ผลการปรับสภาพบรรยากาศต่อคุณภาพและอายุการเก็บรักษาถั่วแขก (*PHASEOLUS VULGARIS* L.)

EFFECT OF MODIFIED ATMOSPHERE ON THE QUALITY AND SHELF LIFE OF GREEN BEANS (*PHASEOLUS VULGARIS* L.)

คาร์มา ดอร์จิ[1] *ธนะบูลย์ สัจจาอนันตกุล*[2*] *ภัทรินทร์ ลีลาภิวัฒน์*[3]

Karma Dorji[1], *Tanaboon Sajjaanantakul*[2*], *Pattarin Leelaphiwat*[3]

[1]*ศูนย์ปฏิบัติการหลังการเก็บเกี่ยวแห่งชาติ กระทรวงเกษตรและป่าไม้ เมืองพาโร ประเทศภูฏาน*

[1]*National Post Harvest Center, Ministry of Agriculture and Forests, Paro, Bhutan.*

[2]*ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์*

[2]*Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University.*

[3]*ภาควิชาเทคโนโลยีการบรรจุและวัสดุ คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์*

[3]*Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University.*

***Corresponding author, e-mail:** fagitbs@ku.ac.th*

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บทคัดย่อ

ถั่วแขก (*Phaseolus vulgaris* L.) หลังการเก็บเกี่ยวเสื่อมคุณภาพจากการสูญเสียสีเขียว เกิดสีน้ำตาล เสียน้ำหนักและนิ่มลงของเนื้อสัมผัส งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการปรับสภาพบรรยากาศ (ลดออกซิเจน (O_2) ร่วมกับเพิ่มคาร์บอนไดออกไซด์ (CO_2)) ต่อคุณภาพถั่วแขกเก็บที่อุณหภูมิ 8 °ซ สภาวะการศึกษาคือ O_2 ต่ำ (2, 5%) ร่วมกับ CO_2 สูง (4, 7, 10%) และสภาวะ O_2 ปกติ (21%) + 10% CO_2 และสภาวะ 2% O_2 + CO_2 ปกติ (0.03%) โดยพบว่าถั่วแขกที่ 8 °ซ ในอากาศปกติมีอัตราการหายใจ 82.44 $mgCO_2\ kg^{-1}\ h^{-1}$ ขณะที่ตัวอย่างในสภาวะปรับบรรยากาศมีค่าลดลงอยู่ในช่วง 5.52 - 66.70 $mgCO_2\ kg^{-1}\ h^{-1}$ โดยไม่เกิดการหายใจแบบไร้ออกซิเจน ซึ่งมีอัตราการเกิดเอทานอลอยู่ในระดับต่ำมาก 5.52-85.37 $\mu L\ L^{-1}$ ทั้งนี้ผลการเพิ่มความเข้มข้น CO_2 เมื่อมี O_2 ในระดับเดียวกัน ทำให้ค่าสีฮิว (hue) ลดลง (90.83-108.27) ดัชนีสีน้ำตาลเพิ่มขึ้น (52.39-85.33) แต่ผลการลด O_2 เมื่อมี CO_2 ในระดับเดียวกันช่วยคงค่าสีเขียวและลดค่าดัชนีสีน้ำตาล การใช้ O_2 ที่ 2% ร่วมกับ CO_2 ปกติ (0.03%) ทำให้ตัวอย่างมีสีเขียวมากกว่า ค่าความเป็นกรดเบสคงที่ และมีค่าของแข็งที่ละลายน้ำมากกว่าตัวอย่างในสภาวะบรรยากาศอื่น เมื่อเพิ่มความเข้มข้น CO_2 พบว่าการสูญเสียน้ำหนักมีค่าเพิ่มขึ้นในช่วง 4.99 - 7.25% ทั้งนี้ในทุกตัวอย่างมีค่าความเหนียวของเนื้อสัมผัสไม่แตกต่างกัน ดังนั้นการใช้ O_2 ที่ 2% ร่วมกับ CO_2 ปกติจึงเป็นสภาวะที่แนะนำให้ใช้ในการปรับบรรยากาศบรรจุภัณฑ์สำหรับถั่วแขกเก็บรักษาที่ 8 °ซ นาน 21 วัน

คำสำคัญ: ถั่วแขก ปรับสภาพบรรยากาศ คุณภาพ อายุการเก็บรักษา ออกซิเจน คาร์บอนไดออกไซด์

Abstract

Harvested green bean (*Phaseolus vulgaris* L.) deteriorates through loss of greenness, browning, weight loss, and softening of texture. The study aimed to investigate the effects of modified atmosphere (reduced oxygen (O_2) combined with increased carbon dioxide (CO_2)) on the quality of green beans stored at 8°C. Atmospheres of reduced O_2 (2, 5%) and increased CO_2 (4, 7, 10%), with additional treatment of normal O_2 (21%) + 10% CO_2, and 2% O_2 + normal CO_2 (0.03%) were studied. Respiration rate of green beans at 8°C in normal air storage was 82.44 mgCO_2 kg^{-1} h^{-1} while respiration of all gas treatments were much reduced to 5.52-66.70 mgCO_2 kg^{-1} h^{-1} without anaerobic respiration. Ethanol production for all samples was very low (5.52-85.37 μL L^{-1}). Increasing CO_2 concentration at equal O_2 level caused reduction in hue value (90.83-108.27), and increased browning index (52.39-85.33). However, reduction of O_2 concentration at equal CO_2 level retained greenness and minimized browning index. Use of 2% O_2 + normal (0.03%) CO_2 resulted in more greenness, stable pH and higher total soluble solids as compared to other treatments. Weight loss increased (4.99-7.25%) with increasing CO_2 concentration. There was no significant difference in toughness of beans for all treatments. Therefore 2% O_2 combined with normal CO_2 is recommended for modified atmosphere packaging of green beans stored at 8°C for 21 days.

Keywords: Green Beans, Modified Atmosphere, Quality, Shelf Life, Oxygen, Carbon Dioxide

Introduction

Green bean (*Phaseolus vulgaris* L.) is a good source of nutrients i.e., protein, fiber, calcium, magnesium, phosphorous and vitamins [1]. Loss of green bean qualities after harvest is mainly due to the high respiration rate of the pod's immature seeds [2]. Cantwell and Suslow [3] reported that water loss was another major post-harvest problem. Browning from physical handling injuries, loss of green color from chlorophyll degradation and texture softening are also the symptoms of quality deterioration of green bean. An optimum storage temperature of 5-7.5°C and a relative humidity of 95-100% has been recommended with an expected shelf-life of 8-12 days [3]. Modified atmosphere packaging and controlled atmosphere storage [4] has been used for prolonging shelf life of fresh produce through reduction of its physiological activity and minimization of handling and mechanical damages. The respiration rates and ethylene production of apple and pear under controlled atmosphere storage were suppressed, which help maintained quality and shelf life of the fruits [5].

For green beans, storage condition up to 30% CO_2 treatment for 24 hours at 27°C gave better color, flavor and less sloughing. Small discoloration was due to less phenolase and phenolic activity [6]. Shelf life of green

bean prolonged to 22 days at 1% O_2 + 3% CO_2 at 8°C due to retention of chlorophyll, carotenoids and pod appearance [7]. A gas combination of 3% O_2 + 3% CO_2 at 8°C and 98% RH retained higher carbohydrates, total sugars and vitamins during 18 days of storage without any risk of anaerobic processes [2]. Groeschel, *et al*. [8] and Cantwell and Suslow [3] reported that the greatest advantage of controlled atmosphere storage of green beans was color retention by reducing the rate of chlorophyll degradation. A range of gas concentration of 2-3% O_2 and 4-7% CO_2 at temperature range of 5-10°C has been recommended as an ideal for green beans [8]. Previous works [2-3], [6-8] on green beans were focused on controlled atmosphere conditions for long term storage, with little information on modified atmosphere effects. Finding the optimum gas mixing concentration of oxygen and carbon dioxide that effectively retains green bean qualities would be important criteria in designing modified atmosphere packaging for retail market of green bean.

Objectives

This study aimed to determine the effects of different combinations of reduced O_2 and increased CO_2 concentrations on the quality and shelf life of green beans stored at 8°C.

Methods

Fresh green beans of the Royal Project Foundation were harvested at Chiangmai Province, Northern Thailand and delivered within 24 hours by refrigerated truck to a laboratory at Kasetsart University, Bangkok. Fresh green beans were soaked in 200 ppm sodium hypochlorite solution for 10 minutes to disinfect any soil microbial. Surface water was then removed and the beans (500 g) were transferred to glass jars which was connected to various gas combination. Controlled atmosphere gas mixing system (ICA 61 CA system, Tonbridge, Kent, UK) was used to control and regulate the concentrations of O_2 and CO_2 gas with a flow rate of 100 mL min^{-1} through the sample glass jars. Gas sample was drawn from the jars regularly for determination of respiration rate using a gas chromatography 6890N (Agilent Technologies, USA). Molecular sieve 5A 80/100 and HayeSep Q 100/120 (Alltech Associates, Inc., USA) columns were used with a TCD detector. A 20% CO_2 + 5% O_2 with balanced nitrogen (Linde Group, Thailand) was used as a standard gas. All samples were stored at 8°C for 21 days.

Ethanol production of the samples (for an anaerobic respiration indicator) was measured according to Khan, *et al*. [9]. Twenty grams of green bean were blended with 10 mL distilled water, filtering through a muslin cloth. Ten mL of aliquot was transferred to 20 mL headspace vial and frozen until used. Frozen bean juice were transferred to 50°C water bath for exactly 20 minutes and 0.5 mL of gas from the headspace of the vial was injected into the GC 6820 (Agilent Technologies, USA) equipped with DB1 column and FID detector. An absolute ethanol was used as an internal standard.

A Hunter Lab colorimeter (Mini Scan XE, USA) was used for determining the CIE L*a*b* values. For each treatment, 5 pods of beans were used and the values were measured from two spots i.e., upper half and lower half, of each bean. Browning index (BI) and hue angle (H) were determined according to the equations by Kasim and Kasim [10].

$$BI = (100 (X - 0.31)) / 0.17 \quad (1)$$

where $X = (a^* + 1.75 L^*) / (5.645 L^* + a^* - 0.012 b^*)$

$$H = \arctan (b^*/a^*) \quad (2)$$

Texture analyzer (TA-XT Plus, Stable Microsystems, UK) equipped with Volodkewich bite jaws (HDP/90) with a test speed of 5 mm/s and a travel distance of 10 mm was used for determining the maximum cutting force as toughness of the green beans [11] with 9 replicates for each sample treatment.

Difference in weight of green beans at the beginning of storage (500 $\pm$ 5 g) was calculated for percentage weight loss as described by Lucera, *et al.* [12]. Total Soluble Solids (TSS) was measured by a hand held refractometer (Atago, N-1α, 0-32% Brix, Japan).

All treatments were done in triplicates and statistical analysis was analyzed by the SPSS software (version 15).

Results

Carbon dioxide production of green beans stored at 8°C ranged between 76.69-89.58 mg CO_2 kg^{-1} h^{-1} during the 9 days of storage with an average respiration rate of 82.44 mgCO_2 kg^{-1} h^{-1} (Figure 1). Respiration rate of green bean at room temperature (30 $\pm$ 2°C) was 175.65 mgCO_2 kg-1 h-1 during 4 days of storage. Carbon dioxide production increased exponentially at first 3 hours at the first day and then declined from second day.

Temperature coefficient (Q_{10}) is a measure of the rate of change in chemical or physiological reaction as a consequence of 10°C rise in temperature [13]. The Q_{10} is expressed as the ratio of a chemical reaction at a given temperature to that of the same reaction at a temperature 10°C lower. The Q_{10} concept allows the calculation of expected respiration rates at a given temperature from a known a known rate at another temperature [13]. Temperature coefficient was in the range of 1.20-1.49 when green beans were studied (data was not shown). It suggested that the respiration rate of the beans increased around 1.20-1.49 times when the temperature increased from 8°C to 30°C.

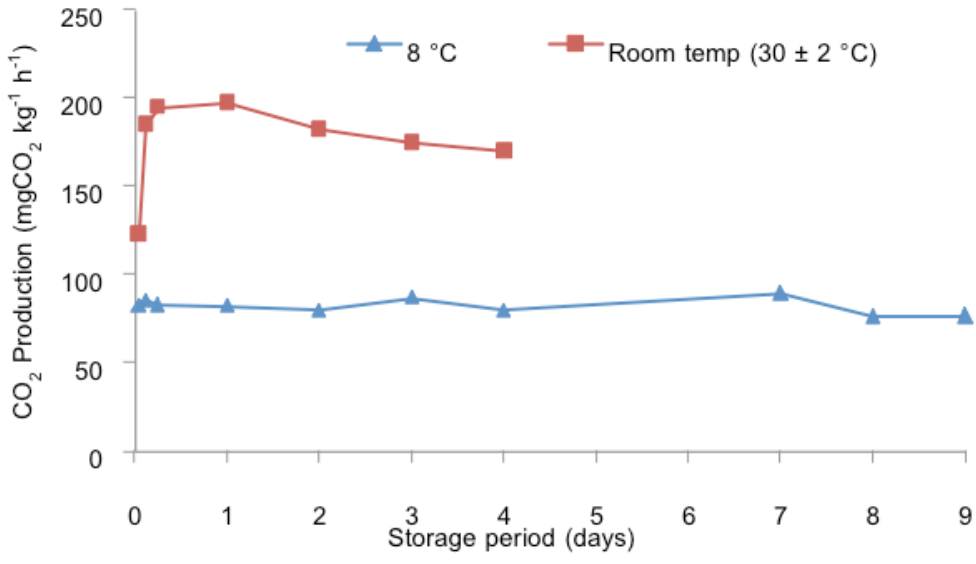

Figure 1. Respiration rate of green bean stored at 8°C and room temperature (30 ± 2°C).

The respiration rate was in the range of 3.68-130.64 $mgCO_2\ kg^{-1}\ h^{-1}$ for all gas combination treatments. The lowest rate was found in 2% O_2 + 0.03% (normal) CO_2 (Table 1). In general, combinations with high CO_2 concentrations caused higher rate of respiration.

Ethanol production from all stored green beans was low (5.52-85.37 $\mu L\ L^{-1}$). Treatments with 2% O_2 did not cause anaerobic respiration (Table 2). Higher ethanol production was found in 10% CO_2 + 21% O_2.

Browning index of fresh green bean was 48.74. Changes of browning index during storage was lowest in 2% O_2 + 0.03% (normal) CO_2 followed by treatments with 2% O_2 + 4% CO_2 and 5% O_2 + 4% CO_2 (Figure 2). Browning index increased with the storage time particularly for treatments containing higher carbon dioxide concentrations. Discoloration of green bean pods were noticed more clearly after 14 days of storage in higher CO_2 treatments due to greater degree of browning.

Hue angle of the treated green bean was 108.60 in the beginning of storage. Hue angle of green bean stored in 2% O_2 + 0.03% CO_2 remained almost constant throughout the storage period with minimal change. Less change was also found in gas combination of 2% O_2 + 4% CO_2 and 5% O_2 + 4% CO_2 on 21st day (Table 3). High CO_2 concentrations caused higher loss of greenness since the highest change of hue was exhibited.

Table 1 Respiration rate ($mgCO_2\ kg^{-1}\ h^{-1}$) of green beans stored at 8°C under different gas combinations

Treatments	Storage time (days)		
	2	12	20
Air (Control)	29.62 ± 2.98^{fA}	23.92 ± 0.80^{eB}	13.80 ± 1.38^{eC}
21% O_2 + 10% CO_2	104.42 ± 7.60^{aB}	130.64 ± 2.76^{aA}	38.64 ± 2.39^{bC}
2% O_2 + 0.03% CO_2	3.68 ± 0.80^{gB}	9.66 ± 0.00^{fA}	4.60 ± 0.80^{fB}
2% O_2 + 4% CO_2	43.24 ± 5.22^{eB}	81.42 ± 1.38^{bA}	48.76 ± 0.80^{aB}
2% O_2 +7% CO_2	65.78 ± 9.19^{cdA}	13.80 ± 2.11^{fB}	15.64 ± 0.80^{deB}
2% O_2 + 10% CO_2	79.58 ± 3.19^{bA}	52.44 ± 2.76^{cB}	27.60 ± 1.38^{cC}
5% O_2 + 4% CO_2	74.52 ± 1.38^{bcA}	23.46 ± 0.00^{eC}	45.54 ± 4.98^{aB}
5% O_2 + 7% CO_2	21.16 ± 4.44^{fC}	52.44 ± 4.98^{cA}	41.40 ± 3.65^{bB}
5% O_2 + 10% CO_2	60.26 ± 14.63^{dA}	34.50 ± 2.39^{dB}	19.32 ± 0.00^{dB}

Means in the same column (treatments) with different small superscript, and in the same row (storage time) with different capital superscript are significantly different at $p < 0.05$ by DMRT

Table 2 Ethanol productions ($\mu L\ L^{-1}$) of green beans stored at 8°C for 21 days under different gas combinations

Treatments	Storage Time (days)			
	4	7	14	21
Air (Control)	38.47 ± 10.00^{a}	9.28 ± 2.00^{bc}	11.99 ± 6.00^{f}	16.79 ± 0.00^{b}
21% O_2 + 10% CO_2	31.87 ± 15.00^{ab}	47.35 ± 7.00^{a}	85.37 ± 3.00^{a}	66.16 ± 10.00^{a}
2% O_2 + 0.03% CO_2	14.84 ± 10.00^{c}	9.05 ± 2.00^{bc}	20.94 ± 4.00^{f}	25.58 ± 20.00^{b}
2% O_2 + 4% CO_2	19.49 ± 5.00^{bc}	15.46 ± 8.00^{b}	25.43 ± 2.00^{ef}	25.05 ± 14.00^{b}
2% O_2 + 7% CO_2	12.92 ± 3.00^{c}	13.02 ± 6.00^{bc}	17.38 ± 5.00^{f}	18.54 ± 10.00^{b}
2% O_2 + 10% CO_2	13.97 ± 2.00^{c}	6.29 ± 1.00^{bc}	42.44 ± 9.00^{cd}	10.76 ± 2.00^{b}
5% O_2 + 4% O_2	10.83 ± 2.00^{c}	9.67 ± 6.00^{bc}	55.08 ± 12.00^{bc}	23.58 ± 16.00^{b}
5% O_2 + 7% O_2	6.78 ± 2.00^{c}	5.52 ± 0.00^{c}	37.71 ± 10.00^{de}	70.81 ± 5.00^{a}
5% O_2 + 10% O_2	42.57 ± 11.00^{a}	10.44 ± 3.00^{bc}	58.40 ± 14.00^{b}	27.91 ± 4.00^{b}

Means in the same column with different superscript are significantly different at $p < 0.05$ by DMRT

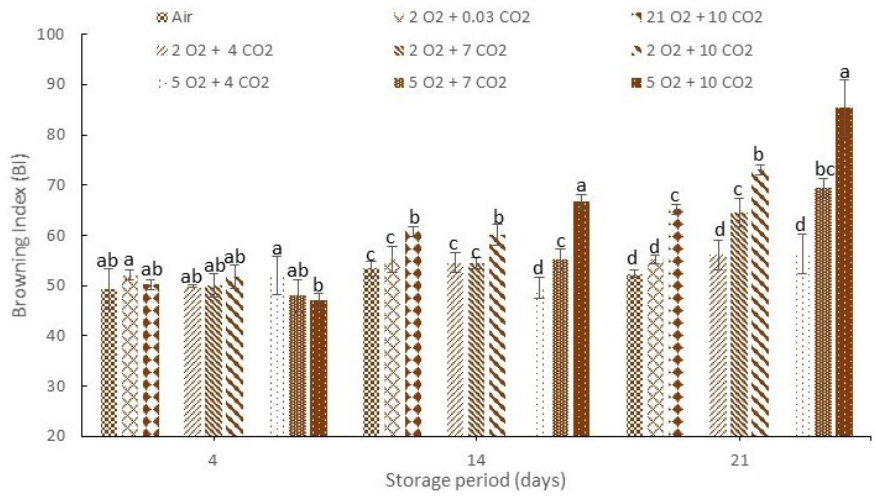

Figure 2. Browning Index of green beans stored at 8°C for 21 days under different gas combinations. Vertical bars represent mean ± standard deviation. The values within a bar with different letters are significantly different in the same group (by day) at $p < 0.05$ by DMRT.

Maximum reduction in total soluble solids was observed in high CO_2 treatments and control (Table 3). Storage condition of 2% O_2 + 0.03% CO_2 had minimal change in total soluble solids on 21 days of storage. This combination exhibited a minimal change in pH compared to other treatments as well.

There was a significant difference of weight loss among the treatments ($p < 0.05$). Weight loss was in the range of 4.99-7.25% for all combinations at the end of storage (Table 4). There was no significant difference of toughness of green beans stored in various gas combinations (Table 4).

Table 3 Hue angle, total soluble solids (TSS) and pH, of green beans stored at 8°C for 21 days under different gas combinations

Treatments/Days	Hue		TSS (°Brix)		pH	
	0	21	0	21	0	21
Air (Control)	108.60^{A}	106.25 ± 0.53^{aB}	2.97^{A}	1.97 ± 0.03^{cdeB}	5.97^{B}	6.29 ± 0.00^{fA}
21% CO_2 + 10% CO_2	108.60^{A}	96.69 ± 1.55^{dB}	2.97^{A}	1.87 ± 0.03^{eB}	5.97^{B}	7.38 ± 0.00^{aA}
2% O_2 + 0.03% CO_2	108.60^{A}	108.27 ± 0.14^{aA}	2.97^{A}	2.20 ± 0.06^{aA}	5.97^{B}	6.57 ± 0.02^{dA}
2% O_2 + 4% CO_2	108.60^{A}	102.21 ± 1.60^{bB}	2.97^{A}	2.13 ± 0.03^{abB}	5.97^{B}	6.44 ± 0.02^{eA}
2% O_2 + 7% CO_2	108.60^{A}	91.38 ± 0.96^{dB}	2.97^{A}	2.13 ± 0.03^{abB}	5.97^{B}	6.93 ± 0.01^{cA}
2% O_2 + 10% CO_2	108.60^{A}	90.94 ± 0.97^{dB}	2.97^{A}	2.07 ± 0.03^{bcB}	5.97^{B}	7.20 ± 0.01^{bA}
5% O_2 + 4% CO_2	108.60^{A}	99.45 ± 2.10^{cB}	2.97^{A}	2.07 ± 0.03^{bcB}	5.97^{B}	6.94 ± 0.02^{cA}
5% O_2 + 7% CO_2	108.60^{A}	91.79 ± 1.79^{dB}	2.97^{A}	2.03 ± 0.03^{bcdB}	5.97^{B}	7.22 ± 0.02^{bA}
5% O_2 + 10% CO_2	108.60^{A}	90.83 ± 0.11^{dB}	2.97^{A}	1.93 ± 0.03^{deB}	5.97^{B}	7.22 ± 0.01^{bA}

Means in the same column (treatments) with different small superscript, and in the same row (days) with different capital superscript for each quality attribute are significantly different at $p < 0.05$ by DMRT

Conclusions and Discussion

Combinations of reduced O_2 (2%) with normal CO_2 (0.03%) was found to be the best condition in retaining greenness and lowering browning index when storing green beans at 8°C for 21 days. It also maintained total soluble solids and pH without causing an anaerobic respiration. At the same reduced O_2 (2%), increase of CO_2 resulted in more loss of greenness, higher browning index, more loss of total soluble solids and greater change of pH.

The respiration rate of green bean in this study (76.69 - 89.58 $mgCO_2\ kg^{-1}\ h^{-1}$) is similar to green beans stored at 8°C for 9 days reported by other study (81.66 mg $CO_2\ kg^{-1}\ h^{-1}$) [14]. Watada and Morris [15] also presented that green beans stored at 5°C and 10°C had respiration rate of 66 $mgCO_2\ kg^{-1}\ h^{-1}$ and 110 $mgCO_2\ kg^{-1}\ h^{-1}$, respectively. Respiration rate of green bean is very high around 70-100 $mgCO_2\ kg^{-1}\ h^{-1}$ at 10°C [15] due to intense metabolic activity of immature seeds inside the pod.

Table 4 Weight loss and toughness of green beans stored at 8°C for 21 days under different gas combinations

Treatments/Days	Weight Loss (%)		Toughness (N)	
	0	21	0	21
Air (Control)	0	4.17 ± 0.12^{e}	368.06 ± 19.97^{A}	341.26 ± 83.45^{aA}
21% CO_2 + 10% CO_2	0	5.51 ± 0.29^{cd}	368.06 ± 19.97^{A}	320.50 ± 61.76^{aA}
2% O_2 + 0.03% CO_2	0	5.92 ± 0.12^{bc}	368.06 ± 19.97^{A}	262.48 ± 24.31^{aA}
2% O_2 + 4% CO_2	0	4.996 ± 0.93^{d}	368.06 ± 19.97^{A}	288.75 ± 117.70^{aB}
2% O_2 + 7% CO_2	0	6.44 ± 0.04^{b}	368.06 ± 19.97^{A}	227.75 ± 112.75^{aB}
2% O_2 + 10% CO_2	0	5.37 ± 0.42^{cd}	368.06 ± 19.97^{A}	263.71 ± 84.41^{aA}
5% O_2 + 4% CO_2	0	5.22 ± 0.39^{cd}	368.06 ± 19.97^{A}	237.69 ± 76.07^{aB}
5% O_2 + 7% CO_2	0	7.25 ± 0.26^{a}	368.06 ± 19.97^{A}	317.68 ± 6.83^{aA}
5% O_2 + 10% CO_2	0	5.50 ± 0.29^{cd}	368.06 ± 19.97^{A}	335.49 ± 51.18^{aA}

Means in the same column (treatments) with different small superscript, and in the same row (days) with different capital superscript for each quality attribute are significantly different at $p < 0.05$ by DMRT

The respiration rate of green beans increases with storage temperature [15]. Decreased respiration at lower temperatures is due to the decrease in tissue metabolism and decrease in activities of enzyme such as polyphenol oxidase, glycolic oxidase and ascorbate oxidase [14]. Most biological reactions have a Q_{10} value of 2 and 3 for temperature between 10 to 30°C [13]. This indicates double or triples increase of reaction rate with every 10°C rise. Temperature coefficient (Q_{10}) values for biological processes, however, are not constant for wide range of temperatures.

Temperature coefficient is usually high between 0-10°C and constantly decreases to around 1 at a higher temperature range. Higher rate of respiration does not necessarily result in higher Q_{10}. For instance, Q_{10} value of green bean remained at 1 when temperature increased from 20-25°C [15]. This indicated that there was no change in reaction in this temperature range.

Treatments with 2% O_2 did not cause anaerobic respiration. Ethanol production from apples stored in controlled atmospheres was 20-25 $\mu L\ L^{-1}$. This level was low and did not give any symptoms of an anaerobic respiration during the storage [17]. Higher ethanol production found in 21% O_2 + 10% CO_2 in our experiment is similar with findings of Khan, *et al.* [9] for longan fruits stored under controlled atmosphere conditions. The increase of ethanol production in the gas combination could be due to stress level caused by controlled atmospheres [18]. Therefore, increase of CO_2 concentrations has a limitation when anaerobic respiration is concerned.

Hue angle of 150°-90° represents green colour while angle below 90° indicates yellowness. Decrease in hue value indicates a reduction in greenness and turns to more yellowness [19]. Modified atmospheres storage of broccoli with reduced O_2 (10% O_2 + 5% CO_2) retained more chlorophyll and resulted in overall maintenance of quality compared to control [20]. Green tomato stored under controlled atmosphere condition of 5% O_2 + 10% CO_2 maintained better greenness [21]. Oxygen is required for synthesis of enzymes pheophorbide 'a' oxygenase and also acts as a substrate in chlorophyll degradation mechanism [22]. This lower oxygen retards the chlorophyll breakdown and retains greenness of the treated beans.

Browning index (BI) indicates chemical change and increase of brown color in fresh cut fruits and vegetables during storage or processing [23]. Effects of CO_2 on browning index is similar to the work of Costa, *et al.* [24] who reported that CO_2 injury in green bean occurred within 14 days of storage at 8°C (20% CO_2 + 2% O_2). But in the present study, CO2 injury occurred when storing in conditions containing more than 7% CO_2 at 8°C during the 21 days storage. This could be due to different variety of green bean. Costa, *et al.* [24] stated that sensitivity to CO_2 injury depended on green bean cultivars. High CO_2 concentration increased brown stain and necrosis on butter head lettuce and the degree of susceptibility differed among the cultivars [25]. Extreme CO_2 atmospheres might reduce the energy status of cells and induce breakage of cellular integrity. This released the polyphenol oxidase enzymes and phenols from the cells and reacted in browning reactions [26].

Guo, *et al.* [14] reported a general drop in total soluble solids due to utilization of sugars in respiration during storage of green beans. All gas combination treatments resulted in reduced total soluble solids in green beans (Table 3). Higher reduction of total soluble solids in higher CO_2 treatments could be due to high CO_2 injury and respiration rate. Increase of hydrolysis and glycolysis

reactions resulted in utilization of sugars [27]. Higher reduction of total soluble solids in control might be due to higher rate of respiration under abundant supply of O_2.

A minimal change of pH in gas combination of 2% O_2 + normal CO_2 could be due to reduction of respiration rate. Higher CO_2 treatments (>7%) induced greater increase in pH (Table 3). This is similar to the work of Sanchez-Mata, *et al.* [28] who reported the reduction in ascorbic acid during the controlled atmosphere storage of green beans. The loss of ascorbic acid during storage was enhanced by the activity of ascorbate oxidase. The change in pH could indicate depletion of acids due to numerous reactions including utilization of acids in respiration. This might also be due to increase of senescence from the physiological stress created under high CO_2 concentrations.

Weight loss (4.99-7.25%) at the end of storage (Table 4) was below the critical level described for green beans. Green beans became unmarketable after 10-12% of their weight loss [3].

There was no significant difference ($p < 0.05$) in toughness of beans (Table 4) for all treatments. Use of blades for cutting in texture determination may not detect small changes in the texture of green beans. The use of other measuring techniques and probes could be taken into consideration for green beans kept in various atmospheric conditions.

The findings indicated that reduced oxygen (2% O_2) combined with normal CO_2 had beneficial effects on reducing physiological respiration and retaining bean qualities. Increase of CO_2 concentration was not effective because it caused undesirable browning and loss of quality. This gas combination is therefore recommended for modified atmosphere packaging of green beans stored at 8°C for 21 days.

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References

[1] USDA. (2017). *FoodData Central, Green bean (branded).* United States Department of Agriculture, Agricultural Research Service. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/372425/nutrients

[2] Sánchez-Mata, M.C., Cámara, M., and Díez-Marqués, C. (2003a). Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.) by controlled atmosphere storage: Macronutrients. *Food Chemistry. 80*(3), 309-315.

[3] Cantwell, M., and Suslow, T. (1998). *Bean, snap-recommendations for maintaining post-harvest quality.* Retrieved from http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English/?uid=3&ds=799

[4] Kader, A.A. (1986). Effects of postharvest handling procedures on tomato quality. *Acta Horticulturae. 190*, 209-221.

[5] Saquet, A. A., and Streif, J. (2017). Respiration rate and ethylene metabolism of 'Jonagold' apple and 'Conference' pear under regular air and controlled atmosphere. *Bragantia*, *Campinas. 76*(2), 335-344.

[6] Buescher, R., and Henderson, J. (1977). Reducing discoloration and quality deterioration in snap beans (*Phaseolus vulgaris*) by atmospheres enriched with CO_2. *Acta Horticulturae. 62*, 55-60.

[7] Cano, M.P., Monreal, M., de Ancos, B., and Alique, R. (1998). Effects of oxygen levels on pigment concentrations in cold-stored green beans (*Phaseolus vulgaris* L. Cv. Perona). *Journal of Agricultural and Food Chemistry. 46*(10), 4164-4170.

[8] Groeschel, E., Nelson, A., and Steinberg, M. (1966). Changes in color and other characteristics of green beans stored in controlled refrigerated atmospheres. *Journal of Food Science. 31*(4), 488-496.

[9] Khan, M.R., Suwanamornlert, P., Leelaphiwat, P., Chinsirikul, W., and Chohenchob, V. (2017). Quality and biochemical changes of longan (*Dimocarpus longan* Lour cv. Daw) fruit under controlled atmosphere conditions. *International Journal of Food science and Technology. 52*(10), 2163-2170.

[10] Kasim, R., and Kasim, M.U. (2015). Biochemical changes and color properties of fresh-cut green bean (*Phaseolus vulgaris* L. Cv. Gina) treated with calcium chloride during storage. *Food Science and Technology. 35*(2), 266-272.

[11] Leadley, C., Tucker, G., and Fryer, P. (2008). A comparative study of high pressure sterilization and conventional thermal sterilization: Quality effects in green beans. *Innovative Food Science & Emerging Technologies. 9*(1), 70-79.

[12] Lucera, A., Conte, A., and Del Nobile, M.A. (2011). Shelf life of fresh-cut green beans as affected by packaging systems. *International Journal of Food Science & Technology. 46*(11), 2351-2357.

[13] Kader, A.A., and Mikal, S. (2003). Respiration and gas exchange. In Bartz, J.A., and Brecht, J.K. eds. *Postharvest Physiology and Pathology of Vegetables*. Marcel Dekker, Inc., New York. pp. 7-31.

[14] Guo, L., Ma, Y., Sun, D.W., and Wang, P. (2008). Effects of controlled freezing-point storage at $0^{\circ}C$ on quality of green bean as compared with cold and room-temperature storages. *Journal of Food Engineering. 86*(1), 25-29.

[15] Watada, A.E., and Morris, L.L. (1967). Growth and respiration patterns of snap bean fruits. *Plant Physiology. 42*(6), 757-761.

[16] Rao, C.G. (2015). Postharvest physiology of fruits and vegetables. In Gopala, R.C. ed. *Engineering for Fruits and Vegetables*. Academic Press, London, UK. pp. 13-38.

[17] Saquet, A.A., and Streif, J. (2008). Fermentative metabolism in "Jonagold" apples under controlled atmosphere storage. *European Journal of Horticultural Science. 73*(1), 43-46.

[18] Kader, A.A. (2003). Physiology of CA treated produce. In International Controlled Atmosphere Research Conference. *Acta Horticulturae. 600*, 349-354.

[19] Pariasca, J.A., Miyazaki, T., Hisaka, H., Nakagawa, H., and Sato, T. (2001). Effect of modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. Var. Saccharatum). *Postharvest Biology and Technology. 21*(2), 213-223.

[20] Argüello Hernández, P.F., Ayuso Yuste, M.C., González Gómez, D., Bohoyo Gil, D., Delgado Adámez, J., and Bernalte Garcia, M.J. (2017). Behavior of fresh cut broccoli under different modified atmosphere conditions. *Emirates Journal of Food and Agriculture*. *29*(3), 188-197.

[21] Sambath, S., Wills, R., Ku, V., and Newman, S. (2018). Retention of green colour of tomatoes marketed as a green vegetable at ambient conditions in Cambodia with modified atmosphere storage and fumigation with 1-methylcyclopropene (1-MCP). *Fruits*. *73*(5), 265-272.

[22] Fang, Z., Bouwkamp, J.C., and Solomos, T. (1997). Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant wild type of *Phaseolus vulgaris* L. *Journal of Experimental Botany. 49*(320), 503-510.

[23] Hirschler, R. (2012). Whiteness, yellowness and browning in food colorimetry: A critical review, In Caivano, J.L., and Buera, M.d.P. eds. *Color in food: Technological and Psychophysical Aspects*. CRC Press, Boca Raton. p. 478.

[24] Costa, M., Brecht, J., Sargent, S., and Huber, D. (1994). Tolerance of snap beans to elevated CO_2 levels. In *Florida State Horticulture Society. 107*, 271-273.

[25] Varoquax, P., Mazollier, J., and Albagnac, G. (1996). The influence of raw material characteristics on the storage life of fresh-cut butter head lettuce. *Postharvest Biology and Technology. 9*(2), 127-139.

[26] Veltman, R.H., Lentheric, I., Van der Plas, L.H.W., and Peppelenbos, H.W. (2003). Internal browning in pear fruit (*Pyrus communis* L. Cv conference) may be a result of a limited availability of energy and antioxidants. *Postharvest Biology and Technology. 28*(2), 295-302.

[27] Bodelón, O.G., Blanch, M., Sanchez-Ballesta, M.T., Escribano, M.I., and Merodio, C. (2010). The effects of high CO_2 levels on anthocyanin composition, antioxidant activity and soluble sugar content of strawberries stored at low non-freezing temperature. *Food Chemistry. 122*(3), 673-678.

[28] Sánchez-Mata, M.C., Cámara, M., and Díez-Marqués, C. (2003b). Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: Micronutrients. *Food Chemistry. 80*(3), 317-322.