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Research article

Effects of high CO_2 and low O_2 on biochemical changes in cut *Dendrobium* orchids

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ABSTRACT

Exportation of cut flowers entails long distance transportation, and the quality of cut flowers deteriorates as the distance and transportation time increase. Low storage temperatures and modified atmosphere are commonly used to extend the life of cut flowers. As a result, this research explored the potential use of high CO_2 and low O_2 to prolong the shelf life of cut flowers. Specifically, this study examined the effects of high CO_2 and low O_2 storage on the biochemical changes in cut *Dendrobium* pink stripe orchid flowers. The experiments were conducted under normal and high CO_2 and low O_2 conditions, and results were compared. Under the normal condition, carbon dioxide (CO_2) and oxygen (O_2) concentrations were 0.03 % and 21 %. In the high CO_2 and low O_2 environment, CO_2 was varied between 5 and 10 %; and O_2 between 2, 4, 6, and 8 %. The storage temperature and relative humidity were 13 °C and 95 %. The originality of this work is the use of high CO_2 and low O_2 storage environments to investigate the biochemical changes in cut *Dendrobium* orchid flowers. The experimental results showed that high CO_2 and low O_2 significantly enhanced the storage life of *Dendrobium* orchid flowers ($p < 0.05$). The longest storage life of 28.33 days was achieved under 5 % CO_2 and 2 % O_2 atmosphere condition, compared with 11.67 days under the normal atmosphere condition. High CO_2 and low O_2 storage also helped to retain total anthocyanin content while lowering fresh weight loss, respiration rate, ethylene production, protein degradation, and protease activity.

1. Introduction

Orchids (*Orchidaceae*) can grow in various topographical areas, from 1,000 m above sea level to sunless humid rain forests epiphytically attached to trees. Thailand grows and exports a variety of orchid species, particularly *Dendrobium* pink stripe cut orchid flowers with an annual export valuation of USD 100 million (Department of Agriculture, 2018). As a result, storage and packaging play an important role in preserving the quality of cut flowers. Modified or controlled atmosphere is typically used to delay postharvest physiological changes during storage, such as wilting and senescence.

According to Rattanapanon and Boonyakiat (2013), modified atmosphere efficiently delayed senescence and extended the postharvest life of ornamentals. Unlike those stored in modified atmosphere, cut orchid flowers stored in normal atmosphere exhibited higher membrane permeability, higher enzyme activity and lower solute uptake capacity, resulting in flower senescence (Poonsri, 2020). Boonyakiat (2020) also documented protein degradation during flower senescence.

High CO_2 and low O_2 packaging has been widely used to delay wilting of fresh produce, including cut flowers (Bishop et al., 2007). High CO_2 and low O_2 restricts the exchange of O_2 and CO_2 and slows the deterioration of produce (Singh and Kumar, 2008). Mitcham and Shelton (1997) investigated the effect of very high to extremely high CO_2 and various lengths of storage time on *Dendrobium* orchid flowers, given 13 °C storage temperature and 95 % relative humidity (RH). The results showed that exposure to excessive CO_2 at any length of time shortened the vase life of orchid flowers.

Matityahu et al. (2016) investigated the effect of controlled atmosphere (CA) on the quality of husks and aril juice of pomegranate cultivars and reported lower husk scald and decay. Ali et al. (2016) studied the effects of different CA on the pericarp browning, biochemical characteristics, and antioxidative activity of *Gola* litchi fruits; and reported that CA could reduce weight loss, pericarp browning, membrane leakage, and malondialdehyde contents. Martins and Resende (2015) investigated the sensory attributes of papaya cv. *Golden* stored under various low O_2

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Figure 1. *Dendrobium* pink stripe orchid flower.

and high CO_2 conditions with ethylene scrubbing; and reported the optimal storage condition of 3 % O_2 and 6 % CO_2.

Atmospheric greenhouse gases (GHG) from human activities contribute significantly to climate change, and the most prevalent atmospheric GHG is CO_2 (Hecht, 2007). Specifically, in addition to extending the storage life of fresh produce and flowers, modified or controlled atmosphere storage also helps lower atmospheric CO_2 since it requires filling the storage room with elevated concentrations CO_2 from the ambient air (Yahia and Singh, 2009; Mditshwa et al., 2017). Besides, controlled atmosphere also reduces the CO_2 production inside the storage room and thus lowers CO_2 emitted into the atmosphere (Eric et al., 2008).

Furthermore, despite the economic significance of *Dendrobium* pink stripe orchids, there exists little research on the effect of high CO_2 and low O_2 storage on the flower quality. Existing research on high CO_2 and low O_2 focused mainly on extending the shelf life of vegetables or fruits.

Table 1. Effect of high CO_2 and low O_2 conditions on storage life of *Dendrobium* orchids.

Condition/Treatment	Storage life (Days)*
Control (0.03% CO_2 + 21% O_2)	$11.67^{g} \pm 0.57$
5% CO_2 + 2% O_2	$28.33^{a} \pm 2.07$
5% CO_2 + 4% O_2	$25.33^{b} \pm 0.97$
5% CO_2 + 6% O_2	$25.33^{b} \pm 1.05$
5% CO_2 + 8% O_2	$23.33^{c} \pm 1.15$
10% CO_2 + 2% O_2	$18.33^{d} \pm 1.15$
10% CO_2 + 4% O_2	$18.33^{d} \pm 0.57$
10% CO_2 + 6% O_2	$16.67^{e} \pm 1.15$
10% CO_2 + 8% O_2	$15.00^{f} \pm 0.57$
Coefficient of variation (%)	4.62
Least significant difference (LSD)	0.82

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

On the contrary, this research explored the potential use of high CO_2 and low O_2 to prolong the shelf life of cut flowers.

Specifically, this research comparatively investigated the storability and quality of cut *Dendrobium* pink stripe orchid flowers under normal (0.03 % CO_2 and 21 % O_2) and high CO_2 and low O_2 conditions. In the high CO_2 and low O_2 environment, CO_2 concentrations were varied between 5 and 10 % and O_2 between 2, 4, 6, and 8 %. The quality metrics of the orchid flowers included storage life, weight loss, anthocyanin content, respiration rate, ethylene production, protein degradation, and protease activity. The originality of this work is the use of high CO_2 and low O_2 storage environments to investigate the biochemical changes in cut *Dendrobium* orchid flowers, unlike existing research which focused primarily on vegetables and fruits.

2. Materials and methods

Dendrobium pink stripe orchid flowers were from a plantation in *Pathumthani* province, Thailand. The orchid flowers were thoroughly washed with water and defective flowers were sorted out. The stem ends were trimmed at 45° angle and inserted into a plastic tube filled with 30 ml distilled water. Figure 1 depicted the cut *Dendrobium* pink stripe orchid flowers used in this research.

The inflorescences (10 flowers/cardboard box) were placed in microclimate-controlled acrylic containers (40 × 40 × 60 cm) equipped with a gas flow system. The atmospheric composition inside the acrylic containers were analyzed on a daily basis using a gas analyzer (PBI-Dansensor; Checkmate II, Denmark).

The experiments were conducted in normal and high CO_2 and low O_2 environments. Under the normal atmosphere, the flowers were retained in 0.03 % CO_2 and 21 % O_2. Under the high CO_2 and low O_2 condition, CO_2 concentration was varied between 5 and 10 % and O_2 between 2, 4, 6, and 8 %. The storage temperature and relative humidity (RH) under the normal and high CO_2 and low O_2 conditions were 13 °C and 95 % (Poonsri, 2015). The experiments were performed in triplicate and results averaged. The flower storability was assessed every five days for the experimental period of 30 days.

2.1. Storage life

Prior to storage life assessment, the vase life of freshly cut *Dendrobium* pink stripe orchid flowers at room temperature (25 °C) was determined and used as reference. The vase life of freshly cut orchid flowers was seven days as more than 50 % of the flowers became visibly wilted.

In the storage life assessment, the orchid inflorescences (on days 5, 10, 15, 20, 25 and 30) were transferred to vases and retained at room temperature. The flowers were visually inspected on a daily basis by a panel of 10 specialists with knowledge of flowers. The storage life ended when the flowers lasted less than seven days at room temperature (i.e., the vase life was shorter than seven days).

2.2. Weight loss

The weight loss of orchid flowers was determined using 10 inflorescences per treatment. Prior to storage, the orchid flowers were weighed using a laboratory digital balance (Mettler-Toledo; PB3002-S, Switzerland) and reweighed every 5 days until end of storage life. The relative fresh weights were calculated and expressed as a percentage (Eq. (1)).

$$Weight\ loss(\%) = \frac{w_1 - w_2}{w_1} \times 100 \quad (1)$$

where w_1 is the initial weight and w_2 is the weight every five days until the end of storage life.

Figure 2. Effect of high CO_2 and low O_2 on *Dendrobium* orchid flowers after 25 days: under normal atmospheric storage (left) and high CO_2 and low O_2 (5 % CO_2 + 2 % O_2; right).

2.3. Anthocyanin content

The anthocyanin extraction followed Rangana (1986) with minor modifications. In the extraction, 0.5 g of orchid petal was crushed and placed in 25 ml acidic ethanol (1.5 M HCl in 95 % ethanol, 15:85 v/v) at 4 °C for 24 h. The solution was filtered using Whatman No.1 paper, and the volume was adjusted to 100 mL with acidic ethanol. Anthocyanin was analyzed by spectrophotometry at 535 nm wavelength (Labome; SPECTRO 23, USA) and converted into mg of anthocyanin per 100 g fresh weight (Eqs. (2) and (3)).

$$\text{Total Absorbance} = \frac{\text{OD xV x 100}}{\text{W}} \quad (2)$$

$$\text{Total anthocyanin content} = \frac{\text{Total Absorbance}}{98.2} \quad (3)$$

where *OD* is the absorbance value at 535 nm, *V* is the solvent volume, *W* is the weight of petal tissues and *98.2* is the constant value.

2.4. Respiration rate

Orchid samples (1 g) were placed in hermetic flask at room temperature for 1 h. Gas samples (1 mL) were drawn through silicone septum using a syringe and analyzed by gas chromatography (Agilent Technologies; 6820, USA) equipped with a capillary column at 100 °C. Hydrogen (100 kPa) was used as carrier gas. The respiration rate of orchid flowers is in mg CO_2 kg^{-1} h^{-1}.

2.5. Ethylene production

The ethylene production was measured by ethylene analyzer (ICA; 56, UK). The ethylene concentrations are expressed in parts per million (ppm).

2.6. Protein degradation in dendrobium orchids

2.6.1. Protein extraction

In protein extraction, orchid petals were immerged in liquid N_2 and pulverized before adding 5 mL of soluble protein buffer containing 50

Table 2. Effect of high CO_2 and low O_2 conditions on weight loss of *Dendrobium* orchids.

Condition/Treatment	Weight loss (%)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	0	$3.54^{a} \pm 1.35$	$13.34^{a} \pm 1.35$	$23.89^{a} \pm 1.45$	-	-	-
5% CO_2 + 2% O_2	0	$1.70^{b} \pm 2.34$	$2.24^{c} \pm 1.64$	$4.71^{c} \pm 2.14$	$6.11^{c} \pm 1.14$	$7.67^{b} \pm 1/43$	13.67 ± 1.43
5% CO_2 + 4% O_2	0	$1.89^{b} \pm 0.98$	$2.57^{c} \pm 1.20$	$5.77^{c} \pm 1.20$	$7.93^{c} \pm 1.30$	$12.10^{a} \pm 1.98$	-
5% CO_2 + 6% O_2	0	$1.67^{b} \pm 1.28$	$2.77^{c} \pm 2.33$	$4.50^{c} \pm 2.73$	$8.11^{c} \pm 2.13$	$13.63^{a} \pm 1.28$	-
5% CO_2 + 8% O_2	0	$1.55^{b} \pm 2.34$	$3.26^{b} \pm 2.73$	$5.29^{c} \pm 2.34$	$10.06^{c} \pm 2.13$	$13.75^{a} \pm 2.34$	-
10% CO_2 + 2% O_2	0	$1.63^{b} \pm 1.37$	$3.33^{b} \pm 1.70$	$6.89^{b} \pm 1.17$	$14.24^{b} \pm 1.47$	-	-
10% CO_2 + 4% O_2	0	$1.77^{b} \pm 1.80$	$3.75^{b} \pm 1.20$	$7.63^{b} \pm 1.80$	$15.66^{b} \pm 1.40$	-	-
10% CO_2 + 6% O_2	0	$1.86^{b} \pm 1.00$	$4.12^{b} \pm 0.90$	$8.76^{b} \pm 097$	$16.57^{b} \pm 0.97$	-	-
10% CO_2 + 8% O_2	0	$1.50^{b} \pm 1.77$	$4.50^{b} \pm 0.48$	$8.89^{b} \pm 0.77$	$18.92^{a} \pm 0/98$	-	-
Coefficient of variation (%)	-	6.42	6.02	8.92	4.22	3.66	10.20
Least significant difference (LSD)	-	0.79	0.76	0.85	1.44	1.38	0.62

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

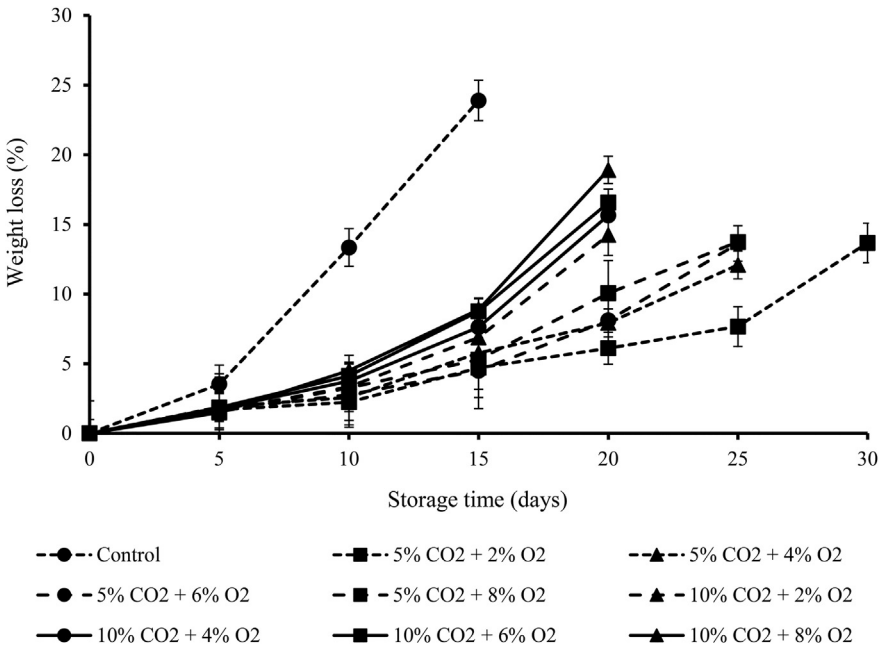

Figure 3. Effect of high CO_2 and low O_2 on weight loss of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

mM Tris HCl pH 7.6, 2 mM disodium ethylenediaminetetraacetate, 2 mM dithiothreitol, and 10 mM $MgCl_2$. The homogenate was incubated for 15 min at room temperature prior to centrifugation at 17,000 g for 20 min at 4 °C. The supernatant was retained and 2 mL of soluble protein buffer added to the extracted petals for second extraction.

In the second extraction, the homogenate was incubated for 15 min at room temperature prior to centrifugation at 17,000 g for 20 min at 4 °C. The supernatant was retained and combined with the first supernatant. The supernatant was soluble protein, and insoluble protein was extracted by adding 7 mL of 0.1 N NaOH into pellet and incubated at 80 °C overnight. The soluble and insoluble proteins constituted total protein.

2.6.2. Protein quantification

Total protein content was absorbance measured at 595 nm using bovine serum albumin as standard protein assay (Bradford, 1976). In the quantification, 50 μL of protein was pipetted into 1.5-mL microcentrifuge tube containing 200 μL protein buffer, and 1 mL of 0.0125 % Coomassie Brilliant Blue (CBB) was added and mixed thoroughly. Samples were left at room temperature for 15 min and the protein content measured by using a spectrophotometer (Labome; SPECTRO 23, USA). The protein content was expressed as mg g^{-1} fresh weight.

2.7. Protease activity of dendrobium orchids

2.7.1. Protease extraction

In protease extraction, orchid petals were homogenized in 3 mL of cold extraction buffer (50 mM Tris HCl, pH 7.6) before adding another 5 ml of the extraction buffer. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C, and the supernatant (crude extract) was retained at -70 °C for protease assay.

2.7.2. Protease assay

Assay of protease activity followed Nieri et al. (1998) with minor modifications. In protease assay, 200 μL of crude extract was placed in 1.5 ml microcentrifuge tubes containing 400 μL incubation buffer (50 mM Na-acetate, pH 5.0 with 0.5 % azocasein). The reference tube containing the incubation buffer without azocasein and crude extract was added with 100 μL 50 % (w/v) trichloroacetic acid (TCA) before incubation. The incubation was carried out at 37 °C for 24 h, and 100 μL of 50 % TCA was added to stop the reaction. The experimental tubes were then placed in ice tub for 1 h before centrifuging at 10,000 g for 3 min at 4 °C. The supernatant was alkalinized by adding 100 μL 10 M NaOH and absorbance measured at 492 nm. Protease activity was the difference

Table 3. Effect of high CO_2 and low O_2 conditions on total anthocyanin of *Dendrobium* orchids.

Condition/Treatment	Total Anthocyanin (mg/100 g fresh weight)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	$4.60^{a} \pm 0.54$	$3.76^{a} \pm 0.13$	$2.42^{b} \pm 0.35$	$2.15^{c} \pm 0.31$	-	-	-
5% CO_2 + 2% O_2	$4.60^{a} \pm 0.54$	$4.50^{a} \pm 0.43$	$4.21^{a} \pm 0.43$	$4.15^{a} \pm 0.43$	$3.97^{a} \pm 0.43$	$3.46^{a} \pm 0.58$	3.14 ± 0.39
5% CO_2 + 4% O_2	$4.60^{a} \pm 0.54$	$4.21^{a} \pm 0.40$	$4.21^{a} \pm 0.40$	$3.76^{a} \pm 0.40$	$2.98^{a} \pm 0.20$	$2.87^{a} \pm 0.45$	-
5% CO_2 + 6% O_2	$4.60^{a} \pm 0.54$	$4.38^{a} \pm 0.08$	$4.22^{a} \pm 0.28$	$3.98^{a} \pm 0.08$	$2.76^{a} \pm 0.02$	$2.16^{b} \pm 0.10$	-
5% CO_2 + 8% O_2	$4.60^{a} \pm 0.54$	$4.32^{a} \pm 0.54$	$4.36^{a} \pm 0.34$	$3.90^{a} \pm 0.54$	$2.86^{a} \pm 0.48$	$2.24^{b} \pm 0.58$	-
10% CO_2 + 2% O_2	$4.60^{a} \pm 0.54$	$3.96^{a} \pm 0.34$	$3.50^{a} \pm 0.74$	$3.16^{b} \pm 0.34$	$2.39^{b} \pm 0.49$	-	-
10% CO_2 + 4% O_2	$4.60^{a} \pm 0.54$	$4.22^{a} \pm 0.38$	$3.69^{a} \pm 0.30$	$3.14^{b} \pm 0.48$	$2.36^{b} \pm 0.23$	-	-
10% CO_2 + 6% O_2	$4.60^{a} \pm 0.54$	$3.90^{a} \pm 0.48$	$3.46^{a} \pm 0.50$	$2.90^{b} \pm 0.48$	$2.21^{b} \pm 0.28$	-	-
10% CO_2 + 8% O_2	$4.60^{a} \pm 0.54$	$3.78^{a} \pm 0.38$	$3.14^{b} \pm 0.38$	$3.00^{b} \pm 0.48$	$2.11^{b} \pm 0.48$	-	-
Coefficient of variation (%)	-	2.07	2.27	5.83	3.77	11.19	4.95
Least significant difference (LSD)	-	0.74	0.23	0.14	0.78	0.67	0.56

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

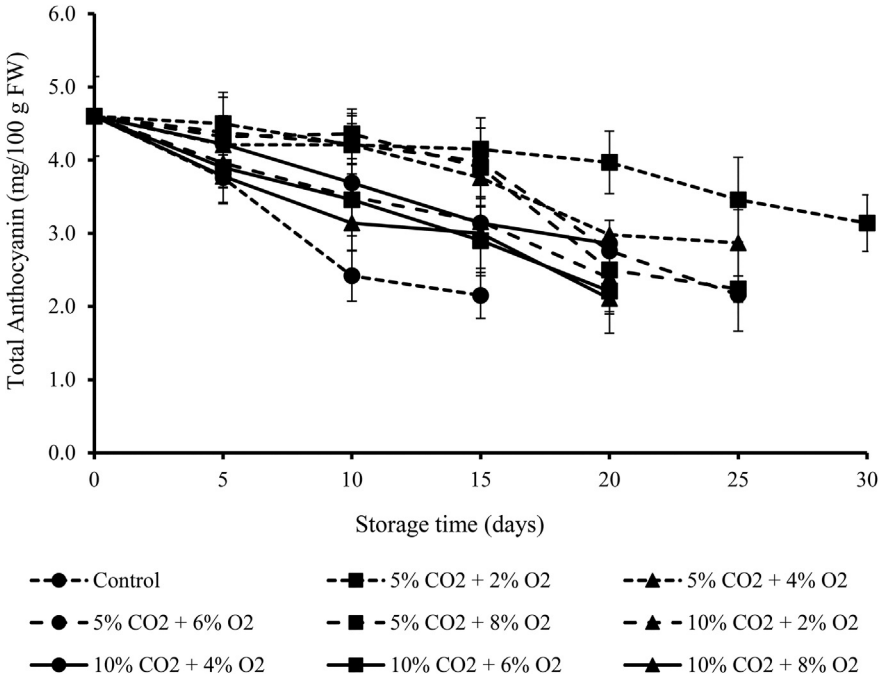

Figure 4. Effect of high CO_2 and low O_2 on total anthocyanin of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

between absorbance at 492 nm of the experimental and reference tubes. Protease activity was an arbitrary unit in which one unit is equal to a change of 0.01 absorbance unit h^{-1} at 492 nm.

In this research, Tukey's multiple comparison test was used to determine statistical differences between experimental treatments and the control (Statcel3, OMS, Tokyo, Japan), given the 5 % significance level.

3. Results and discussion

This section discusses the experimental results of orchid flowers stored under normal (the control) and controlled atmosphere conditions.

3.1. Storage life

The longest storage life of orchid flowers of 28.33 days was achieved under 5 % CO_2 + 2 % O_2 condition (i.e., the optimal condition), while that of orchid flowers stored in normal atmospheric packaging was 11.67 days. Table 1 tabulated the average storage life of orchid flowers under normal and high CO_2 and low O_2 conditions.

The results indicated that high CO_2 and low O_2 significantly prolonged the storage life of orchid flowers, compared to the normal condition ($p < 0.05$). The extended storage life could be attributed to reduced respiration rates, ethylene production, and oxidation of orchid flowers under high CO_2 and low O_2 conditions (Beaudry, 1999). Therefore, controlled atmosphere storage effectively extended the postharvest life of orchid flowers, consistent with studies by Reid (2002), Kader (2002), and Andrew et al., (2009).

In Table 1, at 10 % CO_2, the storage life of orchid flowers was significantly reduced ($p < 0.05$) as higher CO_2 suppressed respiratory metabolism. Zagory and Kader (1989) attributed the lower respiration rate to the inhibitory effect of higher CO_2 on phosphofructokinase in the glycolytic pathway. However, CO_2 beyond a tolerance threshold induces an accumulation of acetaldehyde and ethanol, leading to senescence (Boonyakiat, 2020). Yahia and Singh (2009) documented that tropical plants were susceptible to physiological damage under low O_2 (<2 %) and high CO_2 (>10 %). Poonsri (2015) reported that elevated CO_2 concentrations significantly shortened the storage life of *Dendrobium* orchid flowers.

The incidence and severity of physiological disorders were subjected to O_2 and CO_2 concentrations, temperature, and storage duration (Kader,

Table 4. Effect of high CO_2 and low O_2 conditions on respiration rates of *Dendrobium* orchids.

Condition/Treatment	Respiration Rate (mg CO_2/kg/h)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	$35.18^a \pm 0.54$	$34.50^a \pm 1.24$	$36.58^a \pm 2.35$	$30.15^a \pm 2.23$	-	-	-
5% CO_2 + 2% O_2	$35.18^a \pm 0.54$	$34.20^a \pm 1.55$	$29.98^b \pm 1.43$	$28.55^b \pm 1.80$	$26.98^b \pm 1.83$	$30.22^a \pm 2.33$	27.11 ± 2.58
5% CO_2 + 4% O_2	$35.18^a \pm 0.54$	$34.15^a \pm 1.28$	$30.10^b \pm 1.98$	$27.64^b \pm 0.34$	$28.38^a \pm 1.48$	$26.54^b \pm 1.98$	-
5% CO_2 + 6% O_2	$35.18^a \pm 0.54$	$35.02^a \pm 2.16$	$32.18^b \pm 2.28$	$29.00^b \pm 0.25$	$30.11^a \pm 2.78$	$28.09^b \pm 2.28$	-
5% CO_2 + 8% O_2	$35.18^a \pm 0.54$	$33.58^a \pm 2.16$	$31.22^b \pm 2.34$	$28.58^b \pm 0.50$	$30.98^a \pm 2.93$	$28.44^b \pm 2.34$	-
10% CO_2 + 2% O_2	$35.18^a \pm 0.54$	$32.55^a \pm 2.28$	$30.58^b \pm 1.74$	$28.64^b \pm 0.78$	$27.54^b \pm 1.87$	-	-
10% CO_2 + 4% O_2	$35.18^a \pm 0.54$	$33.99^a \pm 2.28$	$33.05^b \pm 1.96$	$28.32^b \pm 0.57$	$26.80^b \pm 1.20$	-	-
10% CO_2 + 6% O_2	$35.18^a \pm 0.54$	$34.18^a \pm 1.58$	$32.15^b \pm 1.97$	$26.42^b \pm 2.43$	$25.15^b \pm 2.90$	-	-
10% CO_2 + 8% O_2	$35.18^a \pm 0.54$	$31.22^b \pm 1.24$	$29.80^b \pm 1.77$	$26.32^b \pm 2.58$	$23.60^c \pm 2.77$	-	-
Coefficient of variation (%)	-	11.45	18.28	8.28	14.16	12.81	0.24
Least significant difference (LSD)	-	0.74	0.26	0.59	0.97	0.93	0.72

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

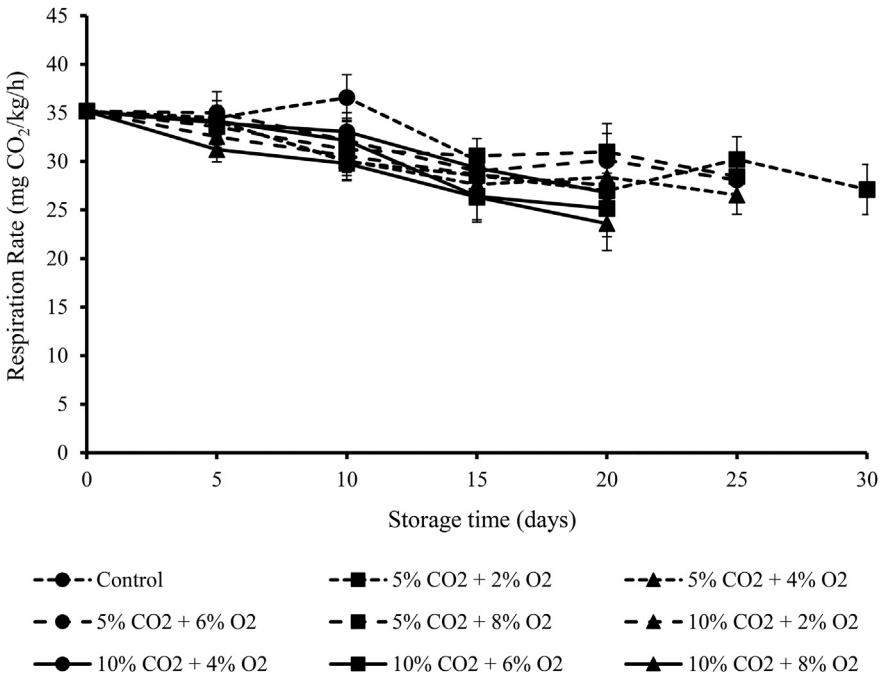

Figure 5. Effect of high CO_2 and low O_2 on respiration rates of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

Table 5. Effect of high CO_2 and low O_2 conditions on ethylene production of *Dendrobium* orchids.

Condition/Treatment	Ethylene (ppm)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	$0.2^a \pm 0.10$	$3.76^a \pm 0.99$	$13.53^a \pm 0.77$	$8.35^a \pm 0.85$	-	-	-
5% CO_2 + 2% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.2^b \pm 0.88$	$0.5^c \pm 0.55$	$1.3^b \pm 1.23$	$2.4^a \pm 0.93$	1.0 ± 0.75
5% CO_2 + 4% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.3^b \pm 0.88$	$0.6^c \pm 0.28$	$1.5^a \pm 1.82$	$1.1^b \pm 1.22$	-
5% CO_2 + 6% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.4^b \pm 0.88$	$0.5^c \pm 0.82$	$1.7^a \pm 1.53$	$0.9^b \pm 0.52$	-
5% CO_2 + 8% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.3^b \pm 0.88$	$0.7^c \pm 0.82$	$2.2^a \pm 1.73$	$1.8^b \pm 0.92$	-
10% CO_2 + 2% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.5^b \pm 0.88$	$1.9^b \pm 0.28$	$0.6^b \pm 1.35$	-	-
10% CO_2 + 4% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.4^b \pm 0.88$	$1.3^b \pm 0.28$	$0.8^b \pm 1.78$	-	-
10% CO_2 + 6% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.5^b \pm 0.88$	$2.4^b \pm 0.58$	$1.5^a \pm 1.57$	-	-
10% CO_2 + 8% O_2	$0.2^a \pm 0.10$	$0.1^b \pm 1.23$	$0.6^b \pm 0.88$	$2.8^b \pm 0.84$	$1.7^a \pm 1.43$	-	-
Coefficient of variation (%)	-	0.42	0.32	0.27	0.33	0.24	0.25
Least significant difference (LSD)	-	0.70	0.95	0.79	0.97	0.72	0.75

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

2002). Lower O_2 and higher CO_2 condition helped preserve the quality of postharvest produce and ornamentals (Andrew et al., 2009). Figure 2 showed cut *Dendrobium* orchid flowers under normal (0.03 % CO_2 + 21 % O_2; left) and controlled atmosphere (5 % CO_2 + 2 % O_2; right) conditions at day 25. The orchid flowers stored in high CO_2 and low O_2 environment had a fresh and healthy appearance even after 25 days.

3.2. Weight loss

The weight loss of orchid flowers under high CO_2 and low O_2 conditions were significantly lower than the control ($p < 0.05$) due to lower vapor transmission (Kader, 2006), as shown in Table 2 and Figure 3. Under the normal atmosphere (control), the weight loss increased rapidly after day 5 (Figure 3) and reached 23.89 % after 15 days, resulting in the end of storage life. Under the optimal controlled atmosphere condition (5 % CO_2 + 2 % O_2), the weight loss progressed slowly and the orchid flowers lost only 13.67 % of the weight after 30 days of storage. The results indicated that the controlled atmosphere condition effectively lengthened the storage life of orchid flowers. Nevertheless, under elevated CO_2 (i.e., 10 % CO_2) conditions, the orchid flowers reached the end of storage life after 20 days, with the weight loss between 14.24 – 18.92 %.

The smaller weight loss could be attributed to the inhibitory effect of controlled atmosphere on the respiration, which resulted in lower water loss and oxidation (Baldwin et al., 1995; Robertson, 2006; Wills et al., 2007). In plant respiration, stored organic materials (carbohydrates, proteins, and fats) are broken down into simple end products where O_2 is used and CO_2 is produced (Kader, 2002). The depletion of stored organic materials during respiration results in senescence, flavor change, and lower salable dry weight. Meanwhile, higher CO_2 (5 %–10 %) and lower O_2 (1 %–2 %) at 0 °C–5 °C reduced respiration rates, water loss, and incidence of diseases (Poonsri, 2015; Izumi et al., 1996). Since cut flowers lose water and wilt very rapidly, relative humidity (RH) should be maintained at 95 % or higher and the flowers should be stored in low temperatures to reduce water loss (van Doorn, 1999). The optimal RH for fruits and vegetables were 85–95 % and 90–98 %, respectively, while that of most cut flowers was 95–99 % (Poonsri, 2017; Kader, 2002).

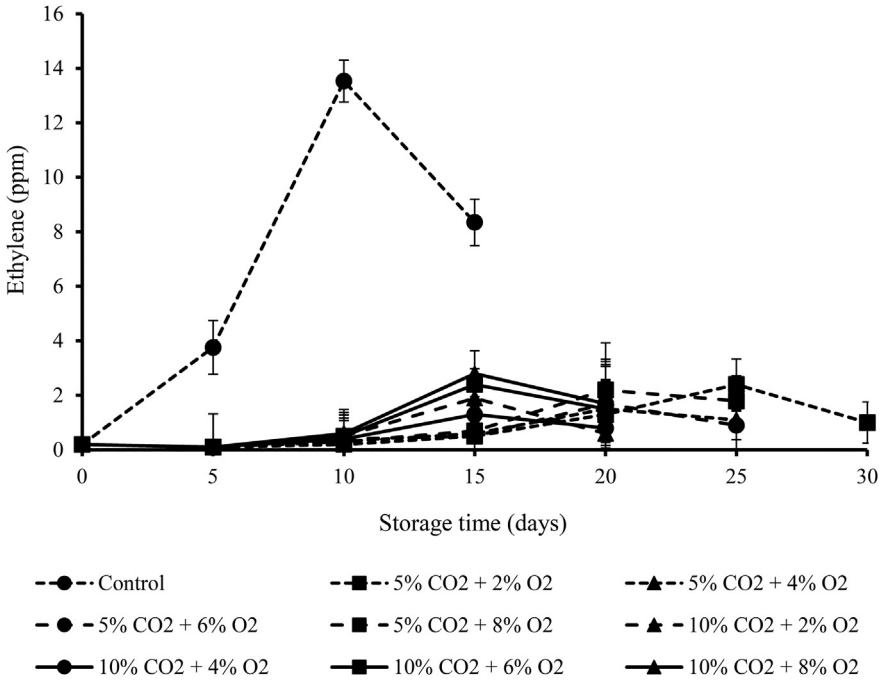

Figure 6. Effect of high CO_2 and low O_2 on ethylene production of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

3.3. Anthocyanin content

Total anthocyanin of orchid flowers under high CO_2 and low O_2 conditions were significantly higher than the control ($p < 0.05$) after 10 days, as shown in Table 3 and Figure 4. Under the normal atmosphere (control), the total anthocyanin noticeably decreased after day 5 (Figure 4) and the remaining anthocyanin content was 2.15 mg/100 g fresh weight after 15 days, reaching the end of storage life of orchid flowers. Under the optimal condition (5 % CO_2 + 2 % O_2), the anthocyanin content decreased very slowly, with the remaining anthocyanin content of 3.14 mg/100 g fresh weight after 30 days, vis-à-vis 4.60 mg/100 g fresh weight at day 0. The results showed that the controlled atmosphere condition helped retain total anthocyanin in the flowers, resulting in the extended storage life.

Controlled atmosphere effectively delayed degradation of anthocyanin pigments due to lower sensitivity to ethylene which is responsible for accelerated biosynthesis of anthocyanin (Bureau et al., 2009; Sass-Kiss et al., 2005). Controlled atmosphere also maintained cellular pH (<7.0) in plant tissues (Siriphanich, 1996), resulting in minimal changes in total anthocyanin. Higher total anthocyanins under controlled atmosphere conditions could be attributed to lower weight loss of the flowers. In other words, higher weight loss increased vacuolar pH (i.e., increased alkalinity), and the higher alkalinity degraded total anthocyanins in orchid flowers by altering the pigments from pink to brown. According to Zhang et al. (2001), anthocyanin degraded and decolorized as the vacuolar pH increased, resulting in senescence.

3.4. Respiration rate

The respiration rates of orchid flowers under normal (the control) and high CO_2 and low O_2 conditions were statistically different after 10 days of storage ($p < 0.05$), as shown in Table 4 and Figure 5. The results showed that the respiration rates under controlled atmosphere were lower than the control.

In Table 4, under the normal atmosphere (control), the respiration rate initially decreased and increased on day 10 and then dropped to 30.15 mg CO_2/kg/h after 15 days of storage. Under the optimal condition (5 % CO_2 + 2 % O_2), the respiration rate steadily decreased and slightly increased on day 25. The respiration rate then decreased to 27.11 mg CO_2/kg/h on day 30. The results indicated that the controlled atmosphere condition effectively decreased the respiration rate of orchid flowers and thereby prolonged their storage life.

Table 6. Effect of high CO_2 and low O_2 conditions on total protein of *Dendrobium* orchids.

Condition/Treatment	Total Protein (mg/g fresh weight)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	$627.0^a \pm 0.54$	$508.4^b \pm 1.24$	$437.2^b \pm 2.35$	$282.9^b \pm 0.23$	-	-	-
5% CO_2 + 2% O_2	$629.0^a \pm 0.54$	$659.0^a \pm 0.55$	$652.0^a \pm 1.43$	$648.0^a \pm 0.80$	$648.5^a \pm 1.83$	$621.0^a \pm 1.33$	561.0 ± 1.58
5% CO_2 + 4% O_2	$630.0^a \pm 1.54$	$650.0^a \pm 0.28$	$650.0^a \pm 1.98$	$646.0^a \pm 0.34$	$635.0^a \pm 1.48$	$580.0^b \pm 0.98$	-
5% CO_2 + 6% O_2	$626.0^a \pm 1.54$	$654.0^a \pm 0.16$	$648.0^a \pm 2.28$	$644.0^a \pm 0.25$	$624.0^a \pm 2.78$	$575.0^b \pm 1.28$	-
5% CO_2 + 8% O_2	$625.0^a \pm 1.54$	$656.0^a \pm 0.16$	$647.0^a \pm 2.34$	$643.0^a \pm 1.50$	$621.0^a \pm 2.93$	$568.0^b \pm 1.34$	-
10% CO_2 + 2% O_2	$631.0^a \pm 1.54$	$645.6^a \pm 0.28$	$631.2^a \pm 1.74$	$621.5^a \pm 0.78$	$625.8^a \pm 1.87$	-	-
10% CO_2 + 4% O_2	$625.0^a \pm 1.54$	$630.9^a \pm 0.28$	$635.8^a \pm 1.96$	$618.7^a \pm 0.57$	$599.0^b \pm 1.20$	-	-
10% CO_2 + 6% O_2	$628.0^a \pm 1.54$	$628.9^a \pm 1.58$	$632.0^a \pm 1.97$	$609.0^a \pm 1.43$	$589.0^b \pm 0.90$	-	-
10% CO_2 + 8% O_2	$623.0^a \pm 1.54$	$622.6^a \pm 0.24$	$637.0^a \pm 0.77$	$611.0^a \pm 0.58$	$570.0^b \pm 0.77$	-	-
Coefficient of variation (%)	26.65	17.50	8.42	8.10	10.29	13.14	14.52
Least significant difference (LSD)	0.62	0.59	0.56	0.56	0.70	0.67	0.59

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

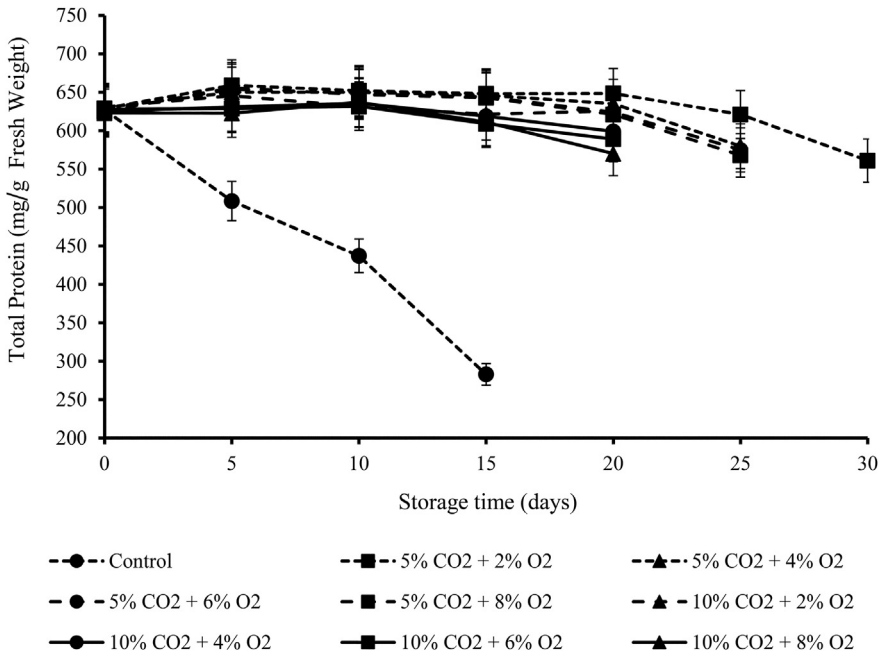

Figure 7. Effect of high CO_2 and low O_2 on total protein of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

According to Poonsri (2015), high CO_2 and low O_2 reduced the respiration rates, ethylene sensitivity, and oxidative processes of cut orchid flowers. In addition, the lower respiration rates under high CO_2 and low O_2 conditions reduced senescence and lower weight loss as the depletion of organic materials was delayed. Higher CO_2 and lower O_2, together with low temperature and high RH, reduced respiration rates, water loss, and total anthocyanin degradation (Yimyong and Soni, 2014; Beaudry, 1999).

3.5. Ethylene production

The ethylene production of orchid flowers under high CO_2 and low O_2 conditions were significantly lower than that of the control ($p < 0.05$), as shown in Table 5 and Figure 6.

In Table 5, under the normal atmosphere (control), ethylene production increased rapidly and peaked at 13.53 ppm on day 10, which is the concentration level that triggers wilting and abscission (Ketsa and Rugkong, 1999). The storage life of the orchid flowers under the normal atmosphere ended after 11.67 days on average. Under the optimal controlled atmosphere condition (5 % CO_2 + 2 % O_2), ethylene production increased very slowly and peaked at 2.4 ppm on day 25. The storage life of the orchid flowers under the optimal controlled atmosphere condition ended after 28.33 days on average. The results showed that the controlled atmosphere condition effectively inhibited ethylene production in the orchid flowers, resulting in the extended storage life.

Modified internal atmosphere (high CO_2 and low O_2) reduced the rates of ethylene production and inhibited ethylene action (Poonsri, 2020). Under High CO_2 and low O_2 conditions, the ethylene production was lower, thus extending the life of ethylene-sensitive plant tissues (Kader, 2002). According to Poonsri (2015), O_2 below 8 % decreased ethylene production and sensitivity to ethylene of fresh ornamentals. Lower O_2 inhibited the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene (Poonsri, 2020; van Altvorst and Bovy, 1995), while higher CO_2 inhibited the ethylene production in cut flowers (Poonsri, 2020).

3.6. Protein degradation and protease activity

Total protein in the orchid flowers under high CO_2 and low O_2 conditions were significantly higher than that of the control ($p < 0.05$), as shown in Table 6 and Figure 7. Under the normal atmosphere (control), total protein rapidly decreased and there remained only 282.9 mg/g fresh weight after 15 days, indicating the end of storage life. Under the

Table 7. Effect of high CO_2 and low O_2 conditions on protease activity of *Dendrobium* orchids.

Condition/Treatment	Protease activity (units/g fresh weight)*						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (0.03% CO_2 + 21% O_2)	$12.75^{a} \pm 1.68$	$15.74^{a} \pm 2.35$	$18.02^{a} \pm 2.54$	$14.25^{c} \pm 2.85$	-	-	-
5% CO_2 + 2% O_2	$12.66^{a} \pm 1.68$	$13.77^{c} \pm 2.14$	$13.61^{c} \pm 3.43$	$14.87^{b} \pm 3.14$	$15.97^{b} \pm 3.14$	$16.59^{a} \pm 3.43$	15.71 ± 2.97
5% CO_2 + 4% O_2	$12.68^{a} \pm 1.68$	$13.61^{c} \pm 1.20$	$14.78^{c} \pm 1.98$	15.97 ± 1.20	$16.59^{a} \pm 2.60$	$15.97^{b} \pm 2.98$	-
5% CO_2 + 6% O_2	$12.76^{a} \pm 1.68$	$13.67^{c} \pm 2.73$	$14.81^{c} \pm 2.28$	$15.99^{a} \pm 2.73$	$16.64^{a} \pm 2.28$	$15.66^{b} \pm 2.28$	-
5% CO_2 + 8% O_2	$12.67^{a} \pm 1.68$	$13.74^{c} \pm 2.43$	$14.99^{b} \pm 2.33$	$16.00^{a} \pm 2.13$	$17.01^{a} \pm 2.64$	$16.22^{b} \pm 2.93$	-
10% CO_2 + 2% O_2	$12.71^{a} \pm 1.68$	$13.93^{b} \pm 1.17$	$15.80^{b} \pm 3.41$	$15.94^{a} \pm 2.64$	$14.76^{c} \pm 3.17$	-	-
10% CO_2 + 4% O_2	$12.73^{a} \pm 1.68$	$13.99^{b} \pm 1.80$	$15.90^{b} \pm 1.79$	$16.02^{a} \pm 1.80$	$14.31^{c} \pm 1.96$	-	-
10% CO_2 + 6% O_2	$12.89^{a} \pm 1.68$	$14.12^{b} \pm 1.97$	$16.21^{b} \pm 2.80$	$16.55^{a} \pm 2.97$	$15.97^{b} \pm 3.93$	-	-
10% CO_2 + 8% O_2	$12.75^{a} \pm 1.68$	$14.44^{b} \pm 2.58$	$16.59^{b} \pm 3.31$	$16.89^{a} \pm 2.88$	$15.71^{b} \pm 2.48$	-	-
Coefficient of variation (%)	11.78	14.94	8.53	12.81	16.75	15.09	12.04
Least significant difference (LSD)	0.65	0.26	0.65	0.93	1.24	0.33	1.38

*The values are mean ± standard deviation; and different superscripts indicate statistical significance at 5% ($p < 0.05$).

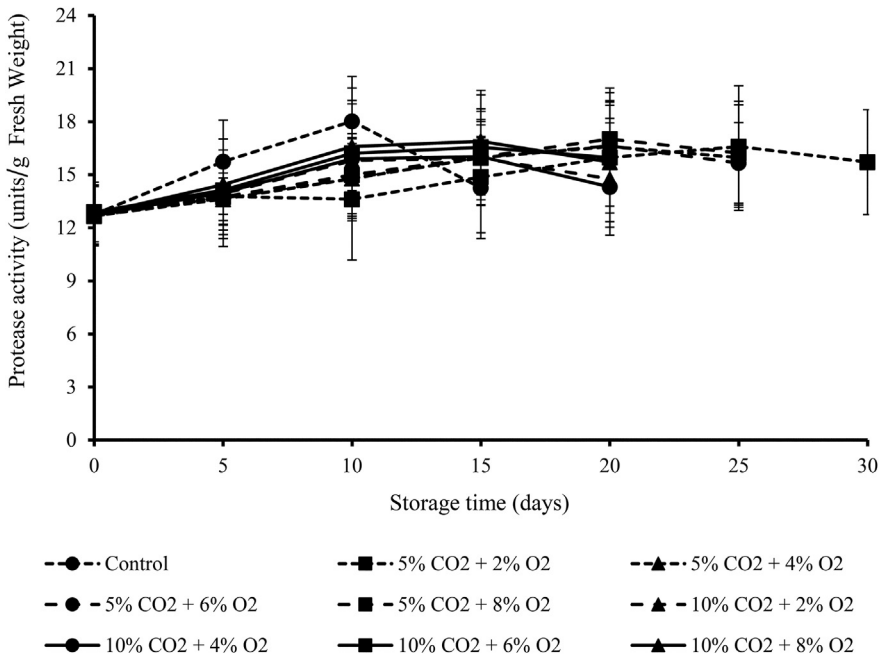

Figure 8. Effect of high CO_2 and low O_2 on protease activity of *Dendrobium* orchid flowers given the storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviation.

optimal controlled atmosphere condition (5 % CO_2 + 2 % O_2), total protein declined very slowly and decreased substantially after 25 days of storage. The results indicated that the controlled atmosphere condition could delay protein degradation and lengthened the storage life of orchid flowers.

The protease activity of orchid flowers under high CO_2 and low O_2 conditions were significantly lower than that of the control ($p < 0.05$), as shown in Table 7 and Figure 8. Under the normal atmosphere (control), the protease activity steadily increased and peaked at 18.02 units/g fresh weight on day 10. The protease activity decreased afterward, consistent with total protein degradation. Under the optimal controlled atmosphere condition (5 % CO_2 + 2 % O_2), the protease activity increased slowly and peaked at 16.59 units/g fresh weight after 25 days of storage. The protease activity was 16.59 units/g fresh weight on the final day of experiment (day 30). The results indicated that the controlled atmosphere condition effectively delayed the protease activity and extended the storage life of orchid flowers.

According to Callis (1995), cellular protein degradation was closely related to endoprotease activity. In addition, protease plays an essential role in the flower senescence (Buchanan-Wollaston, 1997; Nooden et al., 1997). Specifically, the protein degradation increased with increase in the protease activity, which in turn induced the senescence of orchid flowers.

One of the 17 Sustainable Development Goals (SDG) established by the United Nations in 2015 is SDG 13 on climate change which aims to take urgent action to combat climate change and its impacts. Since the controlled atmosphere technology requires filling the storage room with elevated concentrations CO_2 from the atmosphere while reducing the CO_2 production and CO_2 released into the atmosphere, the technology could thus be adopted, alongside other climate actions, to combat climate change and mitigate its impacts.

4. Conclusions

This research studied the effects of high CO_2 and low O_2 on the biochemical changes in cut *Dendrobium* pink stripe orchid flowers. The experiments were carried out under normal and high CO_2 and low O_2 conditions. The results showed that high CO_2 and low O_2 significantly improved the storability of *Dendrobium* orchid flowers ($p < 0.05$). The optimal controlled atmosphere condition was 5 % CO_2 and 2 % O_2, achieving the longest storage life of 28.33 days, vis-à-vis 11.67 days for the orchid flowers under normal atmosphere. The controlled atmosphere also helped to retain total anthocyanin content while lowering fresh weight loss, respiration rate, ethylene production, protein degradation, and protease activity. The floriculture industry, especially cut flower exporters, could adopt the optimal controlled atmosphere (5 % CO_2 and 2 % O_2) to extend the shelf life and preserve the quality of postharvest flowers. Specifically, the extended storage life of *Dendrobium* orchid flowers under the optimal controlled atmosphere condition provides exporters with new opportunities to grow the market share by expanding into new overseas markets.

Declarations

Author contribution statement

Warinthon Poonsri: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The data that has been used is confidential.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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