (SOD), peroxidase (POD), among others (Scandalios, 2005; Zapata *et al.*, 2014). Increased activity of antioxidant enzymes inhibit ethylene biosynthesis and external factors that damage cut flowers and thus prevents aging, which neutralizes the toxic

effects of oxygen resulting from the decomposition of H₂O₂ (MacAdam *et al.*, 1992; Mortazavi *et al.*, 2011).

Ethylene plays an important role in regulating a variety of physiological processes (Abeles *et al.*, 2012) and has been extensively investigated in the floral senescence process (van Doorn 2002). Ethylene is produced in all higher plants from the amino acid methionine. The presence of ethylene immediate precursor, 1-carboxylic acid-1-aminocyclopropane (ACC), formed from S-adenosylmethionine (SAM) by the action of the enzyme ACC synthase (ACS), determines the rate of ethylene production by ACC oxidase (ACO) activity. The activity of these two enzymes can be influenced by many factors, such as temperature and exogenous chemicals (Abeles *et al.*, 2012). Many chemical treatments are used to prolong the life of cut flowers. Citric acid, like other organic acids, can influence the vase life of these flowers. Citric acid reduces bacterial population in vase solution and increases water uptake by xylem (van Doorn, 1997). The positive effect of citric acid on postharvest life of some cut flowers such as lily, tuberose and Lisianthus was reported by Darandeh and Hadavi (2012) and, Azizi and Onsinejad (2015), respectively. Salicylic acid (SA) or methyl salicylate naturally

exists in a wide range of higher plants and functions as a signaling molecule, inducing it to express resistance against microbial attacks. In the postharvest treatment, its action was verified in the possibility of decreasing the ethylene synthesis of the plants and delaying the effects of this hormone, because it can reduce the activity of the ACO, precursor enzyme of its synthesis (Altvorst and Bovy, 1995). It has also been reported that boric acid has chemical properties that inhibit the initial increase in ethylene production (Ahmadnia *et al.*, 2013). Boron plays a role in improving the quality of ornamental plants and in particular the vase life of cut flower (Malakouti, 2003). This element improves carbohydrate metabolism and translocation, whose function is to provide a substrate for cell respiration and cell wall synthesis (Norikoshi *et al.*, 2008). It also acts in the processes of capture and transport (Brown and Hu, 1996), cell wall formation (O'Neill *et al.*, 2004), cell membrane functions (Goldbach *et al.*, 2001) and anti-oxidant defense system (Cakmak and Romheld, 1997).

The use of chemical treatment is an important factor, especially for highly perishable products such as fruits, vegetables and flowers. Among the flowers with short postharvest life, gerberas stand out. Gerberas (*Gerbera jamesonii* Bolus) are categorized as non-climatic flowers as far as ethylene evolution is concerned, but have a limited postharvest life due to high quality loss, affecting some parameters such as color, stem slope and fresh mass decline (Rurigan *et al.*, 2013). In this sense, studies concerning the activity of antioxidant enzymes and the protein and total carbohydrate content during postharvest period have not yet been analyzed in gerbera flowers, which is considered as non-climacteric flower.

Thus, the cultivar 'Piang' was used to investigate whether citric, salicylic and boric acids affect postharvest quality, with particular interest in protein and total carbohydrate levels and enzyme activity.

Materials and methods

Species study: Gerbera (*G. jamesonii* Bolus) stems of cultivar Piang from commercial production in Holambra-SP were packaged, stored and transported under appropriate conditions to the Postharvest Laboratory of the Federal University of Pará – Altamira Campus. In the selection rods that had damage or mechanical injuries caused by transportation were discarded.

Statistical design: The experimental design was completely randomized, in a 4 x 6 factorial scheme, corresponding to the acid concentrations in the maintenance solution (control: potable water, citric acid 200 mg L⁻¹, boric acid 200 mg L⁻¹ and salicylic acid 200 mg L⁻¹) and the analysis times (0, 2, 4, 6, 8 and 10 days after the start of the experiment), with 4 replication and 3 flower stems per experimental unit.

Montage/packing/setting techniques: The stems were standardized at 45 cm in length, with 70 % open inflorescence and then randomly placed in a 400 mL container. They underwent the following treatments: 200 mg L⁻¹ citric acid solution (reagent ACS, ≥ 99,5 % - Sigma-Aldrich), 200 mg L⁻¹ boric acid solution (reagent ACS ≥ 99,5 %), 200 mg L⁻¹ salicylic acid solution (reagent ACS ≥ 99,0 %) and only regular water was used for the control treatment. Each container was sealed with PVC film around the stems to prevent loss of solution to the external

environment by direct evaporation. Maintenance solutions were renewed every two days.

The laboratory was maintained at a controlled temperature of 19 ± 2 °C under white fluorescent light (12 μmole m⁻² s⁻¹), 12 h per day and relative humidity 60-70 % until the end of the experiment in 10 days. During this period, the following analyzes were performed every two days: fresh mass loss (FML), water absorption rate (WAR), luminosity (petals), total protein and carbohydrate, peroxidase enzyme (POD) activity and superoxide dismutase (SOD).

Fresh mass loss (FML %): The stems were weighed on a semi-analytical balance (ARD110 OHAUS). Stem fresh mass loss values were obtained by the percentage difference of the initial fresh mass and the last fresh mass of the day of analysis. The percentage of fresh mass loss was determined by the following formula: $FML = (Ifm - Ffm) / Ffm$, in which fresh mass loss (%) is FML, initial fresh mass Ifm and final fresh mass Ffm, at two-day intervals.

Water absorption rate (WAR; mL): The methodology of van Doorn *et al.* (2002) was used with modifications. At intervals of two days, the containers and stems were weighed and their water volume renewed due to absorption. The values were obtained by the volume of water consumed, calculated by the following formula: $W = (Iwm - Fwm) / Fsm$, where 'W' is the water absorbed (mL g⁻¹), Iwm initial water mass (mL), Fwm final water mass (mL) and Fsm final stem mass (g).

Petal luminosity: The characteristics of the luminosity analysis of the petals were performed with the aid of a colorimeter, CL-3003 (photometer), which does not measure the chromaticity or color angle of the shade. The L * luminance parameters, ranging from zero (black) to 100, were evaluated for white, which means zero absorbance and 100 % transmittance. For this evaluation, four stems were used for each treatment and four readings on the flower petals.

Total longevity: Postharvest quality of gerbera was calculated from the time when about 50 % of the flowers had wilting or senescent tissue symptoms (Larsen and Scholes, 1966).

Peroxidases (POD; EC: 1.11.1.7): Fresh petal tissue was homogenized in 1.3 mL sodium phosphate buffer 0.2 M (pH 6.0), with the aid of liquid nitrogen, previously maintained at 4 °C, (Simões *et al.*, 2015). The extract was centrifuged at 13,000 x g for 21 minutes at 4 °C. The POD was determined by the addition of 300 μL of the supernatant to the reaction mixture containing 1.0 mL of 0.2 M phosphate buffer (pH 6.0), 100 μL of guaiacol (0.5 %) and 100 μL of hydrogen peroxide (0.08 %). Spectrophotometer (SP-22) readings were taken at 470 nm at 30 °C temperature for three minutes. Peroxidase activity was calculated based on the molar extinction coefficient of 26.6 mM cm⁻¹ for guaiacol and expressed in μmol g⁻¹ MF min⁻¹.

Superoxide dismutase (SOD; EC: 1.15.1.1): Extraction was performed with the aid of liquid nitrogen, where 0.2 g of tissue was homogenized in 1.3 mL of 0.1 M sodium phosphate buffer (pH 7.0), according to Cavalcanti *et al.* (2006). The extract was centrifuged at 13,000 x g for 21 minutes at 4 °C. SOD was determined as described by Giannopolitis and Ries (1977). 100 μL aliquots of the supernatant were added to 1660 μL of 50 mM

sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 13 mM methionine, 40 μ L of 2 mM riboflavin and 200 μ L sodium chloride. tetrazolium nitro blue (NBT) at 750 μ M. The means reaction remained under light (*i.e.*, two 18 W fluorescent lamps) for six minutes for further spectrophotometric reading at 560 nm. Activity was determined based on inhibition of NBT reduction, defined as the unit of activity, as the amount of enzyme required to inhibit 50 % photoresistance (Beauchamp and Fridovich, 1971). Superoxide dismutase activity was expressed in UA min⁻¹ g⁻¹ fresh weight.

Total proteins: The petal sample was weighed and placed in a forced air circulation oven at 55 °C with constant weight (dry). From the dry sample, the total nitrogen content was determined by the destruction of organic matter in the micro-kjeldhal flask and distillation in the Kirk apparatus, followed by volumetric determination according to AOAC (1995). Results were expressed as percentage (%).

Total carbohydrates: The total carbohydrate content was determined in the dry matter, using 2.0 g of homogenized petals. These were lyophilized and milled in a Willey mill to pass the 4-40 mesh filter. After sonication of a tissue sample (20 mg), soluble carbohydrates were extracted four times with 80 % hot ethanol. Subsequently, at each extraction, the homogenates were centrifuged at 1,000 x g for 5 min. The supernatant was used for the analysis of soluble sugars. Sugar was colometrically tested by phenol and sulfuric acid technique (Dubois *et al.*, 1956). Results were expressed as percentage.

Data analysis: Data were subjected to analysis of variance (F test at 5 % significance level) by the Assistat statistical program and mean acid concentrations in combination with ethanol and

the control were compared to 5 % probability by Tukey test. For the peroxidase, superoxide dismutase, total protein and total carbohydrate variables were plotted by the SigmaPlot program.

Results and discussion

Under boric acid and salicylic acid treatment higher tendency of fresh mass loss was recorded as compared to other treatments (Fig. 1A). Stems submitted to these two treatments had, on average, 20.18 % and 19.81 % loss, respectively, but did not differ between them. The lowest percentage of fresh mass loss was observed in citric acid treatment (16.61 %) and control (17.79 %).

Concentration of citric acid in a maintenance solution provides maintenance of tissue thickness with postharvest mass gain, but does not indicate increased longevity. This fact is more evident mainly because there was no significant difference when compared to the stems submitted to potable water (Fig. 1A). Scientific studies with citric acid showed positive postharvest results with flowers (Darandeh and Hadavi, 2012). Fresh weight loss in gerbera cut flowers in 200 mg L⁻¹ citric acid solution was less when compared to other concentrations and control (Mehdikhah *et al.*, 2016). These results were also observed in this experiment, as there were more marked reductions from the 4th day after harvest, showing significant differences (Fig. 1D). The loss of fresh mass occurred due to the process of transpiration, increased respiration (Mansouri, 2012) and also the reduction of water conductivity during senescence of flower stems (Dias-Tagliacozzo *et al.*, 2005). The process of transpiration is a major cause of deterioration, which results mainly in altered appearance due to wilting (Ribeiro *et al.*, 2010).

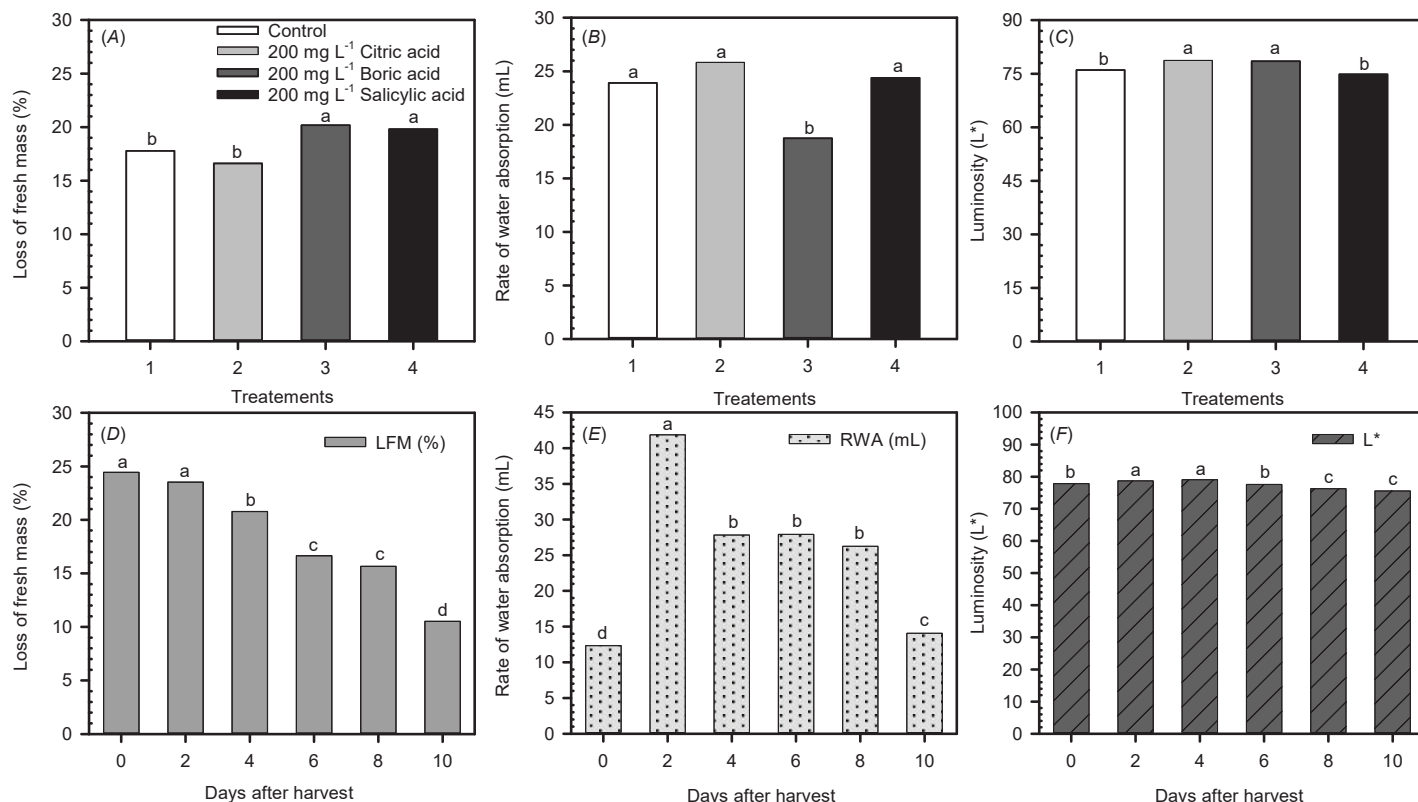


Fig. 1. (A and D). Fresh mass loss (%), (B and E) Water absorption rate (mL), (C and F) Brightness (L*) of gerbera stems (*Gerbera jamesonii* Bolus) treated with acids: citric, boric and salicylic. The flowers were kept at 19 ± 2 °C for 10 days. In each column, for each factor, the means followed by the same letter do not differ from each other by the Tukey test, with a 5 % probability.

This fact was most evident in the treatments with boric and salicylic acids, which showed higher wilting symptoms (Table 1) influencing the longevity of flowers. Still, it can be justified by the inefficiency of these two acids in controlling the microorganisms, which caused the occlusion of the stems. However, in the study by Mehdikhah *et al.* (2016) in gerbera flowers, the results showed that flower quality increased when adequate salicylic acid concentrations were used.

Treatment with 200 mg L⁻¹ citric acid had a higher water absorption rate, which did not differ statistically from treatments with 200 mg L⁻¹ salicylic acid and potable water (Fig. 1B). On the other hand, there was less loss of fresh mass, with better looking stems in 200 mg L⁻¹ citric acid and had higher water absorption rate, with well hydrated petals, which emphasizes the effects of biocidal substances (Capdeville *et al.*, 2003). A similar trend was reported by Azizi and Onsinejad (2015) in a study with application of citric acid in *Lisianthus* (*Eustoma grandiflorum* L.), who found greater efficiency in the water absorption rate in 200 mg L⁻¹ treatment. The water absorption rate was higher on the second day and decreased until the 10th day (Fig. 1E). This phenomenon was also observed in 'boca-de-leão' flowers (*Antirrhinum majus* L.), where the absorption rate in different solutions was higher in the first 24 hours, followed by a decrease over time (Vieira *et al.*, 2010). The water absorption rate of cut flowers depends on their hydraulic conductance and the difference in water potential between the solution and the stem tissues (Spricigo *et al.*, 2010). Water potential is affected by water loss due to transpiration and also by other cellular processes, especially the growth of petals during flowering (van Meeteren and van Gelder, 1999).

For the luminosity variable, represented by a scale of zero (black) and one hundred (white), it was observed that the highest values were found in citric (78.73) and boric (78.49) acid treatment, without significant difference. The floral stems kept in salicylic acid solution and only in potable water, the orange color was intense and changed to yellowish orange between the 8th and the 10th vase day. However, in this experiment, the parameters hue angle and chromaticity, which are essential for color evaluation, were not analyzed. The wide range of different petal colors varies mainly with the combinations of carotenoid and anthocyanin pigments (Kishimoto *et al.*, 2007). The reduction in brightness in this study from the sixth day after harvest indicates the rapid change in petal color, which shows signs of aging. Brightness is an important parameter of color, because the lower the value, the darker the tissue (Lima *et al.*, 2014). According to Moalem-Beno *et al.* (1997), carotenoids and anthocyanins are the two largest classes of pigments responsible for flower color and change significantly during plant development and senescence.

Differences between the treatments mentioned in the previous variables had significant effects on the longevity of gerbera flowers (Table 1). Flowers kept in citric acid solution and in potable water received better evaluation and presented better commercialization condition. However, the floral stems submitted to the maintenance solution with boric and salicylic acids presented lower quality and were not in the conditions of analysis. All the characteristics, turgidity, peduncle inclination and yellowing of the petals are linked to the water balance of the floral stems, an important part in the maintenance of the cellular structures and, consequently, in the visual appearance (data not

Table 1. Average total longevity of Gerbera cut flowers (*Gerbera jamesonii* Bolus) treated with citric, boric and salicylic acids. The flowers were kept at 19 ± 2 °C for 10 days

Treatment	Longevity (Vase life in days)
Control (Potable water)	10.06a
Citric add (200 mg L ⁻¹)	10.22a
Boric add (200 mg L ⁻¹)	8.83b
Salicylic add (200 mg L ⁻¹)	8.93b

Means followed by the same letter do not differ statistically from each other by Tukey's test with 5 % probability

shown). Thus, citric acid has been shown to have a senescence retarding effect and longer floral longevity.

The total protein content in gerbera flowers varied from the first to the last day of analysis between 3 and 1 %, with reduction from the sixth day of evaluation and oscillation on the eighth day (Fig. 2A). Using different postharvest preservatives in the bird of paradise (*Strelitzia reginae*), Vieira *et al.* (2016) observed a decrease in the total protein content from the 8th assessment day. Eason *et al.* (2002) also found that there was a decrease in protein content during senescence of *Sandersonia aurantiaca*. This effect was also observed by Lerslerwong *et al.* (2009) on petals of *Dendrobium* cv. 'Khaosanan'. The decrease in protein levels during senescence is due to inhibition of protein synthesis and/or synthesis in chrysanthemum cv 'Faroe' leaves and flowers after harvest, with increased degradation by proteases, which results in loss of functional capacity of membranes, increased ion production, and ultimately tissue senescence and death (Hörtensteiner, 2006). The influence on protein content in this experiment can be explained by the degradation of macromolecules and organelles (Hörtensteiner, 2006).

Although not very expressive, it can be seen that the treatments applied influenced the total protein content and, from the sixth day after harvest, it was observed that the treatments with citric acid and boric acid had higher total protein contents, respectively, regarding control and treatment with salicylic acid. Such results have also been reported in gerbera flowers and roses under the effect of citric acid (Mehdikhah *et al.*, 2016). However, these authors also found higher levels of total protein in salicylic acid treatment, which contrasts with the data reported in this study. These results may also be related to the activity of antioxidant enzymes, which depend on the application of the product. In the same evaluation period until the last day of analysis, *i.e.*, from 6th to 10th day, the lowest levels were observed in the control, but did not differ statistically with salicylic acid. In the total carbohydrate content, significant differences were greater in gerbera flowers until the second day after harvest, with marked reduction until the last day of analysis (Fig. 2B). On the first day of evaluation, the total carbohydrate content was 35.1 %; on the 10th day, it reached a maximum of 12.50 %. Similar results were also obtained by Olley *et al.* (1996), who found in a single cut flower of the species *Chamaelucium uncinatum*, kept in eppendorf tubes, demonstrated rapid decrease in carbohydrate content. The results were similar to those found in chrysanthemum cv. 'Faroe' (Vieira *et al.*, 2010). Yamane *et al.* (1993) also observed carbohydrate loss during *Gladiolus* perianth senescence. Protein and carbohydrate levels decreased during plant senescence (Eason *et al.*, 1997) due to the action of specific proteases (Shahi and Baker, 2011) and for

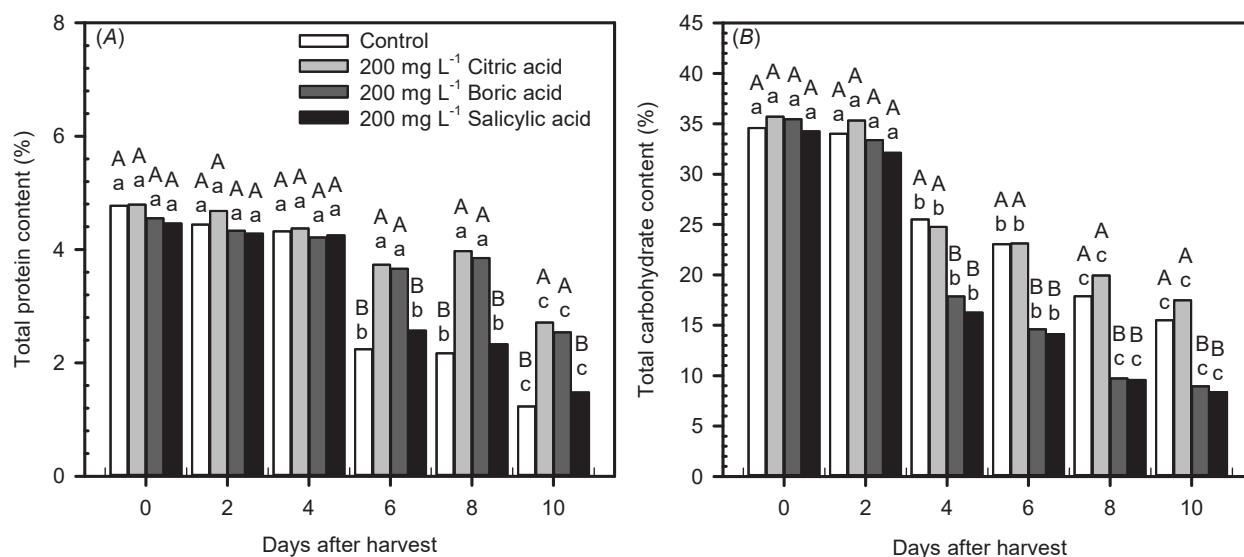


Fig. 2. (A) Total protein content (%) and (B) Total carbohydrate content (%) in gerbera (*Gerbera jamesonii* Bolus) flowers submitted to acid applications. Maintenance solutions containing citric acid (200 mg L⁻¹), boric acid (200 mg L⁻¹), salicylic acid (200 mg L⁻¹) and potable water (control). The flowers were kept at 19 ± 2 °C for 10 days. The averages followed by the same letter do not differ statistically, uppercase in the treatment and lowercase in the different days of the harvest, by the Tukey test with 5 % of probability.

respiration (Siedow and Day, 2001).

Regarding the treatments applied in maintenance solution, the total carbohydrate content in flowers treated with citric acid was higher than the flowers stem submitted to treatments with boric and salicylic acids, differing statistically. In addition, it was observed that flowers maintained without chemical treatment also presented higher total carbohydrate content in relation to these two acids. However, there was no significant difference between citric acid treatments and control. El-Kafie *et al.* (2014) found a positive effect of citric acid on carbohydrate content, but only when applied in combination with sucrose or silver nitrate. Also in this experiment, there was no difference in boric acid and salicylic acid treatment, but it was relatively higher in floral stems treated with boric acid. Regardless of the applied acids, the darkening of the petals was observed during the postharvest evaluation, being more evident in the treatments with boric acid and salicylic acid.

The darkening of plant tissues may be induced by a series of metabolic events associated with the senescence process, initiated

by the consumption of carbohydrate reserves. Lack of available sugars may be a reason for the initial darkening of the petals (Netlak and Imsabai, 2016). In addition, van Doorn and De Witte (1994) indicated carbohydrate metabolism as an important determinant of germination stem inclination, as observed in the present study, but in a small percentage.

Superoxide dismutase (SOD) activity in the citric acid treatment was higher in the first days after harvest (Fig. 3A). From the fourth day, there was a decrease in activity with subsequent oscillation. In the treatment with boric acid, the highest activity was recorded on the second day of evaluation and remained until the end. Also, it was observed that the enzymatic activity was intensified in the treatment with salicylic acid in the sixth and last day of evaluation, which demonstrates greater activity of these acids from the fourth day. However, for peroxidase activity (POD), there was no tendency to decrease and/or increase in activity as in SOD (Fig. 3B). In general, POD and SOD are enzymatic measures to determine the level of oxidative stress in flowers (Mangave *et al.*, 2013). The higher the activity of these enzymes,

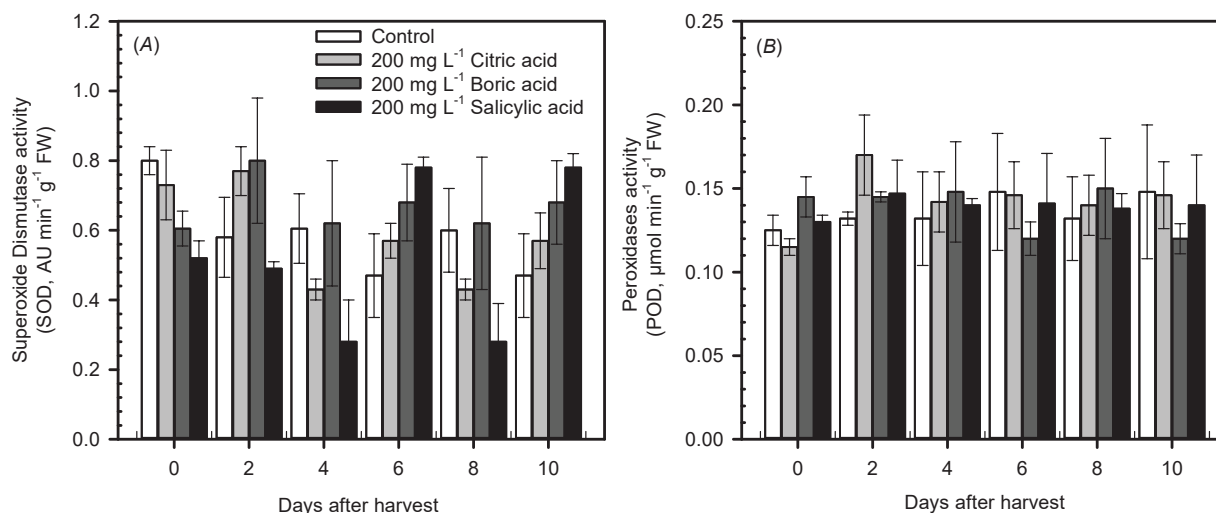


Fig. 3. (A) Superoxide dismutase activity and (B) Peroxidase activity in gerbera flowers (*Gerbera jamesonii* Bolus) submitted to acid application. Maintenance solutions containing citric acid (200 mg L⁻¹), boric acid (200 mg L⁻¹), salicylic acid (200 mg L⁻¹) and potable water (Control). The flowers were kept at 19 ± 2 °C for 10 days. Error bars indicate the standard deviation of the mean.

the higher the stress level of tissues (Shigeoka *et al.*, 2002). This activity was observed on the tenth day of analysis, indirectly evidenced by higher SOD activity (Fig. 3A), since flowers treated with boric acid and salicylic acid had a shorter shelf life, possibly due to greater oxidative damage, not clearly evidenced in POD activity. On the other hand, research shows that salicylic acid has a protective action against the accumulation of reactive oxygen species (ROS) and the regulation of antioxidant enzymes (Shi and Zhu, 2008). Yuping (2009) reported that salicylic acid treatment significantly prolonged the life of gerbera flowers, with increased activity of antioxidant enzyme SOD and decreased ROS production.

Still in the POD activity, the citric acid solution showed higher activity from the second day and then with subsequent oscillation until the last day evaluation (Fig. 3B). Regarding the treatments with boric acid and salicylic acid, there was a tendency to increase the enzymatic activity until the fourth day, with subsequent oscillation. When comparing the results obtained, it is noticeable that all treatments tested followed almost the same trend line, that is, changes in POD activity during the experiment. This variation occurs because the POD enzyme is directly linked to tissue lignification, which polymerizes lignin from hydroxyl oxidation of phenolic groups (Castro and Farias 2005), which makes it important for plant defense and aid. There is evidence that excess compounds, such as lignin, a product of POD activity, and the polyphenoloxidase enzyme, may be related to xylem occlusion, but this relationship is not yet well defined (van Doorn and Vaslier, 2002; Loubaud and van Doorn, 2004). During vase life, POD-promoted salicylic acid activity may be favorable, since POD is a free radical scavenging enzyme (Scandalios, 1993), which allows better membrane maintenance and long-term metabolism, and consequently, benefits the quality of the flowers. However, the results observed in this study are controversial, since treatment with citric acid demonstrated better efficiency in the postharvest quality of gerbera flowers.

The treatment with citric acid presented higher water absorption rate and better appearance in the luminosity of the petals, with 10 days longevity. Also, it presented higher levels of protein and total carbohydrate and lower levels in the activity of enzymes (SDO and POD).

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