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# The Effect of the Long-Term Cold Storage and Preservatives on Senescence of Cut Herbaceous Peony Flowers

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**Abstract:** Recently peonies have become very popular cut flowers. As peony flowering period is short, long term cold storage could assure its prolonged supply and make long distance transport feasible. The effect of dry cold storage, of 8-hydroxyquinoline and nanosilver preservatives on the peony keeping qualities were tested on the most popular cultivar 'Sarah Bernhardt'. The 12 week storage (0–1 °C) shortened flower longevity by 20%, to 8 days and no vascular blockages in the shoots were observed. However, the presence of callose, not considered as a blocking factor, was evident. The sucrose-containing preservatives with either 8-hydroxyquinoline or nanosilver did not extend the flower longevity but they increased flower diameters in both fresh and stored material. Generally, the soluble total and reducing sugars increased in senescing flowers in both non-stored and stored flowers, and they were lower after storage. The free proline increased ca. 20-times during cold storage and at the end of the vase life it remained generally higher in the stored than in fresh flowers. The level of hydrogen peroxide dropped after 12 weeks storage and its contents at the end of the vase life differed depending on the holding solution. Generally it was lower after storage. Storage increased the catalase activity which remained on higher levels in stored flowers from all holding solutions as compared to freshly cut flowers. A five-fold reduction in the peroxidase activity occurred during storage but its activities at the end of the vase life were similar in stored and non-stored flowers. The effects of nanosilver and 8-hydroxyquinoline were similar.

**Keywords:** nanosilver; total soluble and reducing sugars; free proline; hydrogen peroxide; catalase and peroxidase activity

## 1. Introduction

The assortment of cut flowers keeps increasing every year in response to a growing demand for new species with original blooms suitable as cut flowers. The field-grown plants broaden this assortment and offer a serious market advantage because of their lower production costs. Recently, herbaceous peonies with their numerous cultivars featuring large and scented flowers have become popular on the global flower market as they can be used in a wide range of floral arrangements [1]. The most popular species grown for cut flowers is the Chinese peony (*Paeonia lactiflora* Pall.), now on the top of the list of 20 most important cut flowers [2]. However, its use is somewhat limited as cut peonies are usually available only on local markets and for a short harvest time i.e., 3–4 weeks. The largest suppliers of cut peonies are The Netherlands, New Zealand, Israel, USA, Chile, followed by Germany, France and Poland [2].

Peony cultivars form a wide range of flower types [2–4]. For cut flowers, the highest demand is for double and semi-double, and intensely colored flowers, with long and rigid stems, free from

pathogens [5], and capable of withstanding long-term storage [3]. The most popular cultivar grown for cut flower is 'Sarah Bernhardt' with its double, deeply pink flowers. It is high-yielding and resistant to adverse environmental factors so it represents approximately 50% of production of all cut peonies in the world [6,7].

Cold-storage of cut flowers is very important for the growers as it extends the supply period and deliveries can be timed in response to the highest demand. It also makes it possible to prepare large batches of flowers for long distance transport. Depending on the intended use and the method of storage, cut peonies can be stored from several weeks to several months. The flower bud development is slowed down during storage [8]. Low temperature controls the metabolism: it limits respiration and thus the depletion of storage compounds, it reduces transpirational water losses and ethylene biosynthesis, and limits pathogen development [9]. High humidity during storage is crucial to avoid desiccation; water stress is one of the critical factors hastening flower senescence, mainly because the water uptake and transport are impeded by bacteria developing in the xylem. Depletion of reserves is another reason for premature wilting. To counteract these, and to improve keeping qualities, cut flowers preservatives have been developed [10,11]. These not only delay the flower senescence and prolong the vase life but may also improve product quality by increasing the flower diameter and color intensity [12,13]. These preservatives usually contain a sugar as an energy source and a biocide which limits bacteria development in the vase water and hence ensures normal water uptake [14].

Sucrose is the most common energy source in preservatives for cut flowers. It serves as a respiratory substrate and improves water balance by increasing the osmolyte concentration [15]. The exogenous sugar also limits ethylene synthesis and stimulates pigment synthesis thus enhancing the flower color [10,16,17]. However, it may also negatively affect the flower quality by causing leaf desiccation and withering, such as in cut lilies [18].

The exogenous sugar must be applied together with a biocide to avoid an excessive bacteria proliferation in the holding solution. The most common compounds with antibacterial properties used for decades are the esters of 8-hydroxyquinoline-sulphate (8-HQS) or citrate (8-HQC). A combination of sucrose with one of these two is called "the standard preservative" (SP) [19].

Silver is another antibacterial agent used for decades to prolong the flower vase life. It was first used as silver nitrate ($AgNO_3$) [20], later in a complex form as silver thiosulphate (STS) [21–23]. Currently, it is used in the form of nanoparticles which are considered to be much safer for the environment. Silver nanoparticles are potent antibacterial agents [24,25]. Their mechanism of action has not been fully understood [26], but according to Sondi and Salopek-Sondi [27] and Damunupola and Joyce [10] nanosilver (NS) interacts with bacteria membranes and destroys them. It has been reported that nanosilver particles have a high surface/volume ratio therefore they are able to control microorganisms more efficiently [28]. As nanosilver is easy to prepare and shows relatively little environmental toxicity it has become a common substance in postharvest treatments of cut plant material [25,29,30].

Apart from postharvest treatments and storage conditions [2,8,21,31] several other factors contribute to the longevity of cut peonies. One of these is genetics; performance in storage is cultivar-specific [32]. In our earlier trials with 16 peony cultivars, the vase life ranged from 9 to 15 days (unpublished data). Also pre- and postharvest conditions, such as rainfall and temperature during flower bud formation and its further growth, affect the length of vase life [33,34].

The aim of this experiment was to evaluate the effect of the long-term cold storage and flower preservatives on the vase life and certain aspects of senescence in cut peony 'Sarah Bernhardt'. Preservative solutions containing nanosilver were compared with the standard preservative based on the 8-hydroxyquinoline citrate.

## 2. Material and Methods

Cut flowers of the field-grown Chinese peonies (*Paeonia lactiflora* L.) 'Sarah Bernhardt' were used as the experimental material. Several days before harvest plants were sprayed with fungicide 0.1% Teldor 500SC (Bayer) (a single treatment). Flowers were cut in the morning, at the stage of tight, colored bud. For tests of keeping qualities 120 flowers were used; for various analyses 54 flowers were used. One half of the cut flowers were immediately transferred to the laboratory for tests of the holding solution effects on flower keeping quality (60 flowers: 4 treatments × 15 replications). Stems of these flowers were trimmed to 55 cm with three uppermost leaves left, and placed in vases with holding solutions (5 flowers per 1 L vase). Stems placed into distilled water served as a control. The holding solutions were: 2% sucrose (S) with 200 mg $dm^{-3}$ 8-hydroxyquinoline citrate (8-HQC), 2% sucrose with 1 mg $dm^{-3}$ nanosilver (NS) and 4% S with 1 mg $dm^{-3}$ NS. NS used was obtained as a commercial preparation (Altermedica Laboratories, Żywiec, Poland). Neither the preservatives nor water were exchanged during the experiment but they were replenished as needed. Stored flowers were treated in the same way. Each treatment contained 15 stems, individually tagged and treated as single replicates. Longevity was assessed according to their appearance and expressed as the number of days from the harvest or the end of storage until the appearance of deformations due to petal withering.

Flower diameters were measured every 3 days and reported as averages of two measurements of each bud/flower taken at right angles to each other, expressed in cm, with the accuracy of 1 mm.

The experiment was carried out in a room with temperature maintained at 20 ± 1 °C, relative humidity 60%, quantum irradiance of 35 µmol $m^{-2}$ $s^{-1}$ (Fluorescent lamp LF80 36 W/850, Natural Daylight, Pila), and under the 12 h day and 12 h night regime. Flowers for analyses: 3 flowers × 9 sampling dates (the common day 0 sample + 2 dates × 4 treatments) were kept under the same environmental conditions.

The remaining two batches of harvested flowers for tests of the long term storage, 60 destined for the quality evaluation (4 treatments × 15 replications) and another 27 meant for various chemical analyses were placed under 0–1 °C (RH 60%) for 12 weeks. All unnecessary leaves were removed and the flowers were wrapped into perforated plastic foil and packed into cardboard boxes. After storage flowers were treated in the same fashion as the non-stored flowers except that their stem bases were recut by 2 cm before being placed into 1 L vases with the same solutions as the non-stored flowers.

Anatomical observations of stems were done on 1 cm long basal stem sections immediately after harvest and after 12 weeks of storage, as described in Jędrzejuk et al. [35], adapted to perennial stems. Specimen were fixed in 5% glutaraldehyde and 4% paraformaldehyde solution in 0.1 mol $L^{-1}$ sodium cacodylate buffer (pH 7.2–7.3) at 0.8 atm at 20 ± 2 °C and rinsed with the same buffer. Wet samples were observed under a scanning electron microscope (SEM) FEI QUANTA 200 ESEM under low vacuum, at the Analytical Centre, Warsaw University of Life Sciences.

Samples for various composition tests were collected during the experiment. Petal samples were collected on Day 0 (immediately after harvest/end of storage) and on Days 4 and 10 after harvest or end of storage. Petals from three flowers was pooled on each sampling date. For each compound analyzed three extracts were prepared for each treatment on each sampling date and three measurements were made in each extract giving 9 data points for each value. Results were calculated on a dry matter basis. Dry weight of petals was obtained after drying samples at 105 °C according to Strzelecka et al. [36].

Total sugars were measured as described by Dubois et al. [37] and expressed in mg glucose per g of dry weight (DW). The material was homogenized in 80% ethanol. The extracts were incubated for 20 min in a boiling water bath with the 5% phenol and 96% $H_2SO_4$, and the extinction was measured at 490 nm. The total sugar content was calculated from a previously plotted standard curve, prepared for glucose.

Reducing sugars were measured by the Somogyi method as modified by Nelson [38]. The material was homogenized in 80% ethanol. The extracts were incubated for 20 min in a boiling water bath with the copper reagent, the molybdenum arsenic reagent was added and the extinction was measured at 520 nm. The reducing sugar content was calculated from a previously plotted standard curve, prepared for glucose.

The free proline content was tested according to Bates et al. [39] by a colored reaction of proline with ninhydric acid. The absorbance was read at 520 nm. The amount of proline was calculated from a previously plotted standard curve and expressed in μmol $g^{-1}$DW.

The hydrogen peroxide ($H_2O_2$) content of petals was measured spectrophotometrically after the reaction with potassium iodide (KI) as described by Jędrzejuk et al. [40] and expressed at 390 nm as μg of hydrogen peroxide per g of DW.

The catalase (CAT) activity (EC 1.11.1.6) was determined spectrophotometrically as the rate of $H_2O_2$ disappearance at 405 nm according to Goth [41] and expressed as mkatals per g of DW.

The activity of peroxidases (POX) (EC 1.11.1.7) was determined as described by Jędrzejuk et al. [40]. It was estimated spectrophotometrically at 430 nm and expressed in μmols of purpurogalin formed within 1 min per g of DW.

Statistical analyses were done using SPSS (PS Imago PRO 6.0). The analysis of variance (ANOVA) was used to identify significant differences ($\alpha = 0.05$) between treatments followed by Duncan's post-hoc test. Standard deviation (SD) for each mean are shown in each applicable figure.

## 3. Results

Cut peonies placed in water immediately after harvest lasted 10 days (Table 1); those stored for 12 weeks at low temperature and then placed in water lasted for 8 days, a 20% reduction. The sucrose-containing preservatives with either on 8-hydroxyquinoline or nanosilver did not prolong the flower vase life relative to water, but they improved the general flower and leaves appearance (Figure 1). Figure 1 shows that the leaves are not in a good shape after storage and 8 days of vase life, even though there is a difference in their appearance in response to the treatments as compared to the water control. However, the so called "standard preservative" (SP) was significantly more effective than either of the silver-containing solutions in non-stored flowers, with 25% longer vase life (10 vs. 8 days). No significant differences between holding solutions were observed in stored peonies (Table 1).

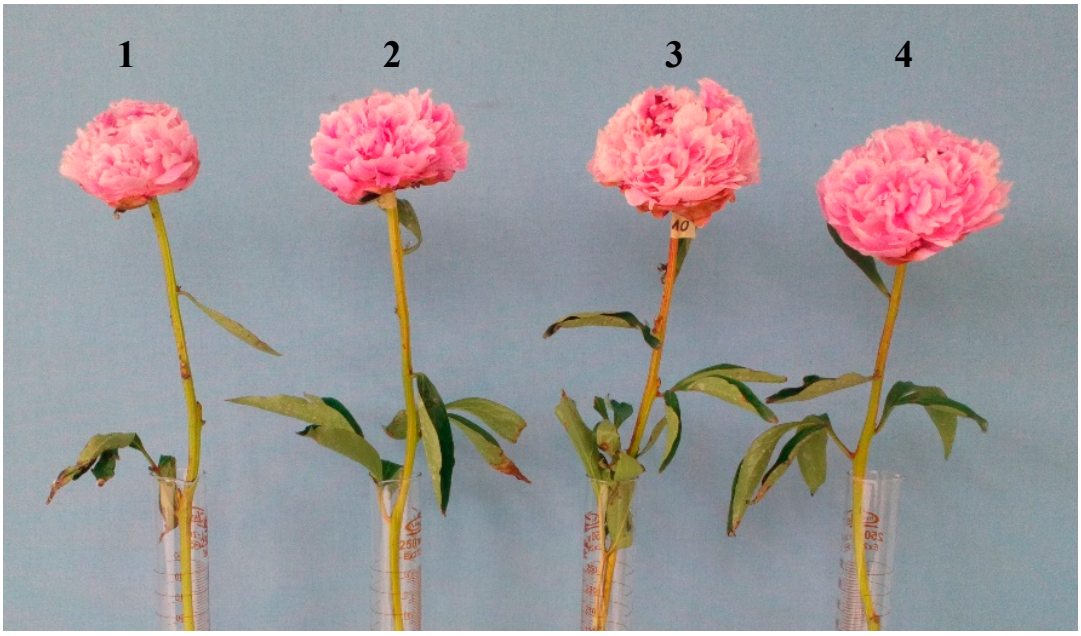


**Figure 1.** The effect of 12 weeks storage and application of holding solutions after storage, on the appearance of cut 'Sarah Bernhardt' flowers after 8 days of vase life. 1 = water; 2 = 8-HQC + 2%S; 3 = NS + 2%S; 4 = NS + 4%S.

Control flowers placed in water immediately after harvest had 10.6 cm in diameter when fully open (Figure 2). Long storage negatively affected flower size: after 12 weeks at low temperature flower diameters were 20–32% lower than those from the non-stored flowers from corresponding

treatments (7.3 cm in water). In addition, about 20% of the flowers did not open after 12 weeks of storage (unpublished data). All the holding solutions increased flowers diameters and the effect was more pronounced in stored flowers where the increase in diameter relative to the respective water ranged from 15% to 33%, with the greatest increase for the NS + 4%S solution (10.1 cm) (Figure 2). In non-stored flowers this increase ranged between 8% and 16%, with those from the silver-containing solutions reaching over 12 cm.

**Table 1.** The effect of holding solutions and storage on the vase life of cut peony 'Sarah Bernhardt' flowers.

| Treatment | Vase Life (Days) | | Mean for Treatment |
|---|---|---|---|
| | Non-Stored | Stored for 12 Weeks | |
| $H_2O$ | 10.0 bc [1] | 8.0 a | 9.0 AB [2] |
| 8-HQC + 2%S | 10.6 c | 8.7 ab | 9.7 B |
| NS + 2%S | 8.7 ab | 7.6 a | 8.2 A |
| NS + 4%S | 8.6 ab | 7.5 a | 8.1 A |
| **Mean for storage** | 9.5 B | 8.0 A | |

[1] Means followed by the same lower case letter do not differ significantly at $\alpha = 0.05$ (Duncan's test). [2] Means followed by the same capital letter (2-way ANOVA) do not differ significantly at $\alpha = 0.05$ (Duncan's test).

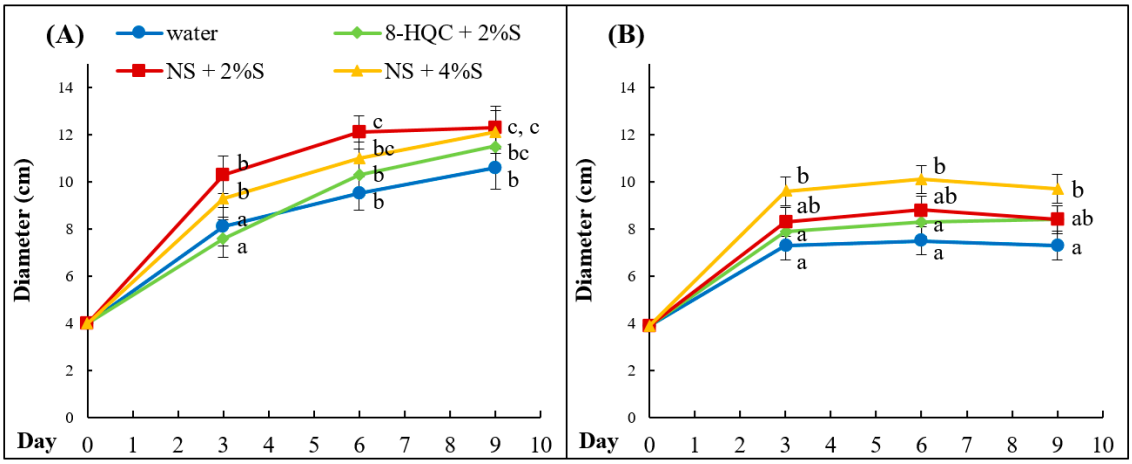


**Figure 2.** Flower diameters of cut non-stored peony flowers (**A**) and cut peony flowers after 12 weeks storage (**B**). Value are expressed as means ±SD of triplicate assays. Vertical bars represent standard deviations of the means. Means followed by the same lower case letter do not differ significantly $\alpha = 0.05$ (Duncan's test). Analyses were done separately for non-stored and stored flowers.

Analyses done immediately after harvest and after 12 weeks of storage show no presence of vascular blockages in the shoots (Figure 3). However, the natural presence of callose, which is not considered as a blocking factor, was evident in shoots after storage (Figure 3D).

The total soluble sugars increased in fresh flowers during first 4 days of vase life, more than 3-fold relative to the initial value (234.1 mg $g^{-1}$DW) in flowers from the SP (753.4 mg $g^{-1}$DW) and the solution of NS + 4%S (810.0 mg $g^{-1}$DW) and approximately 2.5-3-fold in the two other treatments (618.6 and 649.6 mg $g^{-1}$DW in NS + 2%S and water, respectively) (Figure 4A). On Day 10 after harvest the highest amounts of total soluble sugars were found in flowers from NS + 2%S (743.9 mg $g^{-1}$DW) and 8-HQC + 2%S (726. 1 mg $g^{-1}$DW; three times higher than the initial level), while in the flowers from two other treatments the contents dropped significantly, being the lowest in control flowers (451.0 mg $g^{-1}$DW) where it was still almost twice as high as on the day of harvest. Storage had little effect on the sugar content (Figure 4A,B). On Day 0 of vase life it was 202.0 mg $g^{-1}$DW i.e., 86% of the initial level in the freshly cut flowers (234.1 mg $g^{-1}$DW). In stored flowers, the contents of total soluble sugars steadily increased during 10 days, the least so in the control treatment where at the end of vase life it exceeded the initial level by 34%, reaching 271.3 mg $g^{-1}$DW (Figure 4B). In flowers held in the

preservative solutions the total soluble sugars accumulated in petals reaching the levels 2.7–2.8 times higher (544.1–575.5 mg $g^{-1}$DW) than at the beginning of vase life.

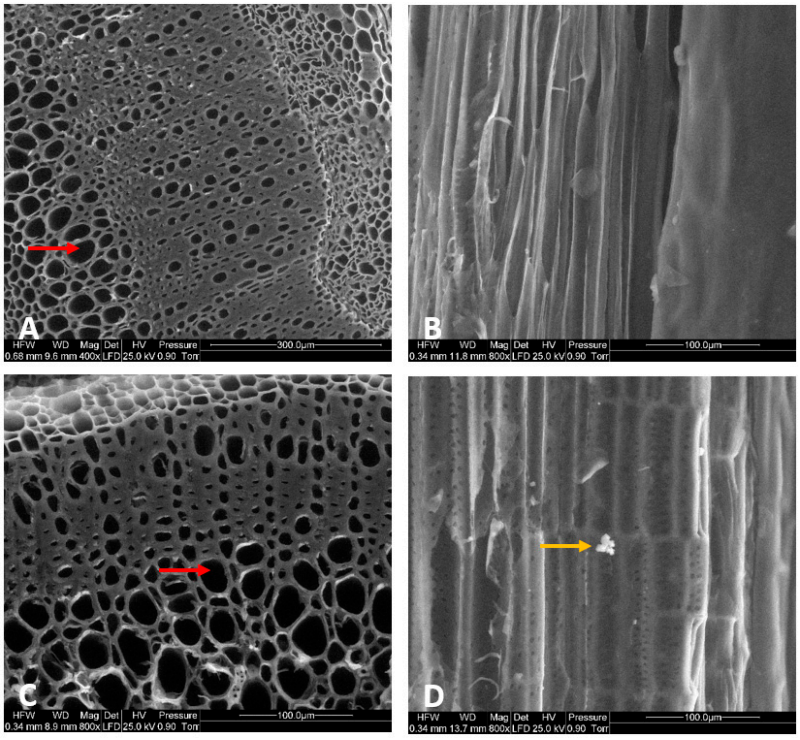


**Figure 3.** Cross section (**A**,**C**) and longitudinal section (**B**,**D**) through the stem of peony 'Sarah Bernhardt': **A**, **B**—immediately after harvest; **C**, **D**—after 12 weeks of storage. ➡ vascular bundles free of bacteria proliferation; ➡ callose.

Changes in reducing sugars showed similar tendencies (Figure 4C,D). In fresh flowers, after a considerable raise on Day 4 from 206.1 to 511.3–700.6 mg $g^{-1}$DW (2.5–3.5-fold relative to the initial value) the sugar contents either remained at this level (632.9 mg $g^{-1}$DW; SP), further increased to 555.4 mg $g^{-1}$DW in water and to 664.2 mg $g^{-1}$DW in NS + 2%S, or dropped to 491.3 mg $g^{-1}$DW in NS + 4%S. The initial content of reducing sugars dropped considerably after storage (Figure 4C,D). It kept increasing in flowers from the preservative solutions, from 112.7 to 366.2–448.2 mg $g^{-1}$DW, reaching the levels 3–4 times higher than at the beginning of the experiment. In control flowers, after an increase on Day 4 to 292.2 mg $g^{-1}$DW, it fell on Day 10 to 131.1 mg $g^{-1}$DW i.e., almost to the initial level.

In freshly cut flowers the free proline level was as low at 1.6 µmol $g^{-1}$DW (Figure 4E). On Day 4 in flowers held in water or in the NS + 2%S solution it increased to 13.7 and 15.1 µmol $g^{-1}$DW, respectively, dropping by one half on Day 10. In flowers placed in SP or NS + 4%S the increase in free proline content on Day 4 was minimal (to 3.3–4.5 µmol $g^{-1}$DW) but on Day 10 over 20 µmol $g^{-1}$DW accumulated in petals in these two treatments. A nearly 20-fold increase in proline content occurred after 12 weeks of storage, from 1.6 at harvest to 31.1 µmol $g^{-1}$DW after storage (Figure 4E,F). On Day 4 of the vase life a further increase—up to ca. 50 µmol $g^{-1}$DW—was observed in flowers held in the SP while in control flowers and those placed into the NS solutions these levels dropped below the initial level (Figure 4F). On Day 10 the proline levels were again higher and ranged between 35 and 44 µmol $g^{-1}$DW. Only in flowers supplied with 4% S this level was lower—19.3 µmol $g^{-1}$DW.

The hydrogen peroxide content increased during first 4 days of the vase life of fresh flowers, in all treatments (Figure 5A). Its highest level was in flowers held in NS + 4%S (573.9 µg $g^{-1}$DW)—3.2 times higher than at the start of the experiment (176.0 µg $g^{-1}$DW). In controls and in flowers placed into NS + 2%S this increase was approximately 2.5-fold, to ca. 440 µg $g^{-1}$DW. The smallest changes in $H_2O_2$ content was in flowers held in the SP. On Day 10 it was comparable to other treatments where it

dropped considerably between Days 4 and 10. After 12 weeks of storage the initial $H_2O_2$ content was lower than in fresh flowers (113.6 vs. 176.0 μg $g^{-1}$DW) (Figure 5A,B). It kept increasing in NS + 4% S where on Day 10 it reached 242.1 μg $g^{-1}$DW i.e., the amount twice as big as on Day 0 (Figure 5B). Also in flowers from three other treatments, where on Day 4 a drop to approximately one half of the initial value was observed, the $H_2O_2$ amounts increased at the end of vase life: in control flowers and those from NS + 2%S back to the initial level, while in those held in the SP it shot up on Day 10 the level of 212 μg $g^{-1}$DW, i.e., almost 2 times higher than on Day 0.

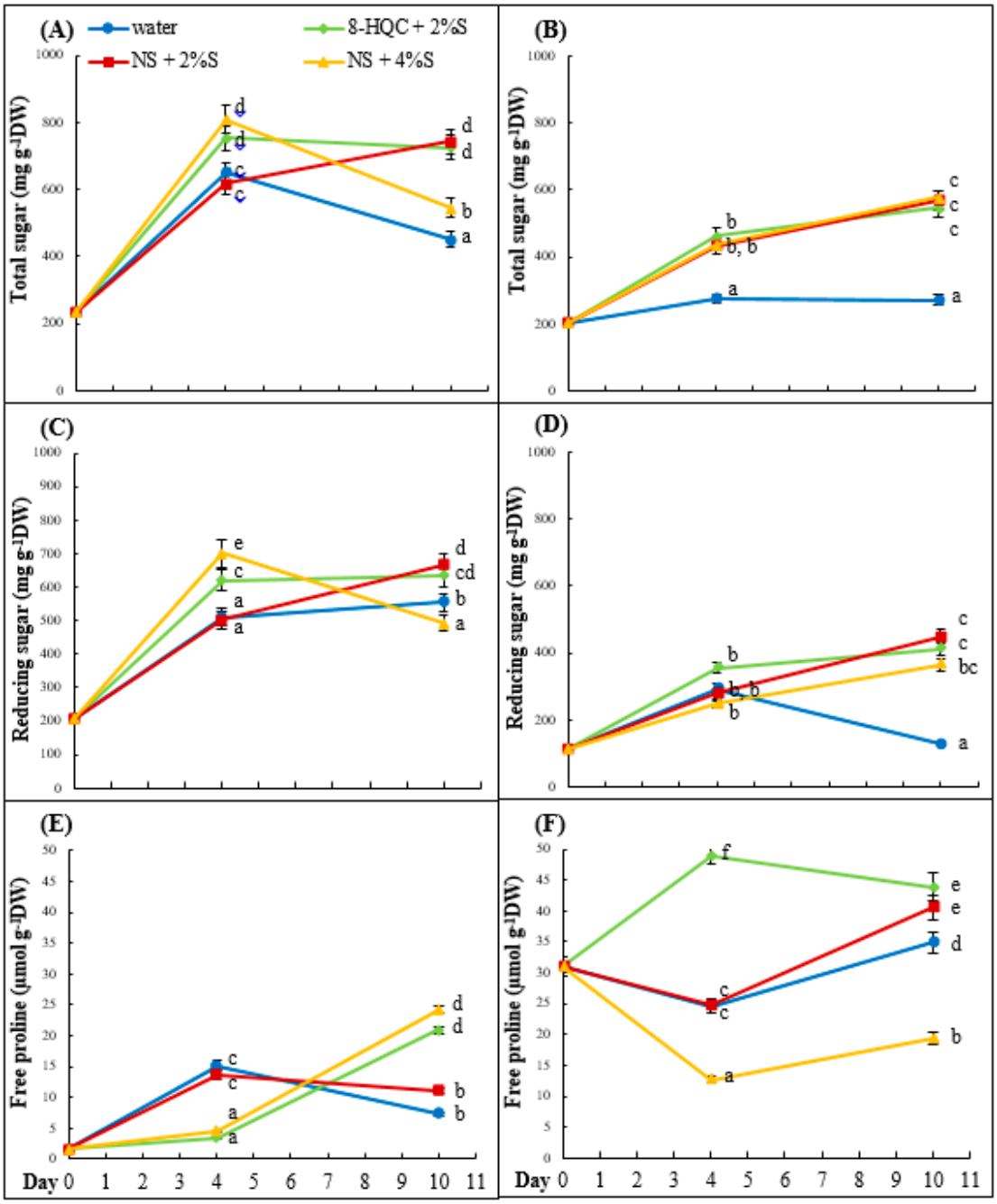


**Figure 4.** Total sugar content (**A**,**B**), reducing sugar content (**C**,**D**) and free proline content (**E**,**F**) in cut non-stored peony flowers (**A**,**C**,**E**) and in cut peony flowers after 12 weeks storage (**B**,**D**,**F**) during 10 days of vase life. Values are expressed as the mean ± SD of triplicate assays. Vertical bars represent standard deviations of the means. Means followed by the same lower case letter do not differ significantly at $\alpha = 0.05$ (Duncan's test). Analyses were done separately for non-stored and stored flowers.

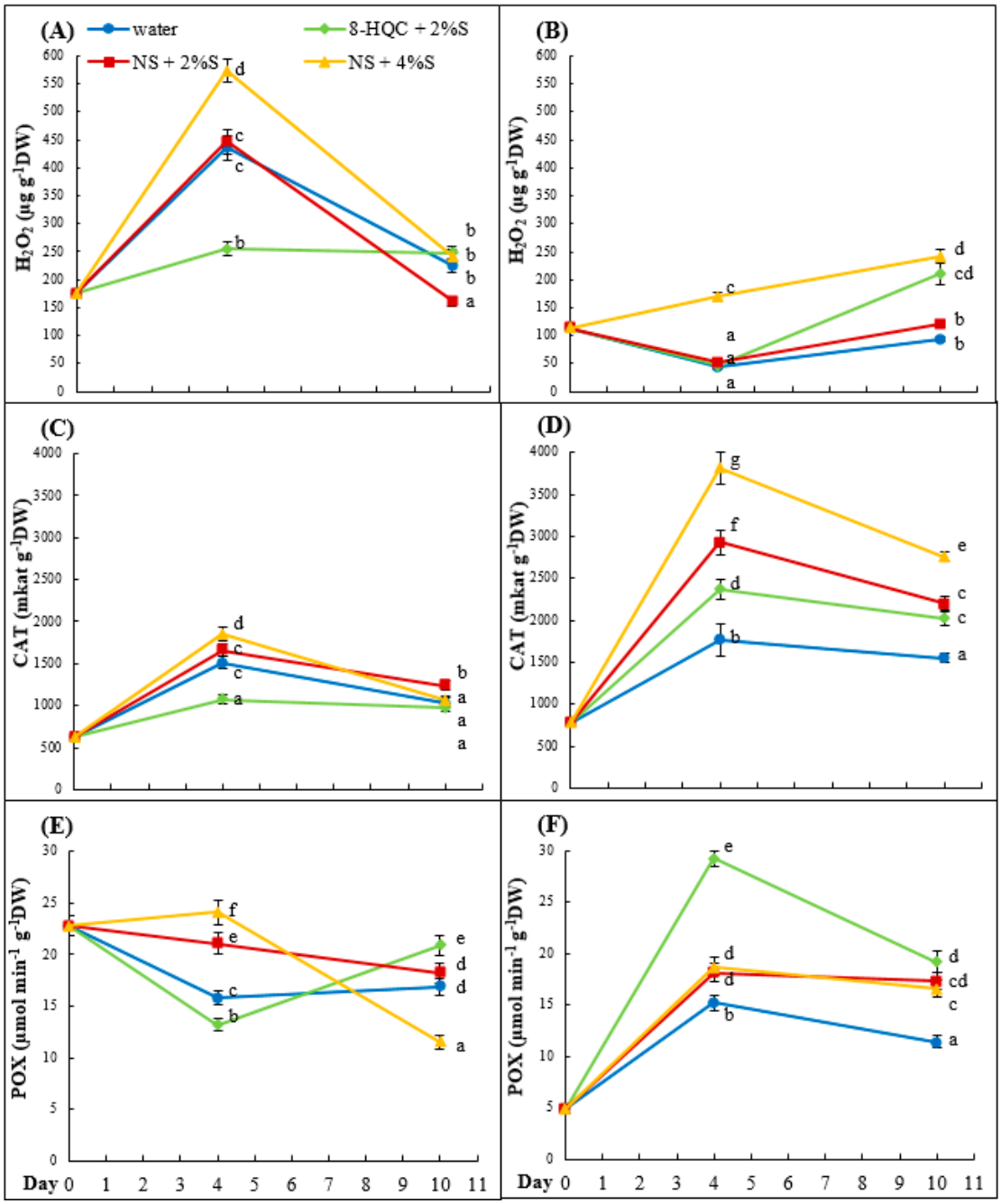


**Figure 5.** Hydrogen peroxide ($H_2O_2$) content (**A**,**B**), CAT activity (**C**,**D**) and POX activity (**E**,**F**) in cut non-stored peony flowers (**A**,**C**,**E**) and in cut peony flowers after 12 weeks storage (**B**,**D**,**F**) during 10 days of vase life. Value are expressed as means ± SD of triplicate assays. Vertical bars represent standard deviations of the means. Means followed by the same lower case letter do not differ significantly at $\alpha$ = 0.05 (Duncan's test). Analyses were done separately for non-stored and stored flowers.

In fresh flowers from all holding solutions the catalase activity increased approx. 1.7–3.0 times between Day 0 and 4, starting at 623 mkat $g^{-1}$DW (Figure 5C). On Day 4 the lowest activity (1075 mkat $g^{-1}$DW) was in flowers from the SP. The catalase activity decreased during the following 6 days, but remained at higher level (976–1239 mkat $g^{-1}$DW) than at the beginning of the experiment. Storage stimulated the CAT activity; at the start of the vase life it was ca. 25% higher than in fresh flowers (782 vs. 623 mkat $g^{-1}$DW) (Figure 5C,D). During the first 4 days of the vase life the activity increased in all the holding solutions—the least so in water (to 1769 mkat $g^{-1}$DW, i.e., over two times

as compared to Day 0) and the most, to 3817 mkat $g^{-1}$DW in NS + 4%S (nearly a 5-fold increase relative to the initial value) (Figure 5D). On Day 10 the activity was lower in all the treatments, being the lowest in the control (1554 mkat $g^{-1}$DW) and the highest in NS + 4%S (2760 mkat $g^{-1}$DW).

The initial peroxidase activity in fresh flowers was approximately four times higher than in the stored flowers (22.8 vs. 4.9 µmol $min^{-1}$ $g^{-1}$DW)(Figure 5E,F). On Day 4 it dropped in fresh flowers in most of the holding solutions (except NS + 4%S) (Figure 5E). Next it dropped in flowers from the NS solutions with 2 and 4%S, to 18.2 and 11.5 µmol $min^{-1}$ $g^{-1}$DW, respectively, and increased in controls, to 16.9 µmol $min^{-1}$ $g^{-1}$DW and in the SP to 20.9 µmol $min^{-1}$ $g^{-1}$DW, but still remaining below the level at the beginning of the experiment. In stored flowers the enzyme activity increased considerably during the first days of the vase life, especially in those in the standard solution: to 29.2 µmol $min^{-1}$ $g^{-1}$DW, i.e., nearly 6 times higher relative to the initial level (Figure 5F). Throughout Day 10 it dropped in all the treatments but was the highest in flowers from the preservatives.

## 4. Discussion

Peony has relatively short vase life. Depending on the cultivar it ranges between 3 and 15 days [5,32,42]. After harvest, cut stems should be rapidly cooled, down to 0–1 °C. Under this temperature and with relative humidity 75–80% (stems should be dry to avoid grey mold infection) they can be stored for several days to several weeks. Longer storage—up to 12 weeks—is also possible but it shortens the vase life [6,8,31]. Before the long term storage, Auer and Holloway [43] recommend 20 min of dry cooling, followed by conditioning in a biocide solution for 15–30 min. According to Walton et al. [31] the vase life of fresh peonies was 5 days longer than that of flowers stored for 8 weeks. The vase life of most peony cultivars—'Sarah Bernhardt' included—has been reported to drop with the length of the storage period [44]. In our trials, the long term storage under 0–1 °C shortened the vase life of 'Sarah Bernhardt' by only 2 days when after storage the stems were placed into water, and by ca. 1 day in flowers placed into the solutions of NS + S. Quite importantly, the long term storage did not affect the vascular system; after 12 weeks of storage no occlusions in the shoots was observed. Callose was present but it is not considered a blocking factor.

The so called "standard preservative" with 8-hydroxyquinoline citrate and sucrose, effectively extends the vase life of many flowers, both of leafless scapes such as gerbera [45] and forced lilac [46], and on leafy stems such as rose [47], lisianthus [48] or lily [49]. Here the solution composed of 8-HQC + 2%S did not affect the length of the vase life of 'Sarah Bernhardt' but it increased the flower diameter and improved the general flower appearance (the aesthetic value of flowers and leaves). We have also compared the effects of well-known and tested 8-HQC with that of nanosilver which has recently emerged as a safe biocide capable of prolonging the vase life of cut flowers, including peonies [50]. In the carnations 'White Liberty' the longevity of flowers held constantly in NS solution (5 ppm) was extended to 26 days [51]. A positive role of sugars in extending the postharvest longevity of cut flowers is also well proven but only when they are combined with a biocide, to control bacterial growth. Here, NS in combination with sucrose did not prolong vase life of 'Sarah Bernhardt' flowers but similarly to the 8-HQC + S combination, it increased flower diameter and improved its general appearance.

Petals serve as nutrient storage for bud development and become energetically depleted in the late phases of senescence [52]. Before petal abscission nutrients are withdrawn to developing organs, including seeds. Using a sugar-containing preservative such as the popular standard preservative, usually prevents such a depletion [53,54]. Here, in fading peony flowers the total soluble sugar content was much lower in flowers held in water than in flowers from other holding solutions, all containing a sugar. That total soluble sugar content dropped even further after 12 weeks storage in flowers held only in water, to a value almost two times lower than in sugar-fed flowers. On Day 10 of the vase life the highest levels of endogenous sugars (total soluble and reducing) in stored and non-stored flowers were observed in the NS + 2%S solution, but these high sugar levels did not translate into a longer postharvest longevity. Nanosilver appeared to stimulate the uptake of the holding solution more than

did 8-HQC (a casual observation; no data shown) so more exogenous sugars accumulated in petals from stems held in the NS solutions. Generally, the longevity of peonies held in biocide solutions with sucrose was comparable to the longevity in water, but carbohydrate reserves appeared higher in the former. This is not surprising as petals showing the first symptoms of wilting may still have high sugar levels [55]. In cut sandersonia and lily flowers held in a sucrose-containing solution the first senescence symptoms are evident before the losses of the soluble carbohydrate take place [56,57]. Walton et al. [31] showed that buds of cut peonies 'Sarah Bernhardt' have enough carbohydrates to drive flower opening and last long even after the 8 weeks storage. Our results confirm this observation as on Day 10 of their vase life, the same 'Sarah Bernhardt' placed into a biocide (8-HQC or NS) with sucrose still showed high sugar contents after 12 weeks in a cold room, higher than immediately after harvest.

Proline defends many organisms—plants included—against the oxidative stress. It participates in numerous biochemical pathways and may control metabolism. Although its effects are associated with different signal pathways, the phenomenon common for all is the generation of the reactive oxygen species (ROS) by oxidation of proline, coupled with the chain electron respiratory transport. Recent research on petal senescence suggests that the free proline content is controlled to satisfy the energetic demands of senescing cells. In petals and leaves, the proline metabolism may affect signal pathways of ROS and delay senescence progression. It has been shown in many ornamental species that the free proline accumulation is associated with flower senescence [58].

The free proline levels in this study appear to have depended on the postharvest treatment. Relative to the initial level immediately after harvest, it was 4.5 times higher on Day 10 of the vase life in non-stored flowers held in water, and 15 times higher in those held in NS + 4%S. After the 12 week storage, a 20-fold increase in the free proline content relative to the initial level was observed before placing flowers into holding solutions. Such a spectacular rise in free proline was reported for other cut flowers, such as roses 'First Red' where it increased 14 times during 6 days of the vase life [59]. According to Bahrami et al. [60] who reported the increase in free proline in senescing lisianthus petals, substances which delay senescence limit proline accumulation while the treatments accelerating senescence stimulate proline synthesis. In cut lisianthus flowers treated with salicylic acid, which prolonged their vase life, the proline level dropped by 75%. Similarly, the standard preservative reduced free proline accumulation in cut lilies [49].

The high hydrogen peroxide content is an indicator of the oxidative stress in cut flowers. In this study, on Day 4 of the vase life, its levels increased considerably in non-stored flowers in all treatments: from over a three-fold rise in NS + 4%S to only 45% in the SP. On Day 10, the levels were generally lower. Similar observations were made in cut day lily flowers where the rise in $H_2O_2$ occurred even before the buds fully opened [61]. The hydrogen peroxide in cut peonies 'Hongyam Zhenghui' increased after conditioning in the NS solution (30 mg $dm^{-3}$ for 36 h) [50]. An opposite reaction was observed in 'Sarah Bernhardt' here: after 12 weeks of cold storage the initial $H_2O_2$ level was lower than in fresh flowers at harvest, perhaps because of a higher catalase activity. This high catalase activity during the vase life here might have reduced the hydrogen peroxide contents on Day 4, but on Day 10 it increased again in all treatments. Hossain et al. [62] made a similar observation in cut gladioli where $H_2O_2$ increased gradually, reaching its maximum in the last phase of the vase life.

Plants developed different protective mechanisms against the oxidative stress effects. During senescence of cut flowers which is induced—among others—by excessive ROS production, antioxidative enzymes such as catalase, peroxidases and superoxide dismutase show increased activity [63]. In non-stored peonies from all treatments here, an increase in catalase occurred during bud opening and the most pronounced (3-fold) increase was observed in flowers from NS + 4%S. After 12 weeks of storage this activity increased 5-fold in the same treatment over the initial value. On Day 10 the CAT activity in stored peonies dropped, but it remained higher than in the corresponding treatments of the non-stored flowers. Similar changes in the CAT and POX activities were reported by Saeed et al. [64] in senescing gladioli where after an initial rise in the activity a drop occurred, but still remained above the initial level. This was also observed by Chakrabarty et al. [65] on cut day lilies.

The POX activity in flowers from three holding solutions decreased on Day 4 of the vase life relative to the initial value immediately after harvest, while in NS + 4%S it increased. This is in line with the observations of Zhao et al. [50] who reported increased activities of defense enzymes such as SOD, CAT and APX in cut peonies held in nanosilver solutions. According to Zhao et al. [50] POX is at first activated to protect flowers from the ROS damage when stress conditions induce the ROS production. Here, after the 12 weeks storage the POX activity was 4.5-fold lower than in non-stored flowers after harvest. They were activated only after the flowers were inserted into holding solutions, probably as a protective reaction.

Generally, cv. 'Sarah Bernhardt' proved quite suitable for long term dry storage. Twelve weeks under 0–1 °C shortened its vase life in water by only 20%, and less so when preservatives were used. Certain senescence parameters such as the free proline level, a decrease in carbohydrates and elevated CAT activity were enhanced by long storage while hydrogen peroxide content and the POX activity fell. Both preservatives used here (8-HQC or NS) stimulated carbohydrate accumulation during senescence but this, however, did not translate into a longer vase life. Moreover, the preservatives enhanced increases in the free proline and hydrogen peroxide contents, and rather unexpectedly, in the activities of both enzymes, catalase and peroxidase. In most cases, the effects of NS and 8-HQC were similar.

## 5. Conclusions

Cut peonies of cv. 'Sarah Bernhardt' proved suitable for long dry cold storage. Twelve weeks under 0–1 °C shortened their vase life by no more than 20%. The sugar-containing preservatives did not prolong the vase life but increased flower diameters. The total soluble and reducing sugar contents in the petals, as well as free proline increased during flower senescence both in non-stored and stored flowers. On all the sampling dates the hydrogen peroxide levels were lower in the stored flowers as compared to the fresh ones. The CAT and POX activities depended on the postharvest treatment and the stage of vase life. No major differences in the effects of NS and 8-HQC were observed so NS can be used as a standard component of preservative solutions for peonies.

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