Effects of Hot Water Treatment on Vase Life and Antioxidant Enzyme Activities of Cut Gerbera Flowers

Guiping Cheng [1], Shenggen He[1,a], Hongmei Li[1], Jiping Liu[1], Xing'an Cheng[2]

[1] College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

[2] College of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

[a] Corresponding author: howtoroot@163.com

Keywords: Hot water treatment, Vase life, Cut flowers

Abstract: Experiments were conducted to investigate the effects of hot water treatment (HWT) on cut gerbera flowers. The stem ends were treated with deionized water at 38°C for 10 min or 45°C for 5 min, and placed immediately in deionized water at room temperature (23 ± 2°C). The vase life, water relations, antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), and the number of bacteria in vase solution were evaluated. Compared with the controls, the treatments with hot deionized water at 38°C for 10 min and 45°C for 5 min significantly prolonged the vase life of cut gerbera 'Crossfire'. However, for other three cultivars, only the treatment with 45°C for 5 min markedly improved their vase lives. Furthermore, hot treatment with 38°C water for 10 min resulted in higher water uptake and water balance value in cut gerbera 'Crossfire' during late vase life. Moreover, higher activities of SOD, CAT and POD also were observed in cut gerbera 'Crossfire' treated with 38°C for 10 min. In addition, the bacteria number of vase solution of hot water-treated gerbera was markedly lower than that of the control after the 7th day of vase period.

1. Introduction

Gerberas (*Gerbera jamesonii* Bolus) belongs to Asteraceae family, the largest family of lowering plants, and is one of most popular cut flowers in the world. Maturity, freedom from defects, stem length, strength and straightness are important quality criteria in gerberas. The main postharvest disorders of cut gerbera flowers are petal wilting and stem breaking/bending, which result in relatively short vase life [1]. Numerous studies had reported positive effects of various chemical additives, such as silver nitrate, 8-hydroxyquinoline citrate, silver nanoparticles, carvacrol, on the longevity of cut gerberas[2-4]. However, the application of these chemical additives have potential environmental pollution.

Hot water treatment (HWT), as a safe, green, non-chemical, less costly and easily applied at commercial scale method, has been widely used in some harvested fruits and vegetables, which can prevent rot development and maintain their quality [5-7]. Unfortunately, there is a shortage of information on the effects of hot water treatment on the vase life and physiology of cut flowers. In the present study, the effects of HWT on the vase life and postharvest physiology of cut gerbera flowers cv. Nevada, Crossfire, Dolcevita and Tonga were investigated.

2. Materials and methods

2.1. Plant materials

Cut gerbera (*Gerbera jamesonii* Bolus 'Nevada', 'Crossfire', 'Dolcevita' and 'Tonga') flowers were purchased from a local market in Guangzhou, China. They were immediately stood upright in tap water and transported within 1 h to the laboratory. The flowers were covered with a plastic film shroud to minimize moisture loss during transportation. In the laboratory, stems were re-cut to 25 cm under deionized water before hot water treatment.

  DOI: 10.25236/iczbe.2018.025

2.2. Hot water treatments

The lowest 5 cm of gerbera stems end were dipped in hot deionized water at 38 °C for 10 min or 45°C for 5 min. Control stems were kept in deionized water at room temperature (23 ± 2°C). After treatment, all the stems were held individually in 250 mL glass vases containing 150 mL deionized water at room temperature. Mouths of the vases were covered with a sheet of low density polyethylene film to minimize evaporation and prevent contamination. Vase solutions were freshly prepared at the beginning of experiment and not renewed in their course. Experiments were conducted at 23 ± 2°C, relative humidity of 60 ± 10% and 12 μmol m^{-2} s^{-1} light intensity under a daily light period of 12 h.

2.3. Experiment 1: effects of HWT on the vase life of four different cut gerberas cultivars

Cut gerberas cv. Nevada, Crossfire, Dolcevita and Tonga were used to evaluate the effects of HWT. Cut gerbera stems were divided into three groups and dipped in deionized water at room temperature (23 ± 2°C, control), 38 °C for 10 min or 45°C for 5 min, respectively. Each treatment comprised 15 replicates. Only vase life was determined in this experiment.

2.4. Experiment 2: effects of HWT on the vase life cut gerbera cv. Crossfire

To confirm the result of experiment 1, cut gerberas cv. Crossfire was selected to repeat the experiment. Similarly, only vase life was determined in this experiment.

2.5. Experiment 3: physiology response of cut gerbera cv. Crossfire to HWT

The experiment was designed, based on the result of experiment 2. Cut gerbera stems cv. Crossfire were treated in deionized water at 38 °C for 10 min. Water relations, antioxidant enzyme activities and the number of bacteria in vase solution were evaluated during vase life.

2.6. Measurements

2.6.1. Vase life

Cut gerbera flowers were observed daily for visual appeal. The vase life was considered to expire if the flower showed symptoms of petal wilting or curling, stems bending or breaking [8].

2.6.2. Water relations

Relative fresh weight, water uptake rate, water loss rate and water balance value were determined according to the described method by Liu et al. [2] .

2.6.3. Enzyme assay

Petals tissues (1 g) from 10 flowers were homogenized in 5 mL of extraction buffer and 0.4 g polyvinylpyrrolidone, and then centrifuged at 15,000 *g* for 15 min at 4 °C. The supernatant was collected as the crude enzyme. The extraction buffer was 0.2 mol/L phosphate buffer (pH 6.8) for peroxidase (POD) and catalase (CAT), and 0.05 mol/L phosphate buffer (pH 7.8) for superoxide dismutase (SOD).

SOD (EC 1.15.1.1) activity was determined by measuring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) following the method of Zeng et al.[9] with some modifications. The reaction solution composed of 0.05 mL of enzyme extract, 1.75 mL of 0.05 mol/L phosphate buffer (pH 7.8), 0.3 mL of 20 μmol/L riboflavin, 0.3 mL of 130 mmol/L methionine, 0.3 mL of 100 μmol/L EDTA and 0.3 mL of 750 μmol/L NBT. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

To measure CAT (EC 1.11.1.6) activity, spectrophotometric procedure was adapted [10]. Reaction mixture (3 mL) contained 0.05 mL enzyme extract, 2.8 mL of 0.05 mol/L phosphate buffer (pH 7.0) and 0.15 mL of 0.1% H_2O_2. One unit of this enzyme activity was defined as an increase of 0.001 in the absorbance at 240 nm per minute.

POD (EC 1.11.1.7) activity was assayed by the method of Duan et al.[10] with slight modifications. In a reaction mixture (3 mL) contained 0.2 mL enzyme extract, 2.2 mL of 0.05 mol/L phosphate buffer (pH 6.8), 0.3 mL of 1% H_2O_2 and 0.1mL of 1% guaiacol. The increase in the absorbance at 470 nm was recorded for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

2.6.4. Number of bacteria in vase solution

The numbers of bacteria in vase solution were determined by the method of Liu et al.[2]. At the vase period of 0, 1, 2, 5, 7 days, 5 mL solution were taken and diluted with 0.9% normal saline to achieve 30-300 bacterial colonies in one Petri dish. Aliquots of 0.1 mL were spread on each nutrient agar plate. They were incubated at 37 ^{o}C for 24 h before count of bacteria.

2.7. Statistical analyses

Completely randomized experiment designs were used. The data were expressed as means ± standard error (SE). Means were compared by the least significant difference (LSD) in SPSS (Version 13, SPSS Inc., USA) test at the 0.05 probability level.

3. Results

3.1. Vase life

In experiment 1, compared with the controls, the vase lives of cut gerbera cv. Nevada, Crossfire, Dolcevita and Tonga were extended by 2.7 d, 1.6 d, 3.2 d and 1.4 d when treated with 45^{o}C hot water for 5 min, respectively (Table 1). Whereas, only the vase life of cut gerbera cv. Crossfire was significantly prolonged by 38^{o}C hot water treated for 10 min (Table 1). For Nevada, Dolcevita and Tonga cultivars, there were no significant differences in vase life between the controls and the treated flowers with 38^{o}C water for 10 min (Table 1). In experiment 2, hot water treatments with 45^{o}C for 5 min, and 38^{o}C for 10 min extended vase lives of cut gerbera cv. Crossfire by 4.9 and 5.3 d, respectively (Table 1).

Table 1 Effect of hot water treatment on the vase life of cut gerbera flowers

Experiments	Treatments	Vase life(days) *			
Experiment 1		Nevada	Crossfire	Dolcevita	Tonga
	Control	6.9 ± 0.3 b	8.3 ± 0.5 b	5.3 ± 0.4 b	5.9 ±0.3 b
	38 ^{o}C 10 min	7.7 ± 0.5 b	11.6 ±0.7 a	6.0 ± 0.4 b	6.7 ± 0.6 ab
	45 ^{o}C 5 min	9.6 ± 0.8 a	9.9 ± 0.6 a	8.5 ± 0.6 a	7.3 ± 0.3 a
Experiment 2		Crossfire			
	Control	12.0 ± 1.7 b			
	38 ^{o}C 10 min	17.3 ± 0.6 a			
	45 ^{o}C 5 min	16.9 ± 0.6 a			

* All values are means ± SE (n=10). Mean values in each column followed by the same letter are not significantly different (P<0.05) by the LSD test.

3.2. Water relations

The changes in relative fresh weight, water uptake rate, and water loss rate and water balance value showed similar trends in the control and the treated cut flower (Fig. 1). Relative fresh weight of cut gerbera increased in initial 2 days and decreased thereafter (Fig. 1A). Hot water treated flowers had relatively higher fresh weight throughout the vase life, compared with the control (Fig. 1A). The tendency of water uptake rate decreased rapidly over the first 3 days, and then declined slowly (Fig.1B). The rate of water uptake of 38^{o}C hot water treated flowers was lower during the first 2 d, and then obviously higher than that of control at the final two days of evaluation period (Fig.1B).

Patterns of changes in water loss rates were nearly the same as those in water uptake rates (Fig. 1C). The changes of water balance value of cut gerbera stems exhibited continual downtrend during the total vase period (Fig. 1D). Hot water treated flowers maintained higher water balance value than control (Fig. 1D).

3.3. Activities of SOD, CAT and POD

SOD activity of control flowers first increased, reached the peak value at day 2, and then decreased. Treatment with 38^{o}C hot water for 10 min slowed the decrease of SOD activity (Fig. 2A). Similarly, CAT activity also increased firstly and then declined, but it reached the maximum at 7th day. HWT resulted in an increase of CAT activity throughout the total vase period, compared with the control flowers (Fig. 2B). Unlike to SOD and CAT, POD activity of control flowers exhibited no obvious change during the vase period. Higher POD activity of HWT treated flowers after the initial two days was observed (Fig. 2C).

3.4. Numbers of bacteria in vase solution

Numbers of bacteria in vase solution of cut gerbera cv. Crossfire increased rapidly from the beginning to the 5th day, and thereafter it declined slightly at the later vase stage (Fig. 3). There was no significant difference between the control and hot water treatment at 38 ^{o}C for 10 min during the first 5 days of vase period (Fig. 3). However, live vase solution bacteria counts were markedly higher in the control than in the HWT at the 7th day of vase period (Fig. 3).

4. Discussions

Previous researches have shown that postharvest heat water treatment could effectively prolong the shelf life of fruits [6, 11]. In the present study, the treatments with hot deionized water at 38^{o}C for 10 min or 45^{o}C for 5 min significantly extended the vase life of cut gerbera cv. Crossfire compared to control (Table 1). Similarly, the prolonged vase life or the delayed senescence by hot water and/or hot air were reported on red ginger [12, 13] and Asiatic hybrid lilies [14]. However, 38^{o}C hot water treated for 10 min could not significantly extend the vase life of cut gerbera cv. Nevada, Dolcevita and Tonga in experiment 1 (Table 1). Seaton and Joyce [15] reported HWT decreased the vase life of Geraldton wax and *Banksia spp*. The differential response to hot water treatment might be associated with the differences in climate conditions of cultivation and/or the variety analyzed.

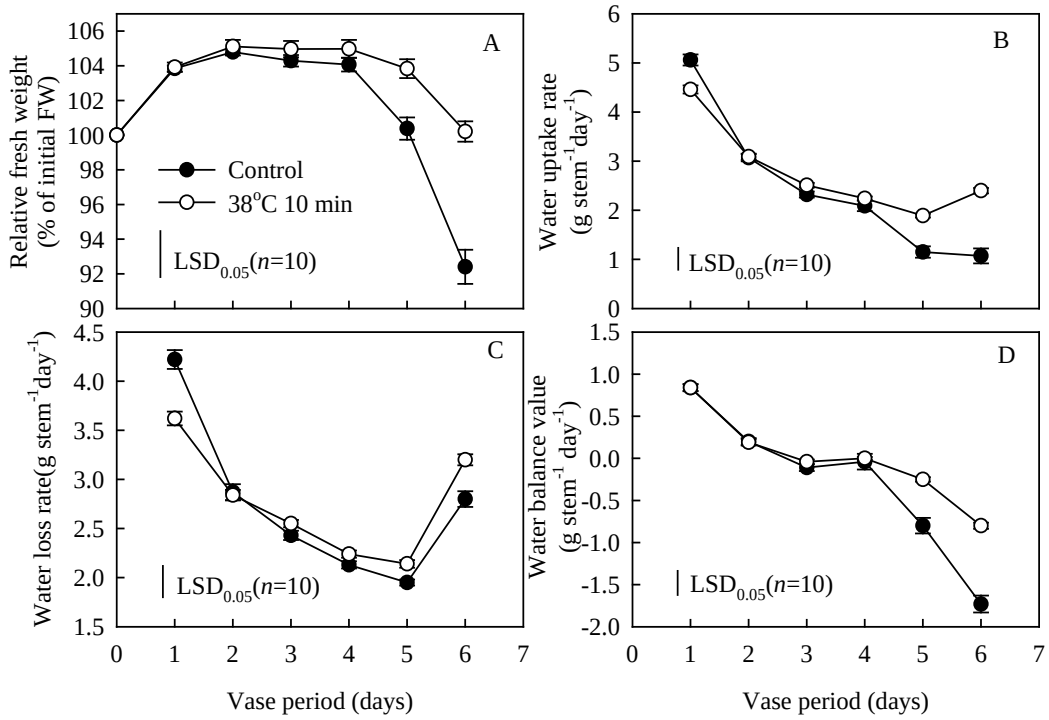

Fig. 1 Effects of hot water treatment on relative fresh weight (A), water uptake rate(B), water loss rate(C) and water balance value (D) of cut gerbera flowers 'Crossfire'

Cut flowers and foliage have limited commercial value because they dehydrate during vase life [16]. Elibox and Umaharan [17] reported that vase life was strongly correlated to water uptake rate which may have a more important role in determining the water balance status of anthurium cultivars. In this study, hot water treated flowers had relatively higher fresh weight and water balance value throughout the vase life evaluation period, compared with the control (Fig. 1A and D). The data suggested that HWT could improve the water holding capacity of cut gerbera flowers. It supported the hypothesis of Woolf *et al.*[14] that HWT stopped or at least delay "active" physiological processes.

The accumulation of reactive oxygen species (ROS) due to the altered balance between ROS production and scavenging capacities will result in lipid peroxidation, which reduced the storage quality and marketability of horticultural products [18]. To deal with the excessive accumulation of ROS and reduce oxidative damage, plant tissues contain several enzymes eliminating ROS, such as SOD, CAT and POD. SOD catalyzes the dismutation of superoxide radical to H_2O_2. CAT and POD are the enzymes mainly responsible for eliminating H_2O_2. Previous study has shown the activities of SOD, CAT and POD were associated with the vase life of cut flowers [10]. In the present study, HWT resulted in the increased activities of SOD, CAT and POD, compared with the control (Fig. 2A, B and C), which was beneficial to eliminate ROS and delay the senescence of cut gerbera.

Bacteria and/or their extracellular products in the stems were the cause of the vascular occlusion and this results in premature wilting and other symptoms of water stress [19]. Bacteria in vase water also could plug the stem and reduce flower longevity. Preservative solutions were used in many researches to prolong the vase life of cut flowers by controlling microbial contamination at the stem base or in the vase solution [2, 19, 20]. Although hot water treated at 38 °C for 10 min could not obviously reduce the number of bacteria in vase solutions during the first 5 days of vase life, but could significantly decrease that at the 7th day (Fig. 2). It means that HWT in cut gerbera flowers could inhibit the growth of bacteria in vase solution at the late vase stage.

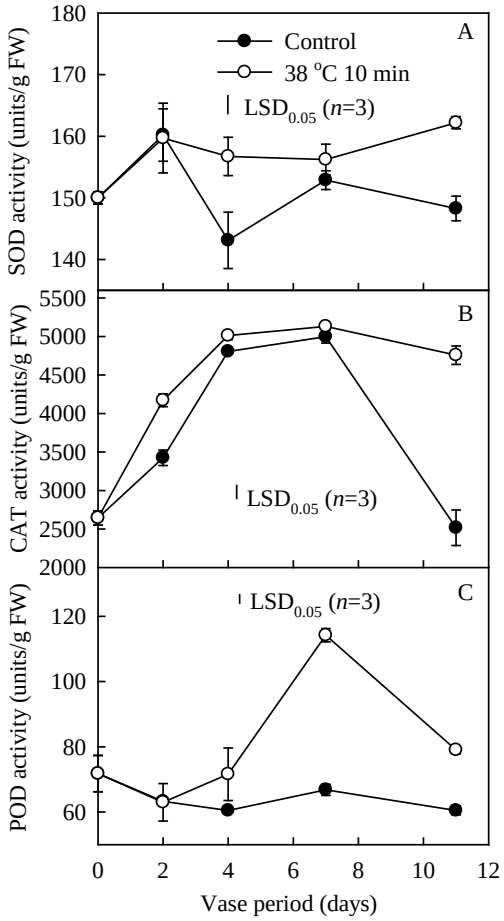

Fig. 2 Effects of hot water treatment on the activities SOD (A), CAT (B) and POD (C) of cut gerbera flowers 'Crossfire'

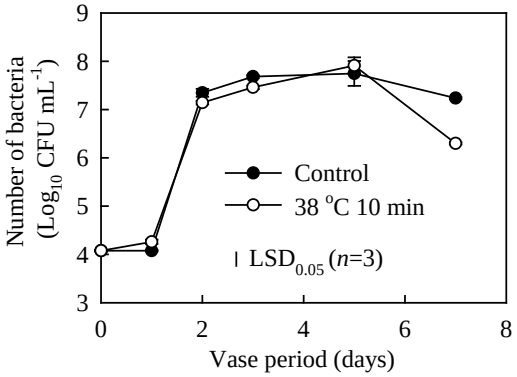

Fig. 3 Effects of hot water treatment on the number of bacteria in vase solution of cut gerbera flowers 'Crossfire'

In conclusion, HWT extended the vase life of cut gerbera flowers. The present data suggested that the cut gerbera flowers treated by hot water could maintain higher relative fresh weight, water balance value and antioxidant enzyme activities during vase period. At the same time, the number of bacteria in vase solution decreased at the 7th day. Thus, hot water treatment could be a promising green technology for improving the postharvest life of cut gerbera.

Acknowledgements

This work was supported by the Science and Technology Project of Guangdong Province

(2016A020210119 and 2014A03011027).

References

[1] R.R.J. Perik, D. Razé, H. Harkema, Y. Zhong, W.G. van Doorn. Postharvest Biol. Technol. **74**, 11–18 (2012).

[2] J. Liu, S. He, Z. Zhang, J. Cao, P. Lü, S. He, G. Cheng, D.C. Joyce. Postharvest Biol. Technol. **54**, 59–62 (2009).

[3] S.A. Nair, V. Singh, T.V.R.S. Sharma. J. Trop Agric. **41**, 56–58 (2003).

[4] H. Mehraj, T. Taufique, M. Shamsuzzoha, I.H. Shiam, A.F.M. Jamal Uddin. J. Biosci. Agric. Res. **09**(02), 804–811 (2016).

[5] T. Djioua, F. Charles, F. Lopez-Lauri, H. Filgueiras, A. Coudret, M.F. Jr, M.-N. Ducamp-Collind, H. Sallanona. Postharvest Biol. Technol. **52**, 221–226 (2009).

[6] T. Sato, H. Saito, K. Maejima, K. Kuba, A. Widiastuti, M. Yoshino. J. JPN Soc. Hortic. Sci. Doi, 10.2503/hortj. OKD-085 (to be published).

[7] M.A. Perini, I.N. Sin, A.M. Reyes Jara, M.E.G. Lobato, P.M. Civello, G.A. Martínez. LWT - Food Sci. Technol. **77**, 314-322 (2017)

[8] D. Geraspolus, B. Chebli. J. Hortic. Sci. Biotechnol. **74**, 78–81 (1999).

[9] C.L. Zeng, L. Liu, G.Q. Xu. Sci. Hortic. **127**, 424–430 (2011).

[10] X.W. Duan, X.G. Su, Y.L. You, H.X. Qu, Y.B. Li, Y.M. Jiang. Food Chem. **104**, 571–576 (2007).

[11] O.A. Karabulut, J.L. Smilanick, C.H. Crisosto, L. Palou. Crop Protection **29**, 903–906 (2010).

[12] T.E. Chantrachit, R. Paull. Postharvest Biol. Technol. **14**, 77–86 (1998).

[13] A.H. Hara, T.Y. Hata, B.K.S Hu, M.C.C. Tsang. Postharvest Biol. Technol. **12**, 101–108 (1997).

[14] A.B. Woolf, S. Combesb, M. Petleya, S. R. Olssona, M. Wohlersa, R.C. Jackmana. Postharvest Biol. Technol. 64, 9–18 (2012).

[15] K.A. Seaton, D.C. Joyce. Sci. Hortic. **56**, 119–133 (1993).

[16] I. Ahmad, D.C. Joyce, J.D. Faragher. Postharvest Biol. Technol. **59**, 258–264 (2011).

[17] W. Elibox, P. Umaharan. Sci. Hortic. **124**, 102–108 (2010).

[18] A.G. Marangoni, T. Paima, D.W. Stanley. Postharvest Biol. Technol. **7**, 193–217 (1996).

[19] W.G. van Doorn, Y. Witte, H. Harkema. Postharvest Biol. Technol., **6**, 111–119 (1995).

[20] J.W. Damunupola, T. Qian, R. Muusers, D.C. Joyce, D.E. Irving, U. van Meeteren. Postharvest Biol. Technol. **55**, 66–69 (2010).