#### **ORIGINAL ARTICLE**



# Designing of modified atmosphere package for enhancement of postharvest quality of *Gladiolus hybridus* Hort. spikes

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#### Abstract

Modified atmospheric packaging (MAP) has emerged as a leading postharvest technique to minimize losses and maintain quality of cut products. The present investigation was conducted to design passive MAP for gladiolus spikes to enhance their postharvest life. The harvested spikes (tight bud stage) were packed in low-density polyethylene (LDPE, 150 guage) and polypropylene (PP, 100 gauge) at 5 °C and 10 °C from 4 to 24 days. The MAP design was based on respiration rate and weight of gladiolus spikes, storage temperature, package gas exchange area and desirable in-pack  $O_2$  and  $CO_2$  concentrations ( $O_2$ : 3–5%,  $CO_2$ : 5–8%). The headspace  $O_2$  and  $CO_2$  concentrations (%) in PP package were, respectively, 5.05 and 7.35 at 5 °C, whereas 4.55 and 8.05 at 10 °C after storage for 8 days. Further, the vase life of spikes in these PP package was 13.86 days after 8 days of storage and declined to 8.86 days after 12 days of storage. The gladiolus spikes packed in PP sleeve (120 cm length, 18 cm width and 50 perforations) could be best stored vertically in cold room (5±0.5 °C) for 10 days with acceptable flower quality and vase life up to 13 days as supported by higher membrane stability index, relative water content, total soluble sugars and proteins, and lower lipid peroxidation. Thus, designed MAP gives a window of 7 days (postharvest life of unpacked spikes 16 days and packed 23 days) for market regulation during glut period to earn remunerative prices without any adverse effect on quality.

Keywords Passive package · Polymeric sleeves · Storage temperature · Vase life

# Introduction

Floriculture has emerged as a major diversification and profitable option in agri-business in recent years (Ezhilmathi et al. 2007). Gladiolus, queen of bulbous flowers, occupies fourth position in international trade of cut flowers after carnation, rose, and chrysanthemum (Bhande et al. 2015). The flower has high demand at both domestic and international fronts in decoration jobs because of its availability in almost

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all colours, appropriate size, and good vase life. However, the constraint faced by the growers is decrease in postharvest quality restricting their efficient marketability. The gladiolus spikes show 80–90% postharvest losses after 9–10 days of harvest that might vary with prevailing environmental conditions (Jhanji and Dhatt 2022). This triggers for a constant hunt for development of technologies, especially storage and packaging to preserve flowers in pristine condition for extended periods.

The low-temperature storage reduces rate of respiration and metabolism that results in proper handling, packaging, and marketing of flowers (Jhanji and Dhatt 2022). The flowers are packed in water retentive plastic films and stored at low temperature for required period in modified atmosphere packaging (MAP). The objective of MAP is to modify the atmosphere (increase in concentration of  $CO_2$  and decrease in  $O_2$  concentration) within the package in minimal time that is best for extending storage period of product (Tinebra et al. 2021). The packaging delays the biochemical and physiological spoilage mechanisms and thus imparts a preservative effect without the

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use of any chemical (Czerwiński et al. 2021). The desirable equilibrium concentration of CO<sub>2</sub> and O<sub>2</sub> within the package could be achieved by matching film permeability for CO<sub>2</sub> and O<sub>2</sub> with the respiration rate of packed product (Mangaraj and Goswami 2011). As different products have different metabolic behaviour and MA packages will be exposed to dynamic environment, so each package has to be optimized for specific product with specific demands (Jacxsens et al. 2002). Several studies pertaining to use of films of different permeability to gases like CO<sub>2</sub>, O<sub>2</sub>, and water vapor etc., to establish modified atmosphere at low temperature during storage has been done in flowers (Jhanji and Dhatt 2017) but designing of mathematical model for MAP of gladiolus needs to be focused. Keeping in view, the demand of gladiolus in floriculture trade, postharvest losses during glut periods and transportation, and effectiveness of MAP in enhancing postharvest life of fresh products, efforts were made to develop MAP for extending postharvest life of gladiolus.

# Materials and methods

#### Location and climate

The study was conducted at the Research Farm, Department of Floriculture and Landscaping, PAU, Ludhiana. Ludhiana is situated at latitude 30.91°N, longitude of 75.48°E and the mean height of 247 m above sea level. The annual average temperature is 23.5 °C with an average rainfall of 700 mm during monsoon season. The corms were sown in October and harvesting of spikes was done in January and corms and cormels in April. Laboratory studies were conducted with spikes harvested at tight bud stage. The spikes of Punjab Glad1 had 15-16 florets and Punjab Glad 3 had 16-17 florets.

#### Design of MAP for gladiolus spikes

The design of MAP is based on the theory that modification of in-pack atmosphere could be done with appropriate packaging material, i.e., the permeability of packaging material is matched with the rate of respiration at the selected temperature to attain the desirable in-pack headspace for selected product. In case, the permeability of the available films is not appropriate to arrive at desirable, it can be enhanced by incorporating macro-perforations in the package.

With this background, the design of the package for gladiolus spikes was based on the following parameters:

Spike parameters: True density, rate of respiration (oxygen consumption and carbon dioxide evolution at selected temperatures of storage), desirable O2 and CO2 concentrations for enhanced vase life of spikes (harvested at tight bud stage).

Packaging material parameters: Gas permeability, thickness.

Package parameters: Length of spikes, fill weight of spikes.

Storage parameters: Temperature and relative humidity.

#### **Product parameters**

True density True density of fresh gladiolus spike was measured using water displacement method in which product was submerged in water and the volume displaced was recorded (Rush et al. 2009).

Rate of respiration The respiration rate of spikes was measured by closed system. The flower samples were taken in containers (neck air sealed with grease). The impermeable glass containers were then kept in environmental chamber. Respiration rate was calculated at 5 °C and 10 °C (±1 °C) with 85% RH ( $\pm$  3%). The O<sub>2</sub> and CO<sub>2</sub> concentration of the container headspace was continuously measured at regular intervals by withdrawing a gas sample from container headspace through a silicon septum using a sampling syringe provided with the headspace analyzer (Gas space Advance, Systech Instruments Ltd., UK). The gas analysis was done till the concentration of gases became almost constant.

#### Gas permeability and thickness of packaging film

The  $O_2$  and  $CO_2$  permeability of the selected films, viz., low-density polyethylene (LDPE) and polypropylene (PP) at 25 °C, is presented in Table 1. The gas permeability of films is affected by temperature and the estimated values of  $O_2$  and  $CO_2$  permeability of the selected films at selected temperatures, viz., 10 °C and 5 °C, were estimated using the Arrhenius relationship (Rai et al. 2011). The thickness of packaging film used in the study was determined in micrometers using the thickness tester (Mitoyo, Japan).

<b>Table 1</b> $O_2$ and $CO_2$ permeability of LDPE and PP at	Gas permeability	LDPE			PP		
different temperatures	$(10^{-6} \text{ ml-m/m^2hr kPa})$	25 °C	10 °C	5 °C	25 °C	10 °C	5 °C
	Po <sub>2</sub>	82.126	32.70	23.53	26.331	9.42	6.52
	Pco <sub>2</sub>	444.076	192.64	142.94	88.9	39.40	29.46

#### Theoretical design of package

The required permeability of package for gladiolus spikes was calculated on the basis of respiration rate of gladiolus spikes, fill weight, storage temperature, and package gas exchange area for the selected storage temperature and a range of desirable in-pack O<sub>2</sub> and CO<sub>2</sub> concentrations (O<sub>2</sub>: 3-5%, CO<sub>2</sub>: 5-8%) recommended for fresh products especially flowers (Rai 2006). As the required permeability was found to be more than the available permeability for selected PP and LDPE films of selected thickness and gas exchange area, it was decided to enhance the available permeability of packaging film by incorporation of macro-perforations of 0.5 mm diameter. The balance of available permeability to be attained through the perforations was estimated as a difference between the required permeability and available permeability through the package gas exchange area at selected temperature. Thus, package was designed for two sleeves (PP and LDPE), two temperatures, and two varieties and two number of spikes in each package (2 spikes and 10 spikes).

# Experimental set up for storage of Gladiolus spikes in designed packages

There were two storage experiments.

#### **Experiment A**

The fresh gladiolus (Gladiolus hybridus Hort) spikes (Punjab Glad 3) were procured from the Research Farm, Department of Floriculture & Landscaping, PAU, Ludhiana at tight bud stage. The spikes were kept at 4 °C in the walk-in cold rooms (Motherson Zannoti, India) in the Department of Processing & Food Engineering, PAU, and Ludhiana for equilibration of temperature for about 2 h before packaging. Healthy spikes free from any damage were selected. Ten spikes were packed in PP sleeves package with dimensions as obtained from theoretical design & ten spikes were kept unpacked. These spikes were then stored at two temperatures, i.e., 5 °C and 10 °C with 85% RH in walk-in cold rooms for different storage durations (0, 4, 8, 12, 16, 20, and 24 days). Thus, total treatments were 28 that comprised of 2 packaging types, viz., packed in PP sleeves and unpacked; 2 temperature regimes and 7 storage durations. All treatments were kept in three replicates. The spikes were evaluated for postharvest keeping quality after each storage duration by unpacking them and keeping in water.

#### **Experiment B**

This experiment was conducted based on the results of experiment 1. Out of the two storage temperatures (5 °C and 10 °C) in experiment 1, the results were better at 5 °C so

further studies were conducted at this temperature. In experiment 1, different storage durations (0, 4, 8, 12, 16, 20 and 24 days) were examined and results depicted that packaging can be done up to 8 days as quality started deteriorating after 12 days so to precisely have data on storage duration we proceeded with storage durations of 0, 8, 10, and 12 days in further studies. Further, to evaluate whether different cultivars respond differently in designed package, two varieties, viz., Punjab Glad 3 & Punjab Glad 1, were taken. Thus, this experiment was conducted with two varieties, two packaging treatments (unpacked and MAP) and four storage durations (0, 8, 10, and 12) at one storage temperature  $(5^{\circ} \text{ C})$ . Ten spikes of each variety were harvested at tight bud stage and packed in PP sleeves package with dimensions as obtained from theoretical design in a similar way as in experiment A and following observations were made.

# Headspace analysis for O<sub>2</sub> and CO<sub>2</sub> concentration in packages

The gas  $(O_2 \text{ and } CO_2)$  composition inside the packages was analysed using oxygen and carbon dioxide analyser (Systech Instruments; Model: Gaspace Advance, UK). The probe was entered into the package by piercing it. Each package had a septum pasted on it through which the probe needle was inserted.

# Evaluation of postharvest quality of spikes packed in designed MAP

Following observations were recorded for both Experiments (A & B):

(a) Days to opening of basal floret in vase

The days to opening of basal floret were recorded as the days taken from placing the spike in vase till opening of basal floret.

(b) Opening of florets (%)

The per cent opening was calculated from the number of florets opened out of the total florets of spike.

(c) Floret size (cm)

The maximum diameter of the 2nd fully opened floret from the base was measured in cm and expressed as floret size.

(d) Maximum number of florets opened at one time The maximum number of florets opened at one time were recorded as the number of florets which were fully open prior to wilting of basal floret on spike..

(e) Vase life (days)

The days taken from placing stored spike in water till wilting of 50% of opened florets were recorded as vase life.

(f) Postharvest life (days)

The postharvest life was recorded from the day of harvesting the spike till the wilting of 50% of total number of opened florets, i.e., storage duration + vase life.

#### (g) Membrane stability index

The membrane stability index (MSI) of tepals weighing 100 mg was determined by the standard method of Premchandra et al. (1990). The electrical conductivity of excised tepals was measured after incubation at 25 °C for 30 min (C1) and then after boiling for 30 min (C2). The MSI was calculated as  $C1/C2 \times 100$ .

#### (h) Relative water content

The tepals (100 mg) were kept in distilled water in a preweighed test tube. The test tubes were again weighed and fresh weight (FW) was recorded as increase in the weight of test tube. After 28 h, saturated tepals were weighed (TW) followed by oven drying to record dry weight (DW). The relative water content (RWC) was calculated by the method of Weatherly (1950) as (FW-DW)/(TW-DW) × 100.

(i) Total soluble sugars and total soluble proteins

Total soluble sugars (TSS) were extracted and estimated from 100 mg tepals following standard method of Dubois et al. (1956). The TSS was extracted in 80% ethanol and estimated using 5% phenol and concentrated sulphuric acid. The orange colour developed was read at 490 nm.

Total soluble proteins were extracted and estimated from 100 mg tepals following standard method of Lowry et al. (1951). The extraction was done in 0.1 M sodium phosphate buffer and 20% trichloroacetic acid. The blue colour developed after estimation with different reagents was read at 520 nm. The TSS and TSP content was calculated as (Concentration of standard × OD of test sample × Total volume of extract) /OD of standard × Volume of sample taken from extract × Amount of tissue taken for extraction).

#### (j) Lipid peroxidase activity

The oxidative stress leads to lipid peroxidation that damages the membrane integrity. Lipid Peroxidase Activity, LPO activity, was estimated following standard method of Heath and Packer (1968). The tissue after homogenization with trichloroacetic acid (TCA) was treated with thiobarbituric acid (TBA). The mixture was incubated for 25 min at 95 °C in water bath to initiate the reaction and then terminated by keeping in ice. The absorbance of solution was read at 532 and 600 nm. Non-specific absorbance at 600 nm was subtracted from the value obtained at 532 nm.

# **Data analysis**

The design of experiment was completely randomized design (CRD). The statistical analysis of variance (ANOVA) was done by the SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). Mean comparisons using critical differences (CD) test at 5% probability were done to calculate significant differences between treatments. Mean standard error for comparing relative difference between treatments was done for respiration studies and headspace gas analysis.

# Results

#### **Respiration studies**

True density of freshly harvested spikes was measured and found to be  $1.01 \pm 0.057$  g ml<sup>-1</sup> for Punjab Glad 3 and  $0.92 \pm 0.057$  g ml<sup>-1</sup> for Punjab Glad 1. The rate of oxygen consumption and carbon dioxide evolution increased with increase in temperature in both the varieties (Table 2).The respiratory quotient was found to be more than one for both varieties at 5 °C and 10 °C.

#### Design considerations for MAP of gladiolus spikes

Based on the theoretical design analysis discussed in Section 'Design of MAP for gladiolus spikes', modified atmosphere package (MAP) designs of 2-spikes and 10-spikes for Punjab Glad 3 for experiment A and MAP design of 2-spikes and 10-spikes for Punjab Glad 3 and Punjab Glad 1 for experiment B were worked out and are presented, respectively, in Tables 3 and 4. The package design was formulated on the basis of respiratory behaviour of spikes at selected temperatures of storage; permeability and thickness of the polymeric film available for packaging; package parameters, viz., length and width of package on the basis of spike dimensions and storage conditions.

As the required permeability of the package was more than the available permeability of selected PP and LDPE films of selected thickness and gas exchange area, the available permeability was enhanced through the macro-perforations and

Table 2         Respiratory behaviour           of gladiolus spikes	Variety	Tempera- ture (°C)	Oxygen consumption rate, (ml kg <sup><math>-1</math></sup> h <sup><math>-1</math></sup> )	Carbon-dioxide evolu- tion rate, (ml kg <sup><math>-1</math></sup> h <sup><math>-1</math></sup> )	Respiration quotient, RQ
	Punjab Glad 3	5	$78.48 \pm 2.31$	$81.96 \pm 2.49$	$1.044 \pm 0.0010$
		10	$142.26 \pm 3.07$	149.51 ± 3.44	$1.051 \pm 0.0015$
	Punjab Glad 1	5	$98.16 \pm 2.86$	$109.05 \pm 3.56$	$1.111 \pm 0.004$
		10	$129.59 \pm 3.16$	$145.58 \pm 2.47$	$1.123 \pm 0.008$

S.no

1

Parameter

Product

Length of spike (cm)

 Table 3
 Theoretical design

 considerations for packaging
 and storage of gladiolus spikes

 Punjab Glad 3
 3

the number of perforations thus required was determined by theoretical analysis. The two-spike PP package for storage at 5 °C and 10 °C required 6 and 12 perforations, respectively, whereas 10-spikes PP package required 32 and 60 perforations, respectively, for storage at the same temperatures. Similarly, LDPE packages for 2 spikes required 4 and 8 perforations for attaining desirable headspace at 5 °C and 10 °C, whereas 30 and 56 perforations were required for 10-spike package to be stored under similar conditions.

# Headspace oxygen and carbon dioxide concentration in packages

The desirable concentration of  $O_2$  in the package of gladiolus spikes is 3–5% and  $CO_2$  is 5–8%. The concentration of  $O_2$  continuously decreased and that of  $CO_2$  increased inside the package at both 5 °C and 10 °C (Table 5). The  $O_2$  and  $CO_2$  concentration in the PP package stored at 5 °C was nearest to the desired values even after 12 days in comparison to 8 days at 10 °C. In case of LDPE packages, a desirable in-pack atmosphere was maintained for less than 12 and 8 days at storage temperatures of 5 °C and 10 °C,

respectively. Beyond these, the concentration of  $O_2$  further declined making the headspace anaerobic and  $CO_2$  increased that led to senescence of spikes.

10 spikes: 30 (5 °C), 56 (10 °C)

From the above results, the storage durations were screened. The spikes could be stored for  $\geq 12$  days at 5 °C and  $\geq 8$  days at 10 °C in designed PP packages, whereas in LDPE packages, it was  $\leq 12$  days at 5 °C and  $\leq 8$  days at 10 °C. With the results of our earlier findings with LDPE and PP packages, PP was found to be better than LDPE in terms of quality maintenance of spikes. Therefore, further studies to determine the suitable storage temperature and duration for enhanced postharvest life of gladiolus spikes under optimized package design, the experiment A was conducted with designed PP package for Punjab Glad 3 at 5 °C and 10 °C.

# Postharvest quality parameters (Experiment A)

The postharvest quality parameters of gladiolus spikes were evaluated after taking out the spikes from package and keeping them at ambient temperature in a vase containing water (Table 6).

	Average weight (g)	200
	True density (g ml $^{-1}$ )	$1.01 \pm 0.057$
	Oxygen consumption rate, $Ro_2(ml kg^{-1} h^{-1})$	Variety 1: 5 °C: 78.48, 10 °C: 142.26
	Carbon-dioxide evolution rate, $Rco_2(ml kg^{-1} h^{-1})$	Variety 1: 5 °C: 81.96, 10 °C: 149.51
2	Packaging material	Polypropylene (PP) Low density polyethylene (LDPE)
	$O_2$ permeability (ml 10 <sup>-6</sup> m/m <sup>2</sup> h kPaat 25 °C)	PP:26.331 LDPE:82.126
	$\text{CO}_2$ permeability (ml 10 <sup>-6</sup> m/m <sup>2</sup> h kPaat 25 °C)	PP:88.9 LDPE:444.076
	Thickness (10 <sup>-6</sup> m)	PP: 25 LDPE: 37.5
3	Storage conditions	Temperature: 5 °C, 10 °C RH: 85%
4	Package (based upon size of spikes to be packaged)	
	Length (m)	2 spikes: 1.20 10 spikes: 1.20
	Width (m)	2 spikes: 0.12 10 spikes: 0.30
	Fill weight (g)	2 spikes: 400 10 spikes: 2000
	Desirable headspace concentration (%)	O <sub>2</sub> : 3–5 CO <sub>2</sub> : 5–8
	Perforations (PP) Perforations (LDPE)	2 spikes: 5 °C: 6, 10 °C: 12 10 spikes: 32 (5 °C), 60 (10 °C) 2 spikes: 4 (5 °C), 8 (10 °C)

Consideration

 $92 \pm 1.49$ 

Gladiolus spikes (Punjab Glad 3)

Table 4Theoretical designconsiderations for packagingand storage of gladiolus spikes

S. no	Parameter	Consideration
1	Product	Gladiolus spikes Variety 1: <i>Punjab Glad 3</i> Variety 2: <i>Punjab Glad 1</i>
	Length of spike (cm)	Variety 1: 92±1.49 Variety 2: 94±1.86
	Average weight (g)	200
	True density (g ml <sup>-1</sup> )	Variety 1: 1.01±0.057 Variety 2: 0.92±0.049
	Oxygen consumption rate, $Ro_2(ml kg^{-1} h^{-1})$	Variety 1: 5 °C: 78.48, 10 °C: 142.26 Variety 2: 5 °C: 98.16, 10 °C: 129.59
	Carbon-dioxide evolution rate, $Rco_2(ml kg^{-1} h^{-1})$	Variety1: 5 °C: 81.96, 10 °C: 149.51 Variety2: 5 °C: 109.05, 10 °C: 149.58
2	Packaging material	Polypropylene (PP) Low density polyethylene (LDPE)
	$O_2$ permeability (ml 10 <sup>-6</sup> m/m <sup>2</sup> h kPaat 25 °C)	PP: 26.331 LDPE: 82.126
	$CO_2$ permeability (ml 10 <sup>-6</sup> m/m <sup>2</sup> h kPaat 25 °C)	PP: 88.9 LDPE: 444.076
	Thickness (10 <sup>-6</sup> m)	PP: 25 LDPE: 37.5
3	Storage conditions	Temperature: 5 °C RH: 85%
4	Package (based upon size of spikes to be packaged)	
	Length (m)	2 spikes: 1.20 10 spikes: 1.20
	Width (m)	2 spikes: 0.12 10 spikes: 0.30
	Fill weight (g)	2 spikes: 400 10 spikes: 2000
	Desirable headspace concentration (%)	O <sub>2</sub> : 3–5 CO <sub>2</sub> : 5–8
	Perforations (PP) Perforations (LDPE)	2 spikes: V1: 6, V2: 8 10 spikes: V1: 32, V2: 44 2 spikes: V1: 4, V2: 6 10 spikes: V1: 30, V2: 40

 Table 5
 Headspace concentration in LDPE and PP packages with gladiolus spikes (Punjab Glad-3) at different storage temperatures

Storage	5 °C				10 °C				
period (days)	LDPE		PP		LDPE		PP		
	O <sub>2</sub> (%)	CO <sub>2</sub> (%)							
0	$21.10 \pm 0.01$	$0.03 \pm 0.01$	21.10±0.01	$0.03 \pm 0.01$	$20.9 \pm 0.01$	$0.03 \pm 0.01$	$21.10 \pm 0.01$	$0.03 \pm 0.01$	
4	$6.50 \pm 0.23$	$6.40 \pm 0.43$	$6.80 \pm 0.21$	$6.10 \pm 0.49$	$5.60 \pm 0.13$	$6.70 \pm 0.14$	$6.65 \pm 0.16$	$6.25 \pm 0.27$	
8	$4.90 \pm 0.24$	$7.65 \pm 0.58$	$5.05 \pm 0.14$	$7.35 \pm 0.68$	$4.30 \pm 0.15$	$8.15 \pm 0.11$	$4.55 \pm 0.25$	$8.05 \pm 0.14$	
12	$2.90 \pm 0.48$	$8.75 \pm 0.36$	$3.65 \pm 0.45$	$7.95 \pm 0.35$	$2.20\pm0.32$	$9.90 \pm 0.09$	$2.85 \pm 0.14$	$9.05 \pm 0.17$	
16	$1.85 \pm 0.54$	$9.80 \pm 0.92$	$2.75 \pm 0.70$	$9.55 \pm 0.84$	$1.40 \pm 0.28$	$10.05 \pm 0.15$	$1.50 \pm 0.23$	$10.25 \pm 0.29$	
20	$0.90 \pm 0.68$	$11.75 \pm 0.51$	$1.15 \pm 0.81$	$11.35 \pm 1.55$	$0.55 \pm 0.36$	$13.45 \pm 0.23$	$0.60 \pm 0.05$	$12.80 \pm 0.32$	
24	$0.30 \pm 0.05$	$13.90 \pm 0.49$	$0.60 \pm 0.07$	$13.55 \pm 0.56$	$0.20\pm0.05$	$14.35 \pm 0.29$	$0.30 \pm 0.08$	$14.20\pm0.22$	

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Table 6	Effect of storage duration	ns on postharve	st quality paramete	rs of gladiolus	s spikes packed	d in designed MAP	of PP at 5	°C and 10 °	С
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Treatments			Days to open-	Per cent	Floret size (cm)	Maximum florets	Vase life (days)
Temperature (°C)	Packaging treatment	Storage period (days)	ing of basal florets	opening of florets		open at one time	
5 °C	MAP	4	4.05	69.25 (56.30)	9.11	6.36	14.48
		8	3.81	68.13 (55.61)	8.86	5.81	13.86
		12	1.18	61.15 (51.42)	7.23	4.03	8.86
		16	Opened	55.62 (48.21)	5.26	3.53	6.66
	Unpacked	4	2.65	67.66 (55.32)	8.22	4.26	10.25
		8	1.33	66.69 (54.72)	7.00	3.71	8.72
		12	0.60	59.98 (50.74)	6.13	2.15	5.86
		16	Opened	54.65 (47.65)	4.21	1.54	2.76
10 °C	MAP	4	2.54	68.75 (55.99)	8.87	5.31	9.78
		8	1.02	65.73 (54.15)	7.49	4.68	7.89
		12	0.39	59.62 (50.53)	7.00	3.52	4.12
		16	Opened	54.15 (47.36)	3.98	2.78	2.18
	Unpacked	4	1.63	66.85 (54.83)	7.10	4.11	8.79
		8	opened	64.97 (53.69)	5.81	2.98	5.75
		12	opened	58.32 (49.77)	4.96	1.72	1.77
		16	opened	52.19 (46.23)	2.23	1.10	-
Control (fresh spikes)			4.25	72.22 (57.17)	9.33	7.00	16.06
CD (5%)			0.64	1.15	0.89	0.63	1.34

#### Number of days taken to open basal floret

The increased storage duration was significantly decreased the days taken to open basal floret at both temperatures for MAP and unpacked spikes. The spikes stored for 4 days in MAP at 5 °C opened basal floret in 4.05 days that was at par to 8 days of storage (3.81 days) in MAP at 5 °C and control. The unpacked spikes stored at 10 °C opened their basal floret after 8 days, whereas the basal floret was opened in all the treatments after 16 days of storage.

#### Per cent opening of florets

The storage duration, packaging, and temperature was significantly affected the per cent opening of florets. The MAP stored spikes at 5 °C after 4 days showed 69.25% of opened florets which was at par to fresh spikes (72.22%) and MAP stored spikes after 8 days (68.13%). The least per cent of florets opened in unpacked spikes stored at 10 °C for 16 days.

#### Floret size

The floret size which adds to the beauty of spike was significantly affected in all treatments as compared to freshly harvested spikes (9.33 cm). The MAP stored spikes for 4 and 8 days at 5 °C and 4 days at 10 °C, respectively, have floret size of 9.11, 8.86 and 8.87 cm which was at par to fresh spikes. Comparing the storage durations, the unpacked spikes at 10 °C have significantly least floret size after each duration indicating the benefits of MAP.

#### Maximum number of florets opened at one time

The maximum number of florets opened at one time is an important parameter influencing the postharvest quality of spikes. The increase in storage duration adversely affected the number as maximum seven florets were opened at one time in fresh spikes which was at par with 6.36 and 5.81, respectively, after 4 and 8 days of storage in MAP at 5 °C. The unpacked spikes opened significantly least number of florets (just 1–2 florets) after 12 days of storage at 10 °C.

#### Vase life

The postharvest quality of spikes is dependent primarily on vase life. The increase in storage duration significantly decreased the vase life from 16.06 days (control) in both MAP and unpacked spikes at both temperatures. Significantly least decline was recorded in vase life of MAP stored spikes after 4 (14.48 days) and 8 (13.86) days of storage at 5 °C. The unpacked spikes stored for 16 days at 5 °C had vase life of 2.76 days, whereas at 10 °C, spikes were discarded after storage as their florets opened and wilted during storage.

Table 7 Headspace concentration in PP packages with gladiolus spikes at 5  $^{\circ}\mathrm{C}$ 

Storage	Punjab Glad	3	Punjab Glad	1
period (days)	O <sub>2</sub> (%)	CO <sub>2</sub> (%)	O <sub>2</sub> (%)	CO <sub>2</sub> (%)
0	$21.10\pm0.01$	$0.03 \pm 0.01$	$21.10\pm0.01$	$0.03 \pm 0.01$
8	$6.40 \pm 0.22$	$6.25 \pm 0.28$	$6.15 \pm 0.14$	$6.55 \pm 0.68$
10	$5.20 \pm 0.47$	$7.15 \pm 0.28$	$4.95 \pm 0.23$	$7.65 \pm 0.41$
12	$3.35 \pm 0.26$	$8.15 \pm 0.46$	$3.05 \pm 0.45$	$8.40 \pm 0.35$

The results of this experiment revealed that MAP at 5 °C was better than MAP at 10 °C as well as unpacked spikes. Further, all the postharvest quality parameters were found to be best in fresh spikes followed by MAP spikes stored at 5 °C for 4 and 8 days. The data depicted non-significant difference of different parameters in MAP spikes after 4 and 8 days of storage, but after 12 days, there was significant decline in quality of MAP stored spikes. Keeping these results in view and to precise the storage duration, another experiment B was conducted with spikes of two varieties (Punjab Glad-1 and Punjab Glad-3) packed in designed MAP at  $5 \pm 0.5$  °C for 8, 10, and 12 days.

# Headspace oxygen and carbon dioxide concentration in packages (Experiment B)

The desirable concentration of  $O_2$  and  $CO_2$  in packages for 10 gladiolus spikes is, respectively, 3–5% and  $CO_2$  is 5–8%. The  $O_2$  (%) decreased and the  $CO_2$  (%) due to respiration of spikes (Table 7). The spikes remain active, but their metabolic activities slowed down near to their desirable concentration. Therefore, the storage duration up to which the concentration of these gases was close to the desirable concentration, the spikes could be stored up to that duration without significant effects on their quality parameters but beyond this concentration spikes hastened towards senescence and their vase life or acceptability declined. In our studies, the desirable concentration was up to 10 days as after 12 days of storage, and the concentrations of  $O_2$  and  $CO_2$  were respectively close to 3% and 8%.

#### Postharvest quality parameters (Experiment B)

The postharvest quality parameters are presented below:

#### Vase life

The increase in storage duration significantly affected the vase life of spikes. The fresh spikes of both varieties had mean vase life of 15.78 days that significantly declined to 13.24 days after 8 days of storage (Fig. 1). Further storage of spikes for 10 days had vase life of 12.42 days which was



**Fig. 1** Vase life of spikes packed in PP sleeves for different storage durations (0, 8, 10, and 12 days). \*Vertical bars represent mean of vase life of three replicates of a variety  $\pm$  standard error. \*The markers in line graph represent vase life of mean of varieties  $\pm$  standard error. \*Different alphabets in lower case represent significant difference between vase life of a variety at different storage duration and upper case represent significant difference between mean of vase life varieties at different storage duration

at par with that of 8 days storage, but significant decline in mean vase life was recorded when storage duration increased to 12 days.

#### Days to opening of basal florets

Increased storage duration significantly decreased the number of days to opening of basal floret (Table 8). The basal floret of fresh spikes took 4.28 days, whereas 3.33 days after 8, 2.76 days after 10 and 1.04 after 12 days of storage.

#### Maximum number of florets opened at one time

Maximum number of florets opened in fresh spikes was 6.96 (Table 8). The storage of spikes significantly decreased the number of opened florets. The florets opened in a spike were 5.78 after 8 days which were at par with 4.37 after 10 days of storage, but further storage up to 12 days significantly reduced the number of opened florets at one time (3.71).

#### Per cent opening of florets

The increase in storage duration decreased the number of opened florets in both varieties (Table 8). The mean of floret opened decreased from 71.42 per cent in fresh spikes to 65.9 after 8 days, 65.41 after 10 days and 58.18 after 12 days of storage.

#### Floret size

The floret size varies with genotype, but the size is influenced with postharvest quality of florets, i.e., turgidity, etc. The floret size of Punjab Glad-3 in fresh spikes was 9.35 cm compared to 8.75 cm in Punjab Glad-1 (Table 8). The size of floret was significantly affected with increased storage duration. The maximum size of floret was 9.05 cm in fresh spikes and least floret size of 7.34 cm after 12 days of storage in both varieties.

#### Membrane stability index

The membrane stability index (MSI) decreased with increase in storage duration in both varieties (Table 9). Fresh spikes had MSI of 73.67 followed by the mean MSI of both varieties after 8 (70.24) and 10 (68.10) days of storage which were at par followed by significant decline after 12 days (63.92).

# Lipid peroxidase activity

Lipid peroxidation reveals the integrity of membrane that is important component regulating several metabolic processes (Table 9). Thiobarbituric Acid Reactive Substances (TBARS) content, an indicator of lipid peroxidation, increased with increase in storage duration from 21.62 nM g<sup>-1</sup> FW in fresh spikes to 29.13 nM g<sup>-1</sup> FW in spikes after 12 days of storage (Table 9).

#### **Relative water content**

The relative water content (RWC) maintained the turgidity of florets. The relative water content significantly decreased with storage (Table 9). The fresh spikes had RWC of 77.07 that decreased to 69.95, 66.73, and 64.15 after 8, 10, and 12 days of storage, respectively.

# **Total soluble sugars**

Storage duration had a significant effect on total soluble sugar content in florets (Table 10). The content declined significantly from 199.56 mg g<sup>-1</sup> FW to 134.89 mg g<sup>-1</sup> FW in Punjab Glad-3 and from 209.56 mg g<sup>-1</sup> FW to 154.87 mg g<sup>-1</sup> FW in Punjab Glad-1 as storage period increased from 0 to 12 days. The total soluble sugar content in florets stored for 8 and 10 days was at par.

#### **Total soluble proteins**

The content of total soluble proteins significantly decreased with increase in storage duration (Table 10). The mean total soluble protein content in fresh florets was 175.65 mg  $g^{-1}$ 

storage	Days to opening	g of basal florets		Maximum numt	ter of florets open	led	Floret opening (	(%)		Floret size (cm)		
period (days)	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mea
0	4.40	4.17	4.28	7.01	6.91	6.96	73.18 (58.78)	69.65 (56.55)	71.42 (58.32)	9.35	8.75	9.05
~	3.61	3.05	3.33	5.85	5.70	5.78	67.14 (55.23)	64.66 (53.51)	65.9 (54.21)	8.90	8.47	8.68
10	2.69	2.83	2.76	4.49	4.25	4.37	66.65 (54.71)	64.16 (53.21)	65.41 (53.51)	8.33	8.18	8.25
12	1.18	0.89	1.04	3.96	3.45	3.71	59.52 (50.47)	56.84 (48.92)	58.18 (49.42)	7.30	7.37	7.34
CD (5%)	0.96	0.41	0.67	1.46	1.54	1.44	1.04	1.37	1.02	0.72	0.52	0.58

FW that declined to 144.86, 136.37, and 121.98 mg  $g^{-1}$  FW after 8, 10, and 12 days of storage.

#### **Postharvest life**

The postharvest quality parameters of gladiolus spikes stored at different storage durations are summarized in Table 11. Although the vase life of spikes after storage decreased, yet the postharvest life, *i.e.*, sum of storage duration and vase life of stored spikes, was higher than that of freshly harvested spikes. The freshly harvested spikes remain in acceptable form for 15.78 days, whereas the stored spikes remain in acceptable form for 13.24 days when removed from package after 8 days of storage *i.e.* the spikes could be used for 21.68 days and the corresponding values after 10th day and 12th day of storage were, respectively, 22.98 days and 20.29 days. The quality parameters of stored spikes revealed that up to 10 days of storage in PP sleeves (100 gauges), there was no significant difference in quality of stored spikes for 8 days, but significant declination was recorded after 12 days of storage. The slight reduction in quality parameters after 10 days of storage as compared to fresh spikes could be compensated as spikes stored in designed MAP provided additional 7 days for handling the spikes than freshly harvested spikes.

### Discussion

The major challenge to postharvest life of any cut flower is the cascade of biotic and abiotic factors that regulate the process of senescence (Jhanji et al. 2023). The market of cut flowers could be regulated by appropriate postharvest management involving packaging and storage of flowers. The MAP is a system in which rate of respiration and gas exchange of stored commodity is equilibrated inside the package that could extend its postharvest life. The respiration rate varies among climatic conditions, growing

Table 9 Effect of storage durations on membrane stability index, lipid peroxidation, and relative water content of gladiolus spikes packed in designed MAP at 5  $^{\circ}$ C

Storage period	Membrane stability index			Lipid peroxidas (nM g <sup>-1</sup> FW)	Lipid peroxidase activity TBARS content (nM $g^{-1}$ FW)			Relative water content		
(days)	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mean	
0	72.47	74.86	73.67	22.67	20.56	21.62	76.84	77.29	77.07	
8	68.55	71.92	70.24	25.78	22.34	24.06	69.75	70.14	69.95	
10	66.72	69.48	68.10	27.89	25.56	26.73	66.57	66.89	66.73	
12	62.75	65.08	63.92	30.82	27.43	29.13	64.42	63.87	64.15	
CD (5%)	3.45	2.89	3.15	1.67	1.87	1.89	2.89	3.24	3.56	

Table 10         Effect of storage
durations on total soluble sugars
and total soluble proteins in
gladiolus spikes packed in
designed MAP at 5 °C

Storage	Total soluble su	gars (mg g <sup>-1</sup> FW)		Total soluble pro	)	
period (days)	Punjab Glad-3	Punjab Glad-1	Mean	Punjab Glad-3	Punjab Glad-1	Mean
0	199.56	209.56	204.56	169.87	181.43	175.65
8	165.78	178.90	172.34	137.45	152.27	144.86
10	156.45	169.56	165.01	129.48	143.26	136.37
12	134.89	154.87	144.88	113.43	130.52	121.98
CD(5%)	6.75	7.89	8.02	5.45	7.29	7.71

Table 11 Overall postharvest quality parameters of gladiolus spikes packed in designed MAP for different durations at 5 °C

Storage dura- tion (days)	Days to opening of basal floret	Floret size (cm)	Maximum florets open at one time	Percent opening of florets	Vase life (days)	Postharvest life (days)
0 (control)	4.28	9.05	6.96	71.42 (58.32)	15.78	15.78
8	3.33	8.68	5.78	65.9 (54.21)	13.24	(13.24+8) 21.68
10	2.76	8.25	4.37	65.41 (53.51)	12.42	(12.42+10) 22.98
12	1.04	7.34	3.71	58.18 (49.42)	8.29	(8.29+12) 20.29

pattern, commodities, varieties, and even cultivar of same commodity (Mangaraj et al. 2014). The permeability of films varies with production process, company, and even with batch of production (Mangaraj and Goswami 2009). Thus, for designing MAP for extending postharvest life of gladiolus spikes, its respiration rate was determined using closed system at different temperatures. The optimum gas composition in MAP could be attained using packaging films [low-density polyethylene (LDPE), polyester (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and saran (PVDC)] with specific ranges of  $O_2$  and CO<sub>2</sub> permeability and perforations might be required to obtain the optimal gas composition inside the package (Del-Valle et al. 2003). Concomitant to this, LDPE and PP sleeves of particular dimensions with particular perforations were optimized for package of 2 and 10 gladiolus spikes in our study (Tables 3 and 4).

The package depending upon spikes respiration rate, packaging material, storage environment especially temperature, and fill weight was designed, and for optimizing the duration, headspace concentration of O<sub>2</sub> and CO<sub>2</sub> was recorded in packages stored for different durations. The increasing concentration of CO<sub>2</sub> and decreasing concentration of O<sub>2</sub> reduces the metabolic activity of the product (Rennie and Tavoularis 2009). The increase in storage duration at 5 °C and 10 °C decreased the concentration of O<sub>2</sub> and increased the concentration of CO<sub>2</sub> within gladiolus package and reached nearest to desired equilibrium concentration after 8 and 12 days suggesting that gladiolus spike could be stored within the designed package up to 12 days as beyond this duration, there was change in O<sub>2</sub> and CO<sub>2</sub> concentrations that had adverse effect. The reduced concentration of  $O_2$  up to 2–5% is needed for slow respiration. The shelf-life of packed product increases when the respiration rate of packed product equilibrates with the permeability of packaging film (Sandhya 2010).

The time of opening of basal floret determines the vase life of spike. Delay in opening of basal floret during storage is favourable as spikes with unopened florets could be easily transported and stored without much damage. The gladiolus spikes packed in PP sleeves offered modified atmospheric (MA) storage that delayed the opening of basal florets during storage in comparison to unpacked spikes (Table 6). The metabolic activity of spikes was reduced to greater extent at 5 °C in comparison to 10 °C in MAP that resulted in delayed opening of basal florets in MAP at 5 °C (Redman et al. 2002).

Increase in storage duration at both temperatures led to decline in the vase life (Table 6). This could be attributed to depletion of carbohydrates, water stress, and/or ethylene sensitivity that provokes postharvest senescence and decline in vase life (Gaur et al. 2022). The ability of buds to open decreased with increase in storage duration (Singh et al. 2002). This was concomitant with our findings as decline was recorded in floret opening and floret size with increase in storage duration (Tables 6 and 8). This decline could also be accounted to low soluble sugar content in spikes at tight bud stage that restricted expansion of upper florets leading to reduced percent of floret opening and reduced floret size (Grover and Singh 2010).

Membrane integrity is a key indicator that signals the onset of senescence and its loss adversely affects the vase life of flowers (Khandan et al. 2021). The results of our study revealed decline in MSI values with progression in storage duration. The decrease in membrane integrity became more pronounced with increase in storage duration as revealed by significant decline in MSI (Table 9). The reduced postharvest quality, viz., floret opening, floret size, and vase life, could be accounted to this decline in MSI with increased storage duration. The MSI values were inversely related to LPO activities in the tepals as increase in storage duration resulted in decrease in MSI and increased the LPO activity prompted breakdown of membrane lipids and altered the membrane integrity (Shabanian et al. 2019).

Detachment of spikes from mother plant deprives them from incessant supply of water and nutrients (Patel et al. 2018). The fresh tepals had greater water retention accounting for higher relative water content (Mittal and Jhanji 2019). The water retained decreases with increase in duration of storage that could account for decline in observed RWC in our results.

The metabolic reserves, especially total soluble sugars and total soluble proteins, are the direct indicators of metabolic status of plant. These metabolites act as osmaticum and play a leading role in regulating osmotic changes, membrane permeability, and oxidative changes (Mittal et al. 2021). Maintenance of metabolite reserves in tepals, maintained the tepal turgidity due to optimum water content that could be further related to prolonged vase life (Hemati et al. 2019). There is decrease in content of metabolites as stored spikes used these metabolites as respiratory substrate and affected vase life (Gomez-Merino et al. 2020).

The instigation of postharvest senescence in stored spikes resulted in decline in water content, membrane lipid peroxidation, ion leakage, and decline in sugar and protein content that adversely affected the quality parameters and declined the vase life (Lone et al. 2021). The packed spikes (PP sleeves) had better postharvest quality in terms of vase life, number of days to open basal floret, percent floret opened, floret size, and maximum number of florets open at one time as compared to unpacked spikes. The packaging of gladiolus spikes in PP sleeves improved their postharvest quality (Jhanji and Dhatt 2017). The packaging minimizes the carbohydrate losses through respiration and water losses through transpiration. This reduction in carbohydrate and water loss provided adequate energy to the florets resulting in their higher percent opening, floret size, and vase life (Kader and Saltveit 2003).

# Conclusions

The MAP of spikes led to maintenance of postharvest quality of spikes by modulating physiological and biochemical attributes such as protein and sugar. The gladiolus spikes packed in designed MAP (PP sleeve: 120 cm length, 18 cm width, and 50 perforations) could be best stored vertically in cold room  $(5 \pm 0.5 \,^{\circ}\text{C})$  for 10 days with acceptable flower quality and vase life up to 13 days. Thus, designed MAP gives a window of additional 7-10 days to growers for postharvest handling during glut period to earn remunerative prices without any adverse effect on quality. The current research offers an immense scope for active packaging in modified atmosphere to enhance vase life with better quality retention. Together, these studies will enable to formulate cost effective and eco-friendly technology for postharvest management of gladiolus spikes with huge economic implications.

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Author's contribution Shalini Jhanji and KK Dhatt planned and executed the experiment. Preetinder Kaur designed the MAP model. Shalini Jhanji and Eena Goyal compiled and analysed the data and wrote the manuscript.

**Data Availability** All data supporting the findings of this study are available within the paper and if some additional data is required it can be made available from the corresponding author upon reasonable request.

# Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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