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CHANGES IN CARBOHYDRATES ASSOCIATED WITH SENESCENCE OF CUT GLADIOLUS SPIKES UNDER PULSING AND WET COLD STORAGE DURATIONS

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ABSTRACT

Several biochemical and metabolic changes are associated with senescence of cut gladioli, particularly in relation to quality characteristics, including dynamism of carbohydrates. The objective of this study was to evaluate the effect of pulsing and wet cold storage on the starch and sugar biomarkers on cut Gladiolus (*Gladiolus grandiflorus* L. cv. Fado) vase quality. Pulsing treatments of 600-ppm 8-hydroxyquinoline sulphate, plus 5% sucrose solution, *versus* distilled water, were administered prior to wet cold storage durations of 0 - 5 days, on the cut Gladiolus grown from corms. This was done in the open field at the Horticulture Research and Teaching Field, Egerton University in Kenya, during two successive seasons. There was a significant difference ($P < 0.01$) in total soluble sugars (TSS) and total starch of spikes pulsed with 600 ppm 8 - HQS + 5% sucrose, compared with the control, during the third day in the vase life of the cut flowers. Prolonged vase life of cut spikes was associated with a decrease in total soluble sugars and increase in total starch, as influenced by pulsing and wet storage duration up to 4 days.

Key Words: *Gladiolus grandiflorus,* soluble sugar, starch

RÉSUMÉ

Plusieurs changements biochimiques et métaboliques sont associés à la sénescence des glaïeuls coupés, en particulier en relation avec les caractéristiques de qualité, y compris le dynamisme des glucides. L'objectif de cette étude était d'évaluer l'effet du stockage au froid pulsé et humide sur les biomarqueurs de l'amidon et du sucre sur la qualité des vases de glaïeul coupé (*Gladiolus grandiflorus* L. cv. Fado). Des traitements pulsés de sulfate de 8-hydroxyquinoléine à 600 ppm, plus une solution de saccharose à 5%, contre de l'eau distillée, ont été administrés avant des durées de stockage au froid humide de 0 à 5 jours, sur le glaïeul coupé à partir de bulbes. Cela a été faite dans le champ de Horticulture Research and Teaching Field, Egerton University au Kenya, pendant deux saisons successives. Il y avait une différence significative ($P < 0,01$) dans les sucres solubles totaux (TSS) et l'amidon total des épis pulsés avec 600 ppm 8 - HQS + 5% de saccharose, par rapport au témoin, au cours du troisième jour de la vie en vase de la coupe fleurs. La durée de vie prolongée en vase des épis

coupés a été associée à une diminution du des sucres solubles totaux et à une augmentation de l'amidon total, sous l'influence de la durée de stockage pulsé et humide jusqu'à 4 jours.

Mots Clés: *Gladiolus grandiflorus*, sucre soluble, amidon

INTRODUCTION

Different cut flower species vary in their requirements for carbohydrates necessary for keeping vitality of post-harvest quality (Jain, 2016). Several biochemical and metabolic changes take place in flowers during development and senescence, including activities such as breakdown of starch into sugars, proteins into amino acids and changes in the phenol content. A genetic programme mediated by changes in ethylene, abciscic acid and cytokinin contents (Gupta and Dubey, 2018) regulates plant senescence.

A number of treatments have been undertaken to delay senescence of cut flowers (Schouten *et al*., 2018). It is recommended that freshly cut flowers be placed in clean water, free of fluorides and chlorine to minimise loss of water through transpiration and respiration. Postharvest treatments on cut flowers have included pre-chilling of cut flowers in ice cold water, pulsing treatments using chemical and novel preservatives, maintaining cold chain storage and use of holding solutions, among other things (Manzoor *et al.*, 2018).

Chemical treatments administered during cold storage help to alleviate flower deterioration due to chilling injury and nutrient depletion (Jain, 2016). It has been documented that *Gladiolus* have been cold stored dry at 1-4 ^{o}C for 3-4 weeks; while *carnation* cut flowers were wet cold stored at 4 ^{o}C and dry cold stored at 0-1 ^{o}C for durations of 4 and 4-12 weeks, respectively (Ahmad *et al*., 2016) . Cut flowers/plants experience physiological changes and ethylene accumulation on removal from storage, and hence chemical treatments are employed to preserve quality (Jain, 2016)

Inclusion of growth regulators such as cytokinin, gibberellins and auxin has been found to improve post-harvest quality of cut flowers (Faraji *et al*., 2011). An innovation thatutilised an electrochemically treated solution containing potassium chloride, hypochloric acid and dissolved oxygen, into which cut flower stems were inserted, improved their quality and vase life (Abdolahi *et al*., 2013). Anumber of chemical treatments have been used to improve cut Gladiolus quality parameters (Bhat and Sheikh, 2015), while a similar fate was accomplished in some ornamental plants treated with preservative solutions containing glycerol and surfactants (Dung *et al*., 2017). The objective of this study was to evaluate the effect of pulsing and wet cold storage on the starch and sugar biomarkers on cut Gladiolus (*Gladiolus grandiflorus* L. cv. Fado) vase quality.

MATERIALS AND METHODS

Gladiolus were grown in the open fields from corms, at the Horticulture Research and Teaching Field, in the Department of Horticulture, Egerton University, Kenya, during the period of July to December 2014 (Plate 1). The maximum and minimum field temperature ranges were 19 - 22 ^{o}C day and 5 -8 ^{o}C night, respectively. The average room temperature used for vase study was 18 ^{O}C with a relative humidity of 76%, and 12/12 hours photoperiod as described by Jeptoo *et al*. (2013).

Treatments and experimental design. A two by six factorial experiment embedded in a completely randomised design with four replicates, was adopted. The factors under study were pulsing at two levels (i.e., pulsing and non-pulsing) and cold storage durations at six levels, *viz*. 0, 1, 2, 3, 4 and 5 days.

Plate 1. Gladiolus (*Gladiolus grandiflorus* L. cv Fado) under cultivation using standard agricultural practices.

Total soluble starch. Total starch was determined using the procedure described by Mukherjee and Mukherjee (2017), with minor modifications in the extraction and determination of total soluble sugars. Approximately, 0.2 g of finely powdered dried petal samples of the pulsed and non-pulsed *Gladiolus* were homogenised, with 10 ml of 80% ethanol by stirring thoroughly, allowing it to stand for 5 minutes and then centrifuging at 5000 rpm for 15 minutes. The residue was retained after removing the alcoholic solution to remove the sugars.

To the residue, 10 ml of fresh hot 80% ethanol was added, while stirring, and the contents were centrifuged again, retaining the residue as before by discarding the alcoholic solution. The washing treatment was repeated twice to get rid of the sugars, before the residue was dried over a water bath. To the dried residue, 5 ml of distilled water was added and the contents were cooled in ice before adding 6.5 ml of 52% perchloric with constant stirring for at least 30 minutes.

The contents in tubes were then centrifuged at 5000 rpm for 15 minutes, and the aqueous starch solution was poured into a 100 ml volumetric flask. The extraction was repeated twice and the starch solution pooled together. The volume was made up to 100 ml with distilled water, and was then filtered through Whatman No.1 filter paper.

Starch was extracted by pipetting out 0.2 ml of the filtrate from above into a test tube and bringing the volume to 1 ml with distilled water. Then, into the tube contents, 4 ml of freshly prepared anthrone reagent was added under ice. Each tube contents was thoroughly mixed and then heated in a water bath for 8 minutes at 100 °C. The solutions in the tubes were then rapidly cooled to room temperature, and the intensity of green to dark green colour was read at 630 nm in a digital UV-visible spectrophotometer.

A glucose standard calibration curve was prepared by making a stock glucose solution of 100 mg in 100 ml of water. A 1:10 dilution of the stock glucose solution in distilled water

was prepared. Aliquots of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml of the working glucose standard solution were made to 1 ml with distilled water, to obtain standard glucose concentrations of 0, 10, 20, 30, 40, 50, 60 and 70 µg ml^{-1}. To each millilitre of glucose at different concentrations, 4 ml of freshly prepared anthrone reagent was added under ice and the contents stirred thoroughly. The test tubes were heated for 8 minutes in a water bath at 100 °C, and then cooled rapidly to room temperature. The intensity of green to dark green colour was read in a digital spectrophotometer for the different glucose standards.

A standard curve of glucose was prepared for absorbance against the different glucose concentrations. The amount of glucose in the samples of pulsed and non-pulsed petals and leaf samples was extrapolated from the glucose calibration curve.

The quantity of starch was calculated in terms of glucose equivalents and a factor of 0.9 was used to convert the value of glucose to starch (Mukherjee and Mukherjee, 2017).

Total soluble sugars. Extraction and determination of total soluble sugars from cut Gladioli was according to standard method (Asil and Roein, 2012) with minor modifications. Approximately, 0.2 g of finely powdered dried petal samples was homogenised in 10 ml of 80% ethanol, with constant stirring; and kept overnight. Centrifugation of the contents was then done at 2000 rpm for 20 minutes. Aliquots of 0.04 ml were then pipette to test tubes and the volume of 1 ml 5% phenol was added, followed by 5 ml of concentrated sulphuric acid with concurrent agitation of the tubes vigorously.

The solution in the tubes was left to stand at room temperature for 15 minutes. The golden yellow colour formed in each sample tube was assessed by reading the absorbance at 490 nm after properly setting and calibration of the instrument with a blank. The total sugar content in the flower samples was determined by reference to thestandard curve of sugar (glucose).

A 0.2 g percent stock glucose solution was prepared by dissolving 100 mg of glucose in 50 ml of 80% ethanol. A set of standard glucose solutions of strengths: 0, 20, 40, 60 and 80 µg ml^{-1} was prepared to 0, 0.01, 0.02, 0.03 and 0.04 ml of stock solution and respectively, the following aliquots of 80% ethanol were added to each tube: 0.04, 0.03, 0.02, 0.01 and 0 ml. The final volume in each tube was 0.04 ml, which was equivalent to the volume of the alcoholic aliquot taken from the alcoholic extract of the powdered flower sample.

To each of the test tube contents, 1 ml of 5% phenol was added, followed by the addition of 5 ml of concentrated H_2SO_4 with concurrent stirring to mix. The solution in each test tube was kept at room temperature for 15 minutes for colour development. The optical density (OD, absorbance) of each tube contents was read in a spectrophotometer at the wavelength of 490 nm.

A standard curve of glucose was prepared by plotting a graph of absorbance against the glucose concentration. Glucose concentration from the flower samples from the pulsed and non-pulsed gladioli was read off from graph from the standard calibrated graph (Asil and Roein, 2012).

Data analysis. Pro data collected were subjected to the GLM model in a two way analyses of variance (ANOVA), to determine differences in pulsing and cold storage treatments on the flower biomarkers of quality (i.e., total starch and total soluble sugars) and vase life). Significant differences among treatment means were assessed using Tukey's test at 5 % level of significance. Analysis was done using Java memory profiler (JMP) software.

RESULTS AND DISCUSSION

Total starch and total soluble sugars. Pulsing cut *Gladiolus grandiflorus* spikes with 600 ppm 8- HQS, in combination with 5% SUC solution, had a significant effect (P<0.01) on the total starch (TS) and total soluble sugars on the third day of the vase life (Table 1). The maximum TS (42.29 ± 0.30 µg mg^{-1} dw), in the pulsed spikes was observed in three days' wet cold stored *Gladiolus* flowers, which incidentally registered the highest vase life (11.50 ± 0.21 days). The next best vase life (11.25 ± 0.21 days) was attained in Gladiolus spikes pulsed and subjected to four days of wet cold storage whose mean starch concentration was 41.96 ± 0.136 µg mg^{-1} dw), and registered the next highest vase life (11.25 ± 0.21 days).

The effect of pulsing cut Gladiolus with 600 ppm 8- HQS, in combination with 5% SUC, followed by wet cold storage, elicited a higher mean total starch (37.00 ± 0.136 µg mg^{-1} dw), which was significantly different (34.54 ± 0.136 µg mg^{-1} dw) from that of the non-pulsed cut spikes (Tables 1 and 2). Also, the pulsing treatment enhanced levels of total starch when followed by wet cold storage durations of 1, 2, 3 and 4 days, translated into improved vase life compared with the zero day, stored spikes.

These results are comparable to accumulation of starch in Gladiolus, which was associated with stem maturation that allowed larger storage reserves, an attribute exhibited during the opening stage (da Costa and Finger, 2016). However, in both the pulsed and non-pulsed spikes, levels of total starch dropped in Gladiolus subjected to five days' wet cold storage period prior to vase study, which may have prompted a downward trend in the cut flower vase life (10.25 ± 0.21 days).

Reduction ofstarch content as the storage duration progressed has also been reported in studies on peony flowers (Walton *et al.*, 2010). Similar results were obtained in

TABLE 1. Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose and cold wet storage on the total soluble starch on the third day in the vase of the cut Gladioli (*Gladiolus grandiflorus* L.cv. Fado)

Days of cold storage	Total starch (µg mg^{-1} dry weight)		Vase (days)	
	Third day in vase		Senescence	
	Pulsed	No pulsing	Pulsed	No pulsing
0	25.75^{h}	20.74^{i}	8.75^{b}	4.75^{c}
1	34.41^{fg}	33.56^{g}	10.25^{ab}	9.50^{ab}
2	36.70^{de}	35.26^{ef}	10.00^{ab}	9.50^{ab}
3	42.29^{a}	37.62^{d}	11.50^{a}	9.75^{ab}
4	41.96^{ab}	40.59^{bc}	11.25^{ab}	11.00^{ab}
5	40.92^{abc}	39.47^{c}	10.25^{ab}	10.25^{hi}
Mean	37.00^{a}	34.54^{b}	10.333^{a}	9.125^{b}
CD at 5 %	0.23	0.23	0.21	0.21
Pulsing (P)	< 0.0001		0.0003	
Period of storage (S)	< 0.0001		< 0.0001	
P*S	< 0.0001		0.0041	

CD = Critical difference, LSD = Least significant difference. Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of significance

TABLE 2. Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose and cold wet storage on the total starch at senescence in the cut Gladioli (*Gladiolus grandiflorus* L. cv. Fado)

Days of cold storage	Total starch (µg mg^{-1} dry weight)		Vase (days)	
	Senescence		Senescence	
	Pulsed	No pulsing	Pulsed	No pulsing
0	14.10^{f}	10.10^{f}	8.75^{b}	4.75^{c}
1	19.29^{de}	18.58^{e}	10.25^{ab}	9.50^{ab}
2	21.28^{ed}	19.93^{de}	10.00^{ab}	9.50^{ab}
3	24.05^{b}	22.55^{bc}	11.50^{a}	9.75^{ab}
4	27.98^{e}	24.05^{b}	11.25^{ab}	11.00^{ab}
5	22.95^{bc}	23.99^{b}	10.25^{ab}	10.25^{hi}
Mean	21.60^{a}	19.90^{b}	10.33^{a}	9.13^{b}
C.D at 5 %	0.14	0.14	0.21	0.21
Pulsing (P)	< 0.0001		0.0003	
Period of storage (S)	< 0.0001		< 0.0001	
P*S	< 0.0001		0.0041	

Means followed by the same letter within evaluation period are not significantly different

postharvest study of Chincherinchee (*Ornithogalum thyrsoides* Jacq) cut flowers, which improved in vase life when wrapped in a cellophane paper and stored for three days at 4 °C before vase study (Dastagiri *et al*., 2017). Enhanced vase life, total starch and total soluble sugars parameters were registered in cut rose flowers pulsed with 3% 8-hydroxyquinoline citrate, with subsequent wet storage at 3 °C before vase study (Asghari *et al*., 2014).

The effect of pulsing cut Gladiolus with 600 ppm 8-HQS, in combination with 5% SUC, followed by wet cold storage, elicited higher total starch (37.00 ± 0.14 µg mg^{-1} dw) which was significantly different (34.54 ± 0.136 µg mg^{-1} dw), from that of the non-pulsed cut spikes (Table 1). The pulsing treatment enhanced levels of total starch, when followed by wet cold storage durations of 1, 2, 3 and 4 days, which translated into improved vase life compared with the zero day, stored spikes.

These results are comparable to accumulation of starch in Gladiolus, which was associated with stem maturation that allowed larger storage reserves, an attribute exhibited during opening stage (da Costa and Finger, 2016). However, in both pulsed and non-pulsed spikes, the levels of total starch in the present study dropped in Gladiolus when subjected to five days' wet cold storage period, prior to vase study, which may have prompted a downward trend in the cut flower vase life (10.25 ± 0.21 days). Reduction in starch content, as the storage duration progressed, has also been reported in studies on peony flowers (Walton *et al*., 2010). A small reduction of total starch and tepal soluble starch, as days after harvesting progressed, was also reported in calla lily flowers (Sales *et al*., 2018). The inclusion of sucrose in combination with kinetin and salicylic acid, delayed petal senescence in cut flowers of *Matricaria parthenium* L. andminimised reduction in starch contents (Mukherjee and Mukherjee, 2017).

At senescence, there was a significant difference ($P < 0.01$) in the concentration of

total starch (TS) between the pulsed and non-pulsed Gladiolus (Table 2). There was a progressive increase in the levels of TS as the wet cold storage increased from the one day to the fourth days' wet stored *Gladiolus* spikes. The mean concentrations (21.60 ± 0.209 µg mg^{-1} dw; 19.90± 0.209 µg mg^{-1} dw, respectively) of TS for both the pulsed and non-pulsed spiked were lower at senescence compared with the levels on the third day in the vase (Tables 1 and 2). The pulsed four days' cold stored *Gladiolus* registered the maximum TS (27.98± 0.209 µg mg^{-1}dw), which was significantly different from the zero day's stored *Gladiolus*. Although the trends in concentrations of TS were the same at senescence, as well as during the third day in the vase, these levels weresignificantly ($P<0.01$) affected by the wet cold storage duration in days (Table 1).

There was also significant variance ($P <0.01$) in concentrations of TS due to the interactive effect of the pulsing and wet cold storage treatments (Table 2). These observations are comparable to research on postharvest qualities of cut Chincherinchee (*Ornithogalum thyrsoides*) Jacq cut flowers which recorded improvement after three days',storage at 4 °C (Dastagiri *et al.*, 2017).

The inclusion of exogenous carbohydrates such as mannitol and sucrose in pulsing and holding solutions, promoted quality parameters in cut *Dendrobium* inflorescence, including delay of tepal senescence (Ichimura *et al*., 2016). Starch and sugar stored in the stem, leaves and petals provide much of the needed respiratory reserves for maintenance of cut flowers (Reid, 2009). Senescence comprises of a series of highly regulated cytological and biochemical events that coordinate the degradation of macro molecules among other activities (Ma *et al*., 2018). According to studies done on cut Gladiolus (Waithaka *et al*., 2016), the contribution of starch to the total carbohydrate content of open florets was minimal. Walton *et al*. (2010) averred that the predominant sugars in the Gladiolus perianth were glucose and fructose, and considered that starch in the florets was the primary source of soluble carbohydrate that contributed to early stages of flower expansion. However, according to their study, there was only 2 mg per perianth rise in starch content between the stages of the bud showing colour and corolla exertion; while the sugar content of the perianth was 15 mg. Hence, this observation indicates that some other storage carbohydrate could be hydrolysed during Gladiolus flower opening, other than starch.

Research done by Waithaka *et al*. (2016) demonstrated the export of radioactive sugar from wilting florets to younger buds, raising the possibility that the drying florets may be the major source of carbohydrates for acropetal opening in spike-like flowers.

In the comparative study between the postharvest characteristics of two rose cultivars ('Audio' and 'Black Magic'), the concentration of starch in the petals did not correlate with the corresponding vase life (Nabigol *et al*., 2014). However, pulsing of cut rose flowers (*Rosa damascene* cv. Trigintipetala) with 200 ppm 8-HQS in combination with 7% sucrose solution retarded carbohydrate and chlorophyll degradation (Eldeen *et al*., 2016).

Pulsing cut *Gladiolus grandiflorus* spikes with 600 ppm 8-HQS, in combination with 5% SUC (sucrose) solution also had a significant effect ($P <0.01$) on the total soluble sugars, both on the third day in the vase and at senescence (Tables 3 and 4). The mean concentration for day 0 stored and pulsed Gladiolus cut spikes was higher (31.00 ± 0.16 mg^{-1} dw.) than with 24.01± 0.16 µg mg^{-1} dw. of non - pulsed spikes. The storage durations of zero-five days, also significantly ($P < 0.0001$) affected the mean concentrations of total soluble sugars on the third day in the vase and at senescence.

The interactive effect of pulsing the cut Gladiolus with 600 ppm 8- HQS, in combination with 5% SUC and the cold storage

TABLE 3. Effect of pulsing with 600 ppm 8-HQS plus 5% sucrose and cold wet storage on the total soluble starch and total soluble sugars in cut Gladioli (*Gladiolus grandiflorus* L.cv. Fado)

Days of cold storage	Total soluble sugars (µg mg^{-1} dry weight)		Vase (days)	
	Third day in vase		Senescence	
	Pulsed	No pulsing	Pulsed	No pulsing
0	31.00 j^{ef}	24.01 ^{k}	8.75 ^{b}	4.75 ^{c}
1	47.42 ^{h}	33.54 ^{i}	10.25 ^{ab}	9.50 ^{ab}
2	58.54 ^{f}	38.95 ^{g}	10.00 ^{ab}	9.50 ^{ab}
3	73.62 ^{d}	45.67 ^{e}	11.50 ^{a}	9.75 ^{ab}
4	86.40 ^{b}	58.58 ^{c}	11.25 ^{ab}	11.00 ^{ab}
5	94.40 ^{a}	75.64 ^{a}	10.25 ^{ab}	10.25 ^{hi}
Mean	49.07^{a}	46.07 ^{b}	10.33^{a}	9.13^{b}
C.D at 5 %	0.19	0.19	0.21	0.21
Pulsing (P)	< 0.0001		0.0003	
Period of storage (S)	< 0.0001		< 0.0001	
P*S	< 0.0001		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of significance

TABLE 4. Effect of pulsing with 600 ppm 8-HQS plus 5% sucrose and cold wet storage on the total soluble sugars in cut Gladioli (*Gladiolus grandiflorus* L. cv. Fado)

Days of cold storage	Total soluble sugars (µg mg^{-1} dry weight)		Vase (days)	
	Senescence		Senescence	
	Pulsed	No pulsing	Pulsed	No pulsing
0	39.66^{l}	33.83^{j}	8.75 ^{b}	4.75 ^{c}
1	47.42 ^{h}	42.56^{i}	10.25 ^{ab}	9.50 ^{ab}
2	58.54 ^{f}	53.94^{g}	10.00 ^{ab}	9.50 ^{ab}
3	73.62 ^{d}	67.34^{e}	11.50^{a}	9.75 ^{ab}
4	86.40 ^{b}	79.15 ^{c}	11.25 ^{ab}	11.00 ^{ab}
5	94.40 ^{a}	93.53 ^{a}	10.25 ^{ab}	10.25 ^{hi}
Mean	66.67 ^{a}	61.72 b	10.333^{a}	9.125^{b}
C.D at 5 %	0.17	0.17	0.21	0.21
Pulsing (P)	< 0.0001		0.0003	
Period of storage (S)	< 0.0001		< 0.0001	
P*S	< 0.0001		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of significance

durations of 1-5 days, also significantly ($P < 0.01$) influenced the total soluble sugars in the cut Gladiolus. The levels of total soluble sugars in the cut spikes, on the third day in the vase and at senescence progressively increased, as the storage duration increased from 0-5 days (Table 4). The levels of total soluble sugars in the cut spikes were higher at senescence compared to the third day of the Gladiolus vase life for both the pulsed and non-pulsed spikes (Tables 3 and 4). The increase in total soluble sugars correlated with the enhanced vase life of cut *Gladiolus* flowers compared with the control (Tables 3 and 4).

The results from this experiment show that the pulsing treatment of 600 ppm 8-hydroxyquinoline plus 5% sucrose elevated the vase life (10.333 ± 0.21 days) of the cut spikes compared with that (9.25 ±0.21 days) of the non-pulsed flowers. The enhanced vase life of the cut pulsed spikes coincided with the improved mean starch concentration (37.00 ± 0.23 µg mg^{-1} dry weight), which was significantly ($P < 0.0001$) different from that (34.54 ± 0.23 µg mg^{-1} dry weight) of non-pulsed spikes on the third day in the vase. The same trend of improved quality parameter biomarker total starch was observed at senescence (Table 3). During the process of senescence, disintegration of macromolecules including, proteins, nucleic acids, lipids and polysaccharides such as starch occurs. In this study, the best vase life was attained in 3 days' pulsed spikes, which was significantly different from that of the control.

Cut spikes pulsed with 600 ppm 8-hydroxyquinoline sulphate plus 5% sucrose also showed a trend with marked higher levels of total soluble sugars compared with the non-pulsed flowers (Table 2). These observations could indicate the additive effect of the exogenous carbohydrate source (sucrose), which may have served as respiratory metabolites to improve the vase life of the cut spikes. This study may also indicate that, for this cultivar of Gladiolus, the wet cold storage in combination with pulsing treatment enhanced flower quality, by inhibiting the breakdown of starch in the pulsed spikes compared with the control.

The increase in the concentration of starch as the period of storage progressed could be indicative of novel biosynthesis of this polysaccharide during wet cold storage of 1, 2, 3, and 4 days, in both the pulsed and non-pulsed spikes. The pulsing treatment enlisted higher levels of total soluble sugars in the spikes, which may have been used as respiratory substrates that led to improved vase life compared with the non-pulsed flowers.

The additive effect of sucrose up to 20 per cent to pulse solution of *Leucadendron* leaf samples derailed postharvest decline of soluble starch after storage (Mukherjee and Mukherjee 2017). The trend is, however, contrary to the results of postharvest studies on red gerbera (*Gerberajamesonii* cv. Intenza) in which unstored cut flowers registered higher levels of total soluble sugars (Muniz *et al*., 2016). The preservation of dry matter and retardation in starch hydrolysis has been reported in cut rose flowers treated with carbohydrates and biocide solutions (Reid, 2009). This beneficial effect of sucrose, in combination with the biocidal, chelating and stomatal closing properties of 8-HQS (8-hydroxyquinoline sulphate) may have promoted the cut flower cellular integration. Chemicals with biocidal activities may be promoting cut flower quality due to their inhibitory effect on microbial growth, hence promoting water uptake that sustains metabolic activities (Jowkar *et al*., 2013).

The trend in the reduction in total soluble Sugars (TSS) compared to the control (Tables 3 and 4), signifies the probable decrease in the rate of breakdown of carbohydrates due to the effect of lowered temperatures, leading to reduced cellular metabolic reactions and respiration (Kazuo *et al*., 2016). According to Sales *et al*. (2018) the addition of carbohydrates in storage solutions did not amount to increase in total soluble sugars in the cut spathes of calla lily (*Zantedeschia*

aethiopica) flowers. For the pulsed spikes, this could also be indicative of the improved quality of the cut spikes because of the effect of sucrose in the pulsing treatment that could have enhanced osmotic turgidity and translocation of nutrients in the cut flower thus maintaining the flower integrity. Sucrose metabolite prevents osmotic stress in cut flowers, thereby promoting hydration and thus keeping quality of cut flowers (Mukherjee and Mukherjee, 2017).

The results obtained in this study are synonymous with the study done on cut *Ranunculus asiaticus* L. cut flowers, in which increased concentrations of cycloheximide not only perpetrated senescence, but aided in elevation of total soluble sugars in petals and sepals (Shahri and Tahir, 2010). Snapdragon (*Antirrhinum majus* L. cv. Yellow Butterfly) pulsed for 12 hours with 200-ppm 8-HQS in combination with 2% sucrose, solution projected extension of the vase life and retardation in the degradation of chlorophyll and carbohydrates (Asrar, 2012).

Many species of cut flowers display extension of the vase life when an exogenous carbohydrate source was supplied (Ichimura *et al*., 2005). Increased activity of the enzyme invertase in developing buds, shows raised levels of sucrose hydrolysis, a requisite for maintaining osmotic changes needed for cell expansion in opening Gladiolus florets (Walton *et al*., 2010). In contrast, use of sucrose in vase solution in *Lilium* 'Stargazer'studies did not improve its vase life; while positive quality effect due to addition of exogenous carbohydrates in other studies has been documented (Ichimura *et al*., 2016).

In another study, the use of a vase solution containing either trehalose or sucrose significantly affected the total carbohydrates in the petals of cut *Alstromeria* cv. Mayfair (Hatamzadeh, 2012). In a different study on cut *Gerbera*, long-time preservation in 200-ppm 8-HQS, had the potential to improve flower quality, negating the need for the pulsing treatment (Jafarpour *et al*., 2015). Accumulation of soluble sugars under low water availability maintains and protects the membrane stability and protein functions (Abid *et al.*, 2018).

The role of soluble sugars in cut flowers is four faceted, namely (i) supply of substrates for respiration, (ii) maintenance of an adequate water balance, (iii) decrease in sensitivity to ethylene, and (iv) delay in climacteric ethylene biosynthesis (Pun and Ichimura, 2003). However, research on two cultivars of cut rose flowers showed that accumulation of soluble carbohydrates in the stems and petals translated into enhanced vase life in HQC plus sucrose treated stems (Nabigol *et al*., 2014). Petals from cut spikes of *Gladiolus* cv. Peters Pears, placed in vase solution enriched with 50 mg l^{-1} GA3 + 50 g l^{-1} sucrose solution, also registered the highest concentrations of both reducing and non-reducing sugars in comparison with the control (Milandri *et al*., 2008). This observation is comparable to another study on cut Gladiolus, in which a vase solution containing 2 Mm calcium acetate promoted an upsurge of total soluble sugars in the petals, compared with water control (Ji-gang *et al.*, 2009). Althoughendogenous and exogenous sugar levels have been attributed to post harvest characteristics in cut flower performance, the opposite was reported in cut lotus flowers (*Nelumbo nucifera*) in which lack of opening and petal blackening was solely due to the stage of flower harvesting (Netlak, 2016).

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