African Journal of Biotechnology Vol. 11(16), pp. 3800-3809, 23 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2254
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of pulsing solution, packaging material and passive refrigeration storage system on vase life and quality of cut rose flowers

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Accepted 25 July, 2011

The experiment consisted of four pulsing solutions (s*ilver thiosulfate* + Chrysal clear solution (RVB), *silver thiosulfate* + *8-hydroxyquinoline sulphate*, *silver thiosulfate* + Chrysal clear solution + *hydroxyquinoline sulphate* and H_2O), two packaging types (cardboard box and box with polyethylene bag) and four storage period intervals (0, 2, 3 and 4 weeks) under passive refrigeration system. The treatments were arranged in a completely randomized design and four replications. The storage room was maintained at 20°C and 60% relative humidity. The three-way interaction between pulsing solution, packaging and storage period were significant (P < 0.01) on flower bud opening stage and leaf quality. Two-way interaction between pulsing solution and storage period was also significant (P < 0.01) on *Botrytis* incidence, maximum flower bud opening and vase life. Storage time significantly (P < 0.001) affected the solution uptake, relative fresh weights and TSS contents of petals. Passive refrigeration system and pulsed with mixtures of *silver thiosulfate*, Chrysal clear solution and *8-hydroxyquinoline sulphate* were maintained a fresh-like quality of flowers. Thus, growers can transport roses from Africa to Europe using ships equipped with passive refrigeration system without mush reduction in vase life and quality of flowers.

Key words: Rose flower, vase life, pulsing, packaging, passive refrigeration system.

INTRODUCTION

Roses *(Rosa hybrida* L.*)* are the most important ornamental plants that belong to the family of Rosaceae and native to diverse habitats in the Northern hemisphere. According de Vries and Dubois (1996), there are 120 species belonging to the genus Rosa. The species are found in the northern temperate climate zones and in the tropical and subtropical parts of the world, including Ethiopia (Zlesak, 2006). Air transportation of rose flower is associated with high cost and requires use of other less expensive transportations to assist the emerging cut flower producers in Africa. One of the most important means of transportations could be through shipping, produces like cut flower long distance which usually takes longer periods and in most cases may take greater than three weeks. In order to maintain optimum storage conditions during transportations, a new cooling system known as passive refrigeration system (PRS^{TM}) was developed. PRS is developed for not only cut flowers but also for the preservation of fruits, vegetables and animal products during transportations (Siltal, 2003). A major cause of deterioration in cut flowers is the blockage of xylem vessels by air and microorganisms that might causes xylem occlusion. There are some commercial cut flower preservatives in the market. *8-hydroxyquinoline sulphate* (8HQS) is very important germicide which is currently used as preservatives in many floral industries (Nowak and Rudnicki, 1990). On the other hand, 8HQS acts as an antimicrobial agent (Ketsa et al., 1995) which is known to increase water uptake (Reddy et al., 1995).

Packaging materials also influences the vase life and

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quality of roses. Different packaging materials can be used to pack roses before passive refrigeration of cur flowers, in order to increase the preservative effect which leads to better quality management. The most common packaging material that is commercially being used in Ethiopia is cardboard box packaging. Whereas the most common preservative that is being used in the country is aluminum sulfate and in many farms other commercially existing products are used as cut flower preservatives. However, the efficacy of these preservatives alone or when combined with packaging and passive refrigeration cooling has not been investigated. Furthermore, solutions which are commonly called flower food contains biocide, sugar, acidulate, surfactant and other necessary mixes. The response of rose varieties grown in Ethiopia to these preservatives, flower foods or passive refrigeration system, pulsing solution and packaging materials is not well explained scientifically or unknown (Ethiopian Horticulture Producers and Exporters Association (EHPEA), 2008), although, a volume of export of floricultural produces is growing and currently showing great promise for Ethiopia's economy. Moreover, research activity focusing on postharvest handling of fresh cut flowers does not exist, while it is mandatory for export and to compute in global market. This is, therefore, need to address postharvest handling research of cut rose flowers and investigate the combined effects of packaging preservatives and passive refrigeration system for cut rose flowers so as to reduce the level of losses and boost up the return for the emerging farmers. Thus, this research was initiated to investigate the effect of pulsing solution, packaging type and storage duration under passive refrigeration system on the vase life and quality of roses.

MATERIALS AND METHODS

Experimental design and treatments

The treatment consisted of four pulsing solutions *silver thiosulfate (STS)* + Chrysal clear solution (RVB), *silver thiosulfate* + *8-hydroxyquinoline sulphate (8HQS)*, *silver thiosulfate* + Chrysal clear solution + 8-*hydroxyquinoline sulphate* and H_2O), two packaging types (box and polyethylene bag combined with box) and four storage periods under PRS (0, 2, 3 and 4 weeks). Chrysal clear RVB solution is a clear universal hydrating treatment which balances the pH of all water and stimulates the water uptake, prevents bent-neck and quality of cut flower (Timmerman and Kroon, 2009). The treatments were combined in a factorial design, resulting in a total of 32 treatment combinations with 4 replications. Each treatment consists of 10 cut flower stems.

Experimental procedures

The rose cultivar used in this study was 'red calypso' and has an intermediate bud size (3.0 to 3.5 cm) with red color (Mitterer and de Ruiter, 2008). Cut flowers of 'red calypso' were obtained from AQ Roses Plc. At the time of the experiment, plants of 'red calypso' were grown under greenhouse condition 37.2°c maximum and 19.6°C minimum temperatures. The roses were harvested at opening stage. Harvesting was carried out manually with care to minimize mechanical injuries or damage. Harvested roses were placed in a plastic container with water and transported to the packing house where they were graded and bunched. Flowers with uniform opening stages, similar bud size and stem length were selected. Lower leaves were removed and the flower stems were trimmed to a uniform length of 50 cm and bundled together.

Two milliliters of STS, RVB clear and 8HQS were added into 4 *l* of water to get the required concentrations (0.5%, v/v). Ten bunches of 10 stems of cut flowers were put in buckets with 4 *l* of the pulsing solutions. Then, the samples were placed in cold room (2 to 4°C) for 24 h. After 24 h of pulsing, the sample cut flowers were packed according to the treatments. A cardboard box with a size of 100 x 33 x 21 cm top boxes of 3 ply and a bottom of 5 ply was used as packaging material. The second packaging was a high density (thick) micro-perforated polyethylene bag (1 x 2 m). The temperature inside the storage was recorded using data loggers (KEY TAG, Model KTL-108).

The samples for week 0 analyses were kept for 3 days inside pre-cooling room (10°C). All the other samples for week 2, 3 and 4 were stored under passive refrigeration system. The passive refrigeration system (model DS-TP-001-03) equipped with thermo pallet (0.97, 1.95 and 2.42 m^3) storage area were used to store the remaining samples until weeks 2 (14 days), 3 (21 days) or 4 (28 days). Then, the samples were subjected to quality analysis. The sample cut flowers were taken out of pre-cooling room after 3 days' storage. After unpacking, the flowers were cut (2 cm) from each stems bottom and put in flora life fast dissolving fresh flower transport and storage solution for 24 h at 2 to 4°C and 90 to 95% relative humidity. Finally, the flowers were taken to the laboratory for vase life testing where the room was controlled at 20°C and 60% combined with 24 h lighting. The flowers were re-cut into 1 to 2 cm from the bottom before putting them on a vase to facilitate the stems solution uptake. The Chrysal clear solution (10 g l^{-1}) was used for analysis. In all the vases, flower of 10 stems with Chrysal flower food were used to evaluate the cut flower stalks. The experiment was continued until the entire flower was rotten.

Data collection

Relative fresh weight and solution uptake

The sample cut flowers were weighed every two days until the end of vase life. The sample flowers were taken out of water for a very short time of 20 to 30 s. The fresh weight of each flower was measured using analytical balance (SW-1S with serial number SN 060713167). The fresh weight was expressed relative to the initial weight of sample flowers (Joyce and Jones, 1992). The volume of solution uptake was calculated by subtracting the volume of water evaporated from a flask of the same volume without cut flowers that of the total volume of water lost from the flask with cut flower sample (Chamani et al., 2005). The volume of water lost was calculated by subtracting the increase in fresh weight from the water uptake volume.

Total soluble solids content

A sample of 5 leaves and 10 petals were taken for the determination of total soluble solid (TSS) contents of rose's petal and leaves. TSS content was measured by using hand refractometer (model RCZ, serial number SN 00850) using the procedures as described by Lacey et al. (2001). The petal juice was extracted using juice extractor. A hand refractometer with the range of 0 to 30 °Birx were used to determine TSS by placing 1 to 2 drops of rose petal juices on the prism. Between samples, the prism of the

1-Tight cut stage | 2-Normal cut stage | 3-Buying stage | 4-Buying stage | 5-Final stage

Figure 1. Developmental stage of rose flower.

refractometer was washed with alcohol, rinsed by distilled water and dried using soft tissue paper.

Botrytis incidence level

The *Botrytis* incidence (%) was determined using the procedure described by Phillips et al. (1985). A scale that varies from 1 to 5 with *Botrytis* percentage 1 = free from incidence, 2 = initiation of spores, 3 = outer petals start to be infected, 4 = more infected petals, 5 = fully rotten was employed in this study. In this case, 1 = 0%, 2 = 20%, 3 = 60%, 4 = 80%, 5 = 100% of the diseased area in each petal. Severity was assessed from the area of the petals infected with diseases relative to the total petal area. Before statistical analysis, each severity rating was converted to a disease proportion, in which the midpoints of the intervals were used (Campbell and Madden, 1990).

Flower bud opening stage

Flower developmental stage is shown in Figure 1. The flower bud opening was rated as follows: rating 1: very tight bud, only the outer petals are clearly visible; rating 2: bud starts to open, the tips of most inner petals are visible and the outer petals are starting to stand out; rating 3, bud open, the outer petals are fully expanded, the flower has not yet reached its maximum size; rating 4, bud is fully open; all the petals are fully expanded but the stamen are not clearly visible, the flower has reached its maximum size; rating 5, bud is fully open; all the petals are fully expanded but the stamen are clearly visible, the flower has reached its maximum size. The maximum flower head diameters of the flower were recorded using the procedure as described by Van Doorn et al. (1991). Flower bud diameters were measured daily with Vernier caliper (cm) and maximum flower diameter was used to evaluate the bud size difference between the treatments.

Visual leaf quality and vase life

The leaf qualities of roses were determined using the procedure as described by Timmerman and Kroon (2009).

Rating 1, very poor quality, very big quality problems, all consumers would discard the plant; rating 2, poor quality, big quality problems, most consumers would discard the plant; rating 3, reasonable quality, moderate quality problems, most consumers would not discard the plant; rating 4, good quality, small quality problems, not all consumers would not discard the plant and rating 5, very good quality, no quality problems and all consumers would like it. Vase life (days) refers to the number of days after the flowers were put on a vase until 50 % of the flowers on a vase (bouquet). Vase life was recorded as the number of days on vase (day 0) to until the flowers show symptoms of bent neck or advanced signs of fading on all petals (Liao et al., 2000).

Statistical analysis

The ANOVA was carried out using SAS. Mean comparisons were made using least significance difference (LSD) at 5% level of significance.

RESULT AND DISCUSSION

Relative fresh weight

The interaction effects of pulsing solution, packaging type and storage time on mean relative fresh weight of ‘red calypso’ flowers were not found to be significant ($P > 0.05$). Similarly, packaging and pulsing solutions had no significant effect ($P > 0.05$) on mean relative fresh weight of ‘red calypso’ flowers. Whereas, the storage period had significant effect ($P < 0.001$) on mean relative fresh weight on days 5 and 9 vase life. The mean weights of flowers stored for 4 weeks under PRS were found to be the same statistically with that of the fresh cut flower sample (Table 1). This indi-cates that there were no significant weight losses under PRS storage. Cut flowers stored for 2 weeks under PRS had higher relative fresh weight. On day 9, cut flowers stored for 3 weeks had significantly higher mean fresh weight.

The relative fresh weight of the sample cut flowers varied with the storage period. From 0 to 4 weeks of storage, the actual weights were decreased by 2.4, 6.0, 6.4 and 32.0%, respectively. Flowers stored under PRS had lower relative fresh weights than the fresh ones. This could be associated with a response to prolonged cold storage. Regardless of the storage period under PRS, relative fresh weight of flowers declined with the increase in vase life. This finding is in agreement with the report of Teixeira da Silva (2003), who reported that the duration of dry storage decreases the fresh weight of cut flowers. Similarly, Ichimura et al. (2002) reported that short vase life of cut rose flowers is due to rapid decline in water

Table 1. Effect of pulsing solution, types of packaging and storage time on fresh weight, solution uptake and petal TSS of 'red calypso' flowers stored under passive refrigeration system.

Treatment	Mean relative fresh weight (g)					Solution uptake in ml day^{-1} g^{-1}fresh weight					Petal TSS (°Brix)			
	1	5	9	13	Mean	1	5	9	13	Mean				
Solution														
STS + RVB	100	97.80	71.36	74.68	85.96	0.63	0.49	0.38	0.33	0.46	6.58	5.83	5.20	5.87
STS + HQS	100	100.34	93.46	65.40	89.80	0.58	0.50	0.36	0.31	0.44	6.61	5.52	5.53	5.88
STS + RVB + HQS	100	99.50	90.28	63.39	88.29	0.63	0.50	0.36	0.32	0.45	6.67	5.83	5.48	5.99
Water	100	100	91.16	64.51	89.08	0.62	0.49	0.36	0.33	0.45	6.41	5.36	5.11	5.63
Mean	100	99.57	86.56	66.99		0.62	0.49	0.37	0.32		6.57	5.64	5.33	
$LSD_{(0.05)}$	NS	NS	NS	NS		NS	NS	NS	NS		NS	NS	NS	
Packaging material														
Box	100	99.21	81.87	62.37	85.86	0.60	0.50	0.37	0.33	0.45	6.66	5.69	5.37	5.91
Box + polyethylene	100	99.93	91.27	71.62	90.71	0.63	0.50	0.35	0.32	0.45	6.47	5.57	5.30	5.78
Mean	100	99.57	86.57	66.99		0.62	0.50	0.36	0.33		6.57	5.63	5.34	
$LSD_{(0.05)}$	NS	NS	NS	NS		NS	NS	NS	NS		NS	NS	NS	
Storage period														
0 week	100^{a}	100^{a}	97.75^{a}	91.81^{a}	97.62^{a}	0.79^{a}	0.64^{a}	0.51^{a}	0.51^{a}	0.61^{a}	7.66^{a}	7.25^{a}	6.33^{a}	7.08^{a}
2 week	100^{a}	100^{a}	87.49^{b}	86.70^{a}	94.03^{a}	0.57^{b}	0.50^{b}	0.46^{b}	0.27^{b}	0.45^{b}	6.92^{b}	6.91^{b}	5.86^{b}	6.56^{a}
3 week	100^{a}	99.05^{b}	91.74^{a}	83.52^{a}	93.58^{a}	0.56^{b}	0.50^{b}	0.38^{c}	0.26^{b}	0.42^{b}	6.75^{b}	6.70^{b}	5.56^{b}	6.34^{a}
4 week	100^{a}	96.33^{c}	75.85^{c}	Nd*	68.05^{b}	0.54^{b}	0.48^{c}	0.27^{d}	Nd*	0.33^{c}	5.73^{c}	4.78^{c}	Nd*	3.50^{b}
Mean	100^{a}	99.55^{a}	86.72^{b}	66.99^{c}		0.62	0.53	0.40	0.26		6.72^{a}	6.41^{a}	4.44^{b}	
$LSD_{(0.05)}$	NS	2.673	12.54	16.224		0.048	0.007	0.025	0.018		0.50	0.42	0.38	
Over all mean	100	99.56	86.57	66.99		0.61	0.50	0.36	0.33		6.57	5.63	5.33	
CV (%)	2.87	8.89	9.21	10.86		2.87	5.41	9.34	14.2		8.69	9.49	8.72	

Means followed by the same letter (s) are not significantly different at 5% level of significancy; NS = non significant at 5% level; RFW = relative fresh weight at day 1, 5, 9 and 13**.**

uptake and drying out of stems. The decrease in relative fresh weight with the increase in the storage period could be associated with a continual loss of water by the flowers through transpiration. The other reason could also be attributed to the fact that decreased capacity of flowers to absorb water from the solution or both during storage period.

Solution uptake

The interaction effects between pulsing solution, packaging type and storage period were not significant (P > 0.05) on the mean solution uptake of 'red calypso' sample flowers. Similarly, pulsing solution and packaging type had no significant effect (P > 0.05) on mean solution uptake of 'red calypso' flowers. One the other hand, the storage period had significant (P > 0.001) effect on the mean solution uptake of 'red calypso' flowers (Table 1). Mean solution uptakes of fresh flowers without storage were found to be significantly higher than the solution uptake of sample flowers stored under PRS for few weeks. In general, the mean solution uptake decreased with the increasing storage period. The mean solution uptake reached minimum value in cut sample flowers after 4 weeks of storage under PRS. The mean solution uptake of cut flowers averaged across vase life days from 0 day to 4 weeks decreased by 28.9, 76.5, 88.1 and

139.4%, respectively. This is in agreement with the findings of Teixeira da Silva (2003), who reported that the duration of dry storage decreases the solution uptake of cut flowers. The solution uptake decreased with an increase in the storage period and hence led to decreased water absorption capacity of the sample cut flowers. This could also be due to the sample flowers response to prolonged chilled storage using passive refrigeration system. Regardless of the duration of storage under PRS, solution uptake of 'red calypso' flowers declined with increasing vase life. On the other hand, water absorption by cut flowers decreased with an increase in vase life resulting in dropping of flowers and the premature wilting of both flowers and leaves. Moreover, decrease in water uptake of cut flowers with vase life could be attributed to the blockage of xylem vessels by micro-organisms that enter into the stems which might be the cause of decrease in water uptake of 'red calypso' flowers in this study. Pulsing with mixtures of STS, RVB and 8HQS solutions had no significant effect on water uptake of 'Red Calypso' flowers in this study. However, Knee (2000) reported that STS and 8HQS pulsing solutions had effect on water uptake of cut flowers which is in disagreement with the present findings. Similarly, literature also showed that the rates of water uptake by stems were highly variable but generally decreased over time (Veen and Kwakkenbos, 1983). Burge et al. (1996) also showed that pulsing cut flowers with STS increased water uptake compared with deionized water treatment. In addition, Chamani et al. (2005) reported that pulsing rose flowers with STS for 2 h greatly assisted in maintaining solution uptake.

Total soluble solid contents of petals

Storage period had significant effect ($P < 0.001$) on mean TSS contents of flower petals on 4, 8 and 12 vase life days (Table 1). The mean TSS contents of flower petals stored for 0 week under PRS was significantly higher than TSS contents of cut flowers stored under PRS for 2, 3 and 4 weeks. The TSS contents of flowers stored under PRS decreased with an increasing storage time, the lowest value being recorded at 8 weeks for all the three measurements. Pulsing solution had also a significant effect on TSS contents of flowers on 4 vase life days. On day 4, flowers pulsed with mixtures of STS + RVB, STS + 8HQS and STS + RVB + 8HQS pulsing solutions had significantly ($P < 0.05$) higher TSS than those sample cut flowers that were treated with water only as a control treatment.

TSS contents of petals decreased with increasing storage period which is in positive agreement with the findings of Figueroa et al. (2005). Initiation of senescence in the rose flowers coincided with a decrease in the level of TSS (Coorts, 1973; Kaltaler and Steponkus, 1976). Regardless of storage time under PRS, TSS contents of petals decreased with an increase in vase life (Kaltaler and Steponkus, 1976). All interaction effects of pulsing solution, packaging type and storage time under PRS on mean TSS contents of 'red calypso' flower petals were not significant ($P > 0.05$) at all three measurements.

***Botrytis* incidence**

The two-way interaction between pulsing solution and storage period was found to be significant ($P < 0.001$) on the changes in percentage of *Botrytis* incidence. On day 8, percentage *Botrytis* incidence for 0 week was almost the same regardless of pulsing solution. Percentage *Botrytis* incidence on cut flowers stored for 2, 3 and 4 weeks under PRS varied with type of pulsing solutions (Table 2). Mean percent *Botrytis* incidence associated with sample cut flowers stored using PRS for 3 weeks and subjected water pulsing treatment and a mixture of STS and RVB clear were found to be significantly higher than those treated with STS + 8HQS. The lowest *Botrytis* incidence was being recorded on sample cut flowers that were treated by the mixtures of STS, RVB and 8HQS. After 4 weeks of storage, the highest *Botrytis* incidence was recorded in flowers subjected to water treatment and followed by those treated with the mixtures of STS and RVB; STS and 8HQS; and STS, RVB and 8HQS.

Botrytis incidence level on roses depends on the pulsing solutions and the storage period. *Botrytis* incidence level on cut rose flowers pulsed with all 4 solutions was the highest after 4 week of storage under PRS and decreased with decreasing storage period. *Botrytis* incidence increased with an increase in storage period under PRS both on days 8 and 12 vase life. This result is in line with the findings of Reid (2005), who reported that an increase in incidence of *Botrytis* in stored flowers. Pulsing 'red calypso' flowers with STS, 8-HQS and RVB clear solution reduced gray mold progress. Elad and Volpin (1993) also reported that reduced severity of gray mold on rose and carnations treated by STS.

The two-way interaction effect of pulsing and packaging was found to be significant ($P < 0.05$) on *Botrytis* incidence on days 8 and 12 vase life. On days 8 and 12 vase life, the *Botrytis* incidence on cut sample flowers that were subjected to water treatments showed higher incidence than those pulsed with the mixtures of STS, RVB and 8HQS (Table 2). Across the pulsing solution treatments, the packaging type also had its own contribution on *Botrytis* incidence. Packaging flowers with polyethylene bags seemed to favour the *Botrytis* incidence when compared with cardboard box packaging (Table 3). This could be attributed to the modification of gas composition which might have resulted in slow respiration. This result seems to disagree with the finding of Phillips et al. (1985), who reported that the injection of CO_2 to the storage container significantly reduced the severity of *Botrytis* rot and improved the quality and vase life. The three-way interaction effects of pulsing solution, packaging and storage period on *Botrytis* incidence were

Table 2. Interaction effect of pulsing and storage period under PRS on mean *Botrytis* incidence (%) on 'red calypso' flowers.

Pulsing solution	Day 8 vase life					Day 12 vase life			
	0	2	3	4	Mean	0	2	3	Mean
STS+RVB clear	1.00^{g}	1.00^{g}	2.00^{de}	3.00^{b}	1.75	1.00^{e}	1.25^{de}	2.75^{a}	1.67
STS+ 8 HQS	1.00^{g}	1.38^{fg}	1.63^{ef}	2.50^{c}	1.63	1.00^{e}	1.25^{de}	1.50^{cd}	1.25
STS+RVB clear+ 8HQS	1.00^{g}	1.30^{g}	1.30^{g}	2.38^{cd}	1.50	1.13^{e}	1.75^{c}	1.50^{cd}	1.46
Water	1.13^{g}	1.63^{ef}	2.38^{cd}	3.63^{a}	2.19	1.25^{e}	2.25^{b}	2.75^{a}	2.08
Mean	1.03	1.33	1.83	2.88		1.10	1.63	2.13	
LSD (5%)	0.41					0.44			
CV	9.13					6.54			

Means followed by the same letter(s) are not significantly different at 5% level of significant.

Table 3. Interaction effects of pulsing and packaging on *Botrytis* incidence level of 'red calypso' stems at day 8.

Pulsing solution	Packaging material					
	Box	Box + PE	Mean	Box	Box + PE	Mean
STS+RVB clear	1.50^{b}	1.69^{b}	1.60	0.94^{c}	0.94^{c}	0.94
STS+ 8 HQS	1.50^{b}	1.63^{b}	1.57	1.06^{c}	1.13^{c}	1.10
STS+RVB clear+ 8HQS	1.69^{b}	1.72^{b}	1.71	1.06^{c}	1.44^{b}	1.25
Water	1.88^{ab}	2.31^{a}	2.10	1.44^{b}	1.68^{a}	1.56
Mean	1.64	1.84		1.13	1.30	
LSD (5%)		0.18			0.20	
CV		9.13			6.54	

Means followed by the same letter(s) are not significantly different at 5% level of significant.

not significant ($P > 0.05$).

Bud opening stage

After 4 and 8 of storage, the three-way interactionbetween pulsing solution, types of packaging and storage period had significant ($P < 0.01$) effect on bud opening stage of 'red calypso' flowers. Bud opening stages of flowers stored for 2, 3 and 4 weeks under PRS differed from each other based on pulsing solution treatments (Table 4). Throughout the storage period of 4 weeks, cut flowers samples subjected to water pulsing treatment had lower bud opening values than flowers pulsed with the mixtures of STS, RVB and 8HQS. There was no noticeable difference in bud opening stages of 'red calypso' flowers packed in boxes combined with polyethylene for 0 and 2 weeks storage across all the pulsing solution treatment. However, flowers stored for 3 and 4 weeks showed difference ($P < 0.001$) in bud opening stages across the pulsing solution treatment combinations. Flowers that were pulsed with water had lower mean bud opening values than flowers pulsed with STS, RVB and 8HQS. The maximum bud opening stage was recorded for all the cut flowers subjected to the pulsing solutions treatment after 4 weeks of storage.

Bud opening stage on day 4 responded positively to the duration of storage period and reached maximum in flowers stored for 4 weeks under PRS. On the same day, the mean bud opening stage of roses packed in cardboard box was found to be 2.7 and in box + polyethylene packaging the mean bud opening value was 2.63. In both packaging types, the mean bud opening stage of roses pulsed with water was lower than in cut flowers subjected to the other pulsing solutions. On day 8, bud opening stage of 'red calypso' that was packed in box and stored for 2 week as well as pulsed with the mixtures of STS, RVB and 8HQS solutions were found to be almost equivalent. However, there was a variation in bud opening between sample cut flowers subjected to different pulsing solutions and stored for the same storage period under PRS storage (Table 4). Flowers that were pulsed with water had lower bud opening stage values compared with flowers that were pulsed with STS, RVB and 8HQS.

Pulsing solution had effects on bud opening of cut flowers that were stored for 3 and 4 weeks under PRS. Generally, flowers that were subjected to water pulsing treatment had lower bud opening values compared with those treated with the mixtures of STS, RVB and 8HQS. The maximum bud opening stage value was observed in flowers stored for 2 weeks after packaging in box combined with or without polyethylene for all the pulsing solution treatments. The bud opening stage values

Table 4. Interaction effect of pulsing, packaging and storage period (weeks) on opening stage of 'red calypso' flower buds.

Treatment/pulsing solution	Day 4 vase life					Day 8 vase life				
	0	2	3	4	Mean	0	2	3	4	Mean
Box packaging										
STS + RVB clear	2.50^{c}	2.75^{bc}	2.75^{bc}	3.12^{a}	2.78	3.00^{ef}	4.00^{a}	3.62^{b}	3.38^{bcd}	3.50
STS + 8 HQS	2.50^{c}	2.63^{c}	2.75^{bc}	3.00^{ab}	2.72	3.60^{b}	4.13^{a}	3.38^{bcd}	2.88^{f}	3.47
STS + RVB + 8HQS	2.50^{c}	2.50^{c}	3.00^{ab}	3.00^{ab}	2.75	3.50^{bc}	4.25^{a}	3.62^{b}	3.38^{bcd}	3.63
Water	2.50^{c}	2.50^{c}	2.50^{c}	2.63^{c}	2.53	3.50^{bc}	3.50^{bc}	3.00^{ef}	2.88^{f}	3.19
Mean	2.50	2.60	2.75	2.94		3.41	3.97	3.38	3.13	
Box + polyethylene										
STS + RVB clear	2.50^{c}	2.63^{c}	2.75^{bc}	3.13^{a}	2.75	3.50^{bc}	4.00^{a}	3.50^{bc}	3.50^{bc}	3.63
STS + 8 HQS	2.50^{c}	2.50^{c}	2.50^{c}	2.75^{bc}	2.56	3.50^{bc}	4.00^{a}	3.25^{cde}	3.00^{ef}	3.47
STS + RVB + 8HQS	2.50^{c}	2.50^{c}	2.75^{bc}	3.00^{ab}	2.69	3.50^{bc}	4.00^{a}	3.25^{cde}	3.13^{def}	3.53
Water	2.50^{c}	2.50^{c}	2.50^{c}	2.50^{c}	2.5	3.00^{ef}	3.38^{bcd}	3.00^{ef}	2.88^{f}	3.09
Mean	2.5	2.53	2.63	2.85		3.38	3.85	3.25	3.13	
LSD (5%)	0.29					0.37				
CV	7.68					7.63				

Means followed by the same letter(s) are not significantly different at 5% level of significance.

Table 5. Interaction effect of pulsing and storage period (weeks) on opening stage of 'red calypso' flower buds.

Pulsing solution	Day 12 of vase life				Day 12 of vase life				
	0	2	3	Mean	0	2	3	4	Mean
STS + RVB clear	4.06^{c}	4.18^{ab}	3.60^{d}	3.95	6.50^{c}	8.00^{a}	7.13^{b}	6.86^{bc}	7.12
STS+ 8HQS	4.00^{c}	4.25^{a}	3.31^{e}	3.85	7.13^{b}	8.13^{a}	6.63^{bc}	5.88^{d}	6.93
STS + RVB + 8HQS	4.00^{c}	4.13^{ab}	3.50^{d}	3.88	7.00^{bc}	8.25^{a}	6.75^{bc}	6.62^{bc}	7.14
Water	3.50^{d}	4.00^{c}	2.93^{f}	3.48	6.50^{b}	6.88^{bc}	5.88^{d}	5.90^{d}	6.29
Mean	3.89	4.14	3.34		6.78	7.80	6.60	6.31	
LSD (5%)	0.18				0.2583				
CV	6.52				7.57				

Means followed by the same letter(s) are not significantly different at 5% level of significant.

increased on day 8 and the value reached its maximum after week 2 storage (stage 4) under PRS (Table 5), declined thereafter to the lowest value (2.88) towards end week 4. Pulsing solution had also significant effect on bud opening of cut flowers on day 8. However, the mean bud opening stage of roses subjected to water pulsing treatment had lower bud opening stage when compared to bud opening values of flowers treated with the other pulsing solutions. This result is in agreement with the findings of Timmerman and Kroon (2009).

On day 12, the bud opening stage ranged from 2.93 to 4.25 with the highest stage being recorded for flowers stored for week 2 under PRS storage conditions. The interaction effect of pulsing and storage period on bud opening stage was significant after 12 days of storage ($P < 0.001$). After 12 days of storage, mean bud opening stages of cut flowers pulsed with all the four solutions were found to be different each other (Table 5). Bud opening stage of flowers depends on the pulsing treatment and storage period. Bud opening stage of flowers was found to be the highest after 2 week of storage under PRS. Several studies showed that petal growth is associated with flower bud opening which results from cell expansion (Kenis et al., 1985) and requires the influx of water and osmolytes such as carbohydrates into petal cells (Evans and Reid, 1988).

Maximum flower bud opening

Maximum flower bud opening of 'red calypso' flowers in this study ranged from 5.88 cm to 8.25 cm which is in agreement with literature data ranges. Elias (2009) reported that the maximum flower bud opening for 'red calypso' without storage is 8.7 cm. The effect of packaging was not significant ($P > 0.05$) on the maximum flower bud opening. The two-way interaction between pulsing solution and storage period was highly significant ($P < 0.001$) on bud opening stage. For 0 and 2 weeks storage, the mean maximum flower bud opening stage of 'red calypso' flowers was found to be higher in cut flowers subjected to STS + 8HQS and STS + RVB + 8HQS solutions. The lowest values were recorded in cut flowers treated with STS + 8HQS on 0 week and with

Table 6. Interaction effects of pulsing solution, packaging and storage period on visual leaf quality of 'red calypso' flowers on day 12 of vase life.

Pulsing solution	Storage duration (week)			
	0	2	3	Mean
Box packaging				
STS + RVB	4.00[a]	3.00[bc]	2.50[c]	3.17
STS + 8HQS	4.00[a]	3.00[bc]	2.50[c]	3.17
STS + RVB + 8HQS	3.75[a]	3.00[bc]	2.50[c]	3.08
Water	2.75[cd]	2.50[c]	2.50[c]	2.58
Mean	3.63	2.86	2.50	
Box + polyethylene packaging				
STS + RVB	3.00[bc]	2.75[bc]	2.50[c]	2.75
STS + 8HQS	3.00[bc]	2.50[c]	2.50[c]	2.67
STS + RVB + 8HQS	3.00[bc]	2.75[bc]	2.50[c]	2.75
Water	2.50[d]	2.00[e]	2.50[c]	2.33
Mean	2.86	2.50	2.50	
LSD (5%)	0.29			
CV	7.68			

Means followed by the same letter(s) are not significantly different at 5% level of significant.

STS+ RVB + 8HQS on 2 weeks. In flowers stored for 3 and 4 weeks, the mean maximum flower bud opening were realized in STS + RVB, while the lowest values were recorded in flowers pulsed with water only (Table 5).

Maximum flower bud opening of 'red calypso' depends on the type of pulsing solutions and storage period. Maximum flower bud opening responded positively to pulsing with RVB clear, 8HQS and STS solutions. Pulsing 'red calypso' flowers with STS + RVB clear solution, STS + 8HQS and STS + RVB clear solution + 8HQS increased the flower bud opening size by 13.20, 10.17 and 13.51% when compared with those subjected to water pulsing as a control. Ichimura et al. (2005, 2006) found that maximum flower bud increases when 20 g sucrose l^{-1} plus and 200 mg of HQS l^{-1} pulsing solution are used. Maximum flower bud opening of 'red calypso' stored under PRS decreased with increasing storage life. The maximum flower bud opening decreased with an increasing of storage period.

Visual leaf quality

Visual leaf quality of ''red calypso'' cut flowers was very good. This is similar to the leaf quality scale that was reported by Timmerman and Kroon (2009). The three-way interaction between pulsing solution, packaging material and storage period was significant ($P < 0.001$) on the changes in visual leaf quality on day 12. On this vase life day, the leaf quality of flowers packaged using cardboard box packaging was better compared to the quality at the other storage periods (Table 6). The interaction effect of pulsing solution and duration of storage on visual leaf quality was highly significant ($P < 0.001$) on day 4 of vase life. On this day, the highest scale (5.00) was recorded in flowers stored for 0, 2 and 3 weeks whereas the lowest (3.50) was recorded in flower stored for 4 week. On day 4, visual leaf quality of 'red calypso' which was stored for 0, 2 and 3 weeks and pulsed with the STS + RVB, STS + 8HQS and STS + RVB + 8HQS solutions was statistically the same. Nevertheless, flowers stored for 4 week showed noticeable quality difference (Table 7). Similarly, the interaction effect of pulsing solution and duration of storage on visual leaf quality was highly significant ($P < 0.001$) on day 8 of vase life. On this day, the highest scale which is 4.88 was recorded at storage duration 2 weeks and the lowest was 2.38 at week 4. Leaf yellowing and Leaf drop were the main quality problems observed on the leaf.

Vase life

Mean vase life of 'red calypso' cut flowers ranged from 10 to 17 days. Vase life is 15 and 17 days (Elias, 2009). The vase life of 'red calypso' cut flowers in this study came to an end due to wilting. And some of them especially week 4 storage shows decay from *Botrytis*. Termination of vase life for many cut flowers is characterized by wilting. Generally, wilting is caused by an imbalance between water uptake by flowering stems and water loss via transpiration from their leaves and/or other organs unless despite their stem being held in water continuously (Halevy and Mayak, 1981; van Doorn and Stead, 1997).

Table 7. The interaction effect of pulsing and storage period on visual leaf quality of 'red calypso' flowers on day 4 of vase life.

Pulsing solution	Storage duration (week)									
	Day 4 of vase life					Day 8 of vase life				
	0	2	3	4	Mean	0	2	3	4	Mean
STS + RVB	5.00^{a}	5.00^{a}	5.00^{a}	3.50^{d}	4.62^{a}	3.88^{bcd}	4.88^{a}	4.00^{bc}	3.00^{e}	3.94
STS+ 8HQS	5.00^{a}	5.00^{a}	5.00^{a}	3.63^{d}	4.66^{a}	3.75^{bcd}	4.75^{a}	4.00^{bc}	2.63^{ef}	3.78
STS+ RVB + 8HQS	5.00^{a}	5.00^{a}	5.00^{a}	4.00^{c}	4.75^{a}	3.50^{d}	4.75^{a}	4.00^{bc}	3.63^{cd}	3.97
Water	4.87^{b}	4.75^{b}	4.50^{b}	3.50^{d}	4.40^{c}	3.00^{e}	4.13^{b}	3.88^{bcd}	2.38^{f}	3.35
Mean	4.96^{a}	4.94^{a}	4.87^{b}	3.66^{c}		3.53	4.63	3.97	2.91	
LSD (5%)	0.29					0.42				
CV	6.42					9.61				

Means followed by the same letter(s) are not significantly different at 5% level of significant.

Table 8. Interaction effects of pulsing solution and storage period under PRS on vase life of 'red calypso' flowers.

Pulsing solution	Storage duration (week)				
	0	2	3	4	Mean
STS + RVB clear	17.00^{ab}	14.96^{c}	14.87^{c}	10.10^{d}	14.23^{a}
STS + 8HQS	16.80^{b}	14.95^{c}	14.98^{c}	10.07^{d}	14.20^{a}
STS + RVB clear + 8HQS	17.30^{a}	15.02^{c}	14.84^{c}	10.04^{d}	14.30^{a}
Water	15.00^{c}	14.91^{c}	14.90^{c}	10.18^{d}	13.75^{b}
Mean	16.53^{a}	14.96^{b}	14.90^{b}	10.10^{c}	
LSD (5%)			0.43		
CV			3.1		

Means followed by the same letter(s) are not significantly different at 5% level of significant.

The combined effect of pulsing solution and duration of storage under PRS on vase life of 'red calypso' flowers was found to be significant ($P > 0.001$). At 0 day of storage, mean vase life of 'red calypso' flowers that were pulsed using the mixtures of STS + RVB, STS + 8HQS and STS + RVB + 8HQS were higher compared with the vase life of flowers pulsed with water only. Mean vase life of 'red calypso' flowers decreased with duration of storage but varied very little with pulsing solutions (Table 8). Pulsing with STS + RVB, STS + 8HQS and STS + RVB + 8HQS extended the vase life of fresh 'red calypso' cut roses by 1.5 days compared with those pulsed with water (Table 7). The positive response of vase life to STS, RVB clear and 8HQS could be due to their effect on delaying senescence, carbohydrate source and biocide source, respectively. This response of cut roses to pulsing with STS, RVB and 8 HQS depends on the storage duration under PRS.

Mean vase life of 'red calypso' flowers decreased with the storage period (Table 8). It decreased by 88 and 87.6% between 2 and 3 weeks and 59.41% between 3 and 4 weeks, respectively. Vase life of cut rose flowers of cultivar 'brodiae' in refrigerated storage at 1°C for 0, 2 and 4 weeks decreased by 98.4 and 70.77% from 0 week storage after it was pulsed with 2% sucrose solution (Susan et al., 1990). It is logical that the most important components of the cold-storage technique which might adversely affect quality are: (1) water loss during dry storage; (2) low temperature injury; (3) continued ageing during the time at low temperature (Halevy and Mayak, 1974). The water status of the flower was assumed to be an important determinant of flower quality after cold storage (Halevy and Mayak, 1974). Nevertheless, the effect of passive refrigeration storage has better vase life of 'red calypso' cut flowers in this study.

Conclusion

The three-way interaction between pulsing solution, packaging and storage period was significant ($P < 0.01$) on flower bud opening stage and leaf quality of flowers. Similarly, the two-way interaction between pulsing solution and storage period was found to be highly significant ($P < 0.01$) on *Botrytis* incidence, maximum flower bud opening and vase life of 'red calypso' flowers. Storage time significantly ($P < 0.05$) affected the solution uptake, relative fresh weights and TSS contents of petals. After 2 and 3 weeks storage in PRS, 'red calypso' flowers subjected to the mixtures of STS, RVB and 8HQS pulsing

treatment had vase life of 15 days, which is only 1.5 day lower than fresh control cut flowers. The average solution uptake, relative fresh weights, bud opening stage, maximum flower bud opening, TSS contents of petals, *Botrytis* incidence and leaf quality of 'red calypso' flowers after three weeks in PRS and subjected to the mixtures of STS, RVB and 8HQS pulsed treatments were as good as that of the fresh flowers. Growers can thus, transport roses from Ethiopia to Europe using ships equipped with PRS to reduce their cost with out significant reduction in vase life and quality of flowers. However, more research is needed to include other rose varieties and on alternative pulsing solutions that can further enhance the quality and vase life of roses during PRS storage.

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