

Role of Gases Environment in Short and Long Term Banana Storage

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Bananas begin ripening processes as soon as they are harvested and after ripening they have a relatively short post harvest life, so some time they cannot reach to the ultimate consumers within the specific period and heavy losses occur. A reduction in O₂ and an increase in CO₂ have been shown to reduce the respiration rate and extend the pre-climacteric life of fruit. Therefore, this study was carried out to investigate the effect of low O₂ and increased CO₂ storage on the ripening behaviour and quality of banana fruit. Low O₂ and elevated CO₂ storage markedly enhanced the pre-climacteric life of bananas possibly by suppressing ethylene synthesis and respiratory metabolism. Bananas stored in controlled atmosphere retained their firmness and freshness better than those held in air which is beneficial in reducing the mechanical damage and bruising during shipment. The residual effect of controlled atmosphere storage on delayed ripening is diminished when treated with ethylene. Controlled atmosphere storage was found beneficial to alleviate the problem of Under-Peel Discolouration (UPD) in banana fruit for long term storage. There was a little difference in quality of the ripe fruit after storage in a wide range of O₂ and CO₂ levels. Storage of bananas at 4 % O₂ along with 5 % CO₂ is recommended for controlled atmosphere storage of pre-climacteric bananas to attain the good quality of ripe fruit.

Key Words: Quality, Residual, Ripening, Synthesis, Bruising, Elevated, Ethylene.

INTRODUCTION

The maintenance of fruit quality for a specific period of time before its consumption is an important factor in the postharvest storage of fruits. A major aim of commercial handling of bananas, as with most climacteric fruit, is to keep them unripe throughout a large part of the storage and marketing chain as such fruit is more resistant to physical damage and microbial attack¹. There are various recommendations in the literature for optimum levels of CO₂ and O₂ for banana storage² but

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most commercial practice uses 12 to 16 °C with 2 to 5 % CO₂ and 2 to 5 % O₂³. Pre-climacteric bananas exposed to low O₂ took longer to ripen when subsequently exposed to air than fruits kept in air for the whole period⁴. However, in simulated transport trials, found that controlled atmosphere stored bananas had higher firmness and lower soluble solids content for the first 3 d during subsequent ripening with no effect on peel colour. All differences disappeared as fruit reached eating ripeness⁵.

Little information is available in the literature where efforts have been made to determine the effects of controlled atmospheres on ripening bananas. Ripening of bananas, which had already been initiated by exogenous ethylene, was slowed in low O₂ atmospheres compared to air⁶. Bananas which had been initiated to ripen by exposure to exogenous ethylene and then immediately stored in 1 % O₂ at 14 °C remained firm and green for a 28 d storage period but then initiated to ripen almost immediately when transferred to air at 21 °C⁷. However, it was found that packing bananas in polyethylene film bags (0.03 mm) at 20 °C resulted in fermentation flavour when ripe⁸.

Packaging banana as individual hands in polyethylene film (0.075 mm) bags of 1:1 surface to weight ratio (cm² g⁻¹) with 50 mL of saturated potassium permanganate absorbed into suitable porous matrices could be recommended to increase storage life, but in their experiments they removed the fruit from the bags for final ripening⁹. It is also reported that ethylene treated fruit could be ripened in plastic film, but at a higher temperature (18 °C) the fruit showed signs of fermentation⁹.

Therefore this study was carried out to explore whether the ripening processes which had been depressed in reduced O₂ and increased CO₂ for short/long term storage could be initiated with ethylene treatment and could produce as good quality ripe fruit as a control.

EXPERIMENTAL

Three independent experiments were conducted at Post Harvest Laboratory, Cranfield University, Silsoe College, UK.

Experiment-1: Pre-climacteric Cavendish bananas were obtained from Wilkinson, Bedford. The fruit was stored in 2 % O₂ with 0, 3, 5 and 7 % levels of CO₂ and compared to fruit stored at 21 % O₂ and 0 % CO₂ levels. Healthy banana hands having 8 fingers were selected and marked according to the treatments. The fruits were stored with the required gas mixture of CO₂ and O₂ in closed system inside the controlled temperature rooms. The closed system was consisted of an air tight plastic containers (Model C217, Mailbox International Ltd, Cheshire, UK) of each 75 L capacity. Each container had one inlet and one outlet tube. The tip of the outlets was immersed in water to prevent back flow into the container. The inlets tubes were connected to channel the gas distributor (Mercury, UK serial No. ss13306) by PVC tubing of 6.5 mm internal diameter. The gas distributor was connected to a computer programmed gas blender (Signal Instrument Co. Ltd. Surrey, UK 850 series) which was connected to compressed oxygen and carbon dioxide with a nitrogen

generator (Bolton 75-72). Times and in travels used in each particular controlled atmosphere are specified under as in each experiment. Controlled atmosphere combination between oxygen and carbon dioxide were made according to the gas concentration needed in each experiment. The gas output from the gas blender and controlled atmosphere storage containers was analyzed regularly for oxygen and carbon dioxide levels using an oxystate 2 fruit store analyzer fitted with an infrared gas analyzer and a paramagnetic oxygen analyzer (David Bishop Instrument Sussex, UK type 770). The inlet and outlet gases were analyzed each day in order to check the O₂ and CO₂ levels.

After, 3 weeks fruits were removed from the storage and treated with 1000 ppm ethylene for 24 h to initiate the ripening and then stored at 16 ± 1 °C ambient storage environment for ripening. The experiment was conducted with 4 replications. Fruits were analyzed when they reached colour score 6 (fully yellow). Ripening of banana fruit was assessed according to peel colour changes compared to a colour chart². The quality of ripe fruit was assessed when they reached at colour stage 6.

The peel colour was measured by colorimeter (Minolta Model (CR-200/CR-200)). Results were recorded in a* and b* values. A positive a* corresponding to the degree of redness and negative value corresponds to the degree of greenness. A positive b* value represents the degree of yellowness and negative one represents the degree the blueness. Peel firmness was measured using an Instron Universal Testing Machine (model 2211) with a 8 mm cylindrical probe. Total soluble solids percentage was measured using a refractometer (Atago Co. Ltd refractometer PR-1).

Individual fruit was weighed before storage and then reweighed at score 6 (fully ripe) and then cumulative weight loss percentage was calculated.

Experiment-2 and 3: Pre-climacteric Cavendish bananas from St. Lucia were obtained from Wilkinson, Bedford, UK. A range of O₂ (1, 2 and 4 %) and CO₂ (5, 7 and 9 %) levels were investigated and compared to the fruit stored at 21 % O₂ and 0 % CO₂ as a control. The fruit was stored in controlled atmosphere storage for 3 week (experiment 2) and 3 month (experiment 3) in a cold room at 14 ± 1 °C. The actual gas levels passing through the controlled atmosphere channels were constantly measured. The actual levels of O₂ and CO₂ were as below:

Set gas mixture values	Actual gas mixture values Experiment 2	Actual gas mixture values Experiment 3
1 % O ₂ + 5 % CO ₂	1.1 % O ₂ + 5.3 % CO ₂	1.3 % O ₂ + 5.2 % CO ₂
1 % O ₂ + 7 % CO ₂	1.0 % O ₂ + 7.3 % CO ₂	1.3 % O ₂ + 7.3 % CO ₂
1 % O ₂ + 9 % CO ₂	1.2 % O ₂ + 9.5 % CO ₂	1.3 % O ₂ + 9.5 % CO ₂
2 % O ₂ + 5 % CO ₂	2.0 % O ₂ + 5.5 % CO ₂	2.3 % O ₂ + 5.3 % CO ₂
2 % O ₂ + 7 % CO ₂	2.0 % O ₂ + 7.4 % CO ₂	2.4 % O ₂ + 7.4 % CO ₂
2 % O ₂ + 9 % CO ₂	2.0 % O ₂ + 9.5 % CO ₂	2.4 % O ₂ + 9.4 % CO ₂
4 % O ₂ + 5 % CO ₂	4.0 % O ₂ + 5.5 % CO ₂	4.2 % O ₂ + 5.4 % CO ₂
4 % O ₂ + 7 % CO ₂	4.1 % O ₂ + 7.4 % CO ₂	4.3 % O ₂ + 7.4 % CO ₂
4 % O ₂ + 9 % CO ₂	4.1 % O ₂ + 9.4 % CO ₂	4.2 % O ₂ + 9.5 % CO ₂
21 % O ₂ + 0 % CO ₂	22.1 % O ₂ + 0.1 % CO ₂	21.4 % O ₂ + 0.2 % CO ₂

After, 3 weeks (second experiment) and 3 months (third experiment) fruits were removed from the storage and treated with 1000 ppm ethylene for 24 h to initiate the ripening and then stored at 16 °C in normal air for ripening as in first experiment. All quality parameters were assessed at colour stage 6 as in first experiment. In these experiments one extra quality pear meter (sensory evaluations) was also carried out to test the quality of ripe fruit. A panel of 8 assessors was selected from the college and the tests involved individual in isolated tasting conditions under a standard light source. Judges were asked to assess pulp flavour, sweetness, off flavour, astringency and acceptance on the 5 pointed scale as follows: 1 = Low, 2 = Moderate, 3 = Moderate to high, 4 = Good/High, 5 = Very good/very high.

The averages were calculated and presented in the form of tables. Data were processed and analyzed of variances (ANOVA) was carried out based on Randomized Completely Designs (RCD) using MSTATE, a PC based programme. LSD at $p = 0.05$ was used to test for significant differences of results where applicable.

RESULTS AND DISCUSSION

Storage life (days required to reach colour stage 6): Bananas treated with exogenous ethylene after removal from the controlled atmosphere system and those held as a control ripened simultaneously. All bananas took 9 d to reach colour stage 6 after removal from controlled atmosphere storage in both short term experiments. In long term storage (experiment 3) controlled fruit ripened after 29 d and all quality parameters were assessed at that time. The fruit which were held in controlled atmosphere storage removed from storage after 3 months and reached colour stage 6 after 7 days of ethylene treatments.

The green life of bananas was markedly enhanced when stored under reduced O_2 or elevated CO_2 atmospheres. This effect could possibly be due to suppressing ethylene biosynthesis. These findings are similar to the findings of other workers¹⁰ who mentioned that lowering the O_2 concentration reduced the rate of ethylene production and delayed ripening. It has long been established that reduced O_2 concentration and increased CO_2 concentration in the surrounding atmosphere of bananas can reduce the respiratory rate and increase the storage life or the time to the onset of ripening¹¹. In the present investigations, controlled atmosphere storage substantially increased the green life of bananas from less than 1 month to over 3 months. The current investigations confirm the work of¹¹ who showed that banana ripening was at half the rate at 5 % CO_2 storage relative to air but in that work no ethylene pre-treatment was involved, as in the current investigations. Low O_2 reduces ethylene production by inhibiting activities of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and ACC synthase¹². Elevated CO_2 levels are shown to inhibit the activity of ACC synthase, while ACC oxidase activity is stimulated at low CO_2 levels and inhibited at high CO_2 levels¹³.

One would expect the delayed ripening effect on bananas stored under controlled atmosphere conditions for a comparatively longer period to have a residual effect

after removal from controlled atmosphere storage. This might be true for those that were not treated with ethylene after controlled atmosphere storage. But in the current investigations, it is interesting to note that bananas which were removed after 3 months controlled atmosphere storage took only seven days to reach colour stage 6, compared to 9 days for bananas, in an earlier experiment, stored for three weeks in controlled atmosphere storage. The faster ripening processes of these bananas suggests that the initial metabolic events which precede the production of ethylene required for ripening took place during the longer term storage, but the final steps involving production of ethylene were inhibited.

Weight loss (%) during controlled atmosphere storage: Control fruit held in 21 % O₂ with 0 % CO₂ showed significantly more weight loss than those held in different controlled atmosphere conditions (Tables 1 and 2). It was observed that weight losses of bananas during controlled atmosphere storage decreased with an increase in CO₂ or decrease in O₂ concentrations in the storage atmosphere. There was a trend to increase weight loss when the O₂ level was higher and the CO₂ level lower, which would be accounted for by a lower respiration rate. Same trend of weight loss was found in experiment 2 and 3.

TABLE-1
EFFECT OF DIFFERENT CONCENTRATIONS OF CO₂ ON THE QUALITY OF
BANANA FRUIT IN SORT TERM STORAGE (EXPERIMENT 1)

Treatments	Weight loss % during storage	Weight loss % during ripening	Peel colour		Firmness (N)	TSS (%)	Starch (%)
			a Green	b Yellow			
2 % O ₂ + 0 % CO ₂	1.21	3.29	-1.98	+49.74	2.45	20.95	15.00
2 % O ₂ + 3 % CO ₂	1.06	3.05	-1.98	+49.58	2.53	21.00	16.67
2 % O ₂ + 5 % CO ₂	1.06	2.98	-1.92	+49.65	2.86	21.38	15.83
2 % O ₂ + 6 % CO ₂	0.97	3.05	-1.94	+49.90	3.07	21.40	16.67
21 % O ₂ + 0 % CO ₂	1.88	2.93	-1.87	+49.01	2.05	20.65	18.30
LSD (P=0.05)	0.08	0.14	NS	NS	0.68	NS	NS
CV %	5	4	18	3	6	4	16

TABLE-2
EFFECT OF DIFFERENT CONCENTRATION OF GASES ON WEIGHT LOSS %
DURING STORAGE AND RIPENING IN BANANAS (EXPERIMENT 2 and 3)

	Weight loss % during storage						Weight loss % during ripening					
	Short term			Long term			Short term			Long term		
	CO ₂ %			CO ₂ %			CO ₂ %			CO ₂ %		
	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %
1 % O ₂	2.46	2.41	2.27	10.58	10.46	10.39	2.21	2.40	2.53	3.32	3.80	4.10
2 % O ₂	2.63	2.49	2.37	10.78	10.69	10.59	2.23	2.36	2.47	3.33	3.43	3.76
4 % O ₂	2.79	2.71	2.61	10.95	10.89	10.76	2.34	2.43	2.45	3.29	3.36	3.49
21 % O ₂ + 0 % CO ₂	3.36			3.84			2.15			-		
LSD P = 0.05	0.21			0.60			0.27			0.38		
CV %	6.00			5.00			9.00			8.00		

Weight loss during ripening after controlled atmosphere storage: Bananas held in lower O₂ and higher CO₂ conditions showed higher weight losses than those held in air or in comparatively higher O₂ and lower CO₂ concentrations in the storage atmosphere. The situation was the reverse when bananas were removed from controlled atmosphere storage and kept in normal atmosphere for ripening. This could be due to the fact that respiration rate of these bananas remained suppressed under controlled atmosphere conditions. When they were moved to normal air, they respired faster and lost more weight than those held in normal air. It has earlier been suggested that suppression of respiration by high CO₂ in crops producing ethylene might occur mainly because of inhibition of the action or synthesis of ethylene¹⁴.

Fruit colour: Greenness (a*) and yellowness (b*) of peel colour of bananas held in different CA conditions and those held as a control in 21 % O₂ with 0 % CO₂ were not significantly different (P = 0.05), showing that bananas were at same stage of ripeness when subjected to the analysis (Tables 1 and 3). The control fruits in the long-term storage experiment showed significantly lower yellowness of peel colour but these are not directly comparable with those stored in CA conditions as the control fruit was ripened at 14 °C during storage. These bananas also showed under peel discolouration (UPD). These findings are in agreement with those¹⁵ reported that chilling symptoms of Lacatan bananas were reduced when stored in low O₂ levels of 3 to 4 % and CO₂ levels of 0 to 5 % in high relative humidity (80 to 100 %).

TABLE-3
EFFECT OF DIFFERENT CONCENTRATION OF GASES ON PEEL COLOUR IN
SHORT TERM AND LONG TERM STORAGE OF BANANA FRUIT

	Peel colour a* values (-) greenness						Peel colour b* values (+) yellow					
	Short term			Long term			Short term			Long term		
	CO ₂ %			CO ₂ %			CO ₂ %			CO ₂ %		
	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %
1 % O ₂	4.7	4.8	4.9	2.8	3.4	3.3	48.6	48.5	45.6	52.5	53.7	53.6
2 % O ₂	4.6	4.7	4.7	2.5	2.5	2.5	50.8	49.2	48.0	54.5	53.1	54.1
4 % O ₂	4.3	4.4	4.4	2.9	2.4	2.5	48.8	47.0	47.1	49.7	51.9	53.0
21 % O ₂ + 0 % CO ₂	3.9			3.0			48.0			46.9		
LSD P = 0.05	NS			NS			NS			2.85		
CV %	11			19			7			4.00		

Fruit firmness: Controlled atmosphere treatments significantly (P = 0.05) affected the firmness of bananas compared with those held in air as a control (Tables 1 and 4). Bananas held in controlled atmosphere conditions showed similar peel firmness except those held in 4 % O₂ with 5 % CO₂ and were firmer than those held in air as a control. Firm ripe bananas are believed to be less susceptible to mechanical injury and fungal infection¹⁶ thus the controlled atmosphere storage of fruit would be beneficial in terms of maintaining quality and freshness. The superiority of controlled atmosphere stored bananas over control fruit in retaining firmness and freshness could probably be due to the reduced weight losses of these bananas but other factors

TABLE-4
EFFECT OF DIFFERENT CONCENTRATION OF GASES ON PEEL FIRMNESS AND TSS
OF SHORT TERM AND LONG TERM STORAGE BANANAS (EXPERIMENT 2&3)

	Peel firmness (N)						TSS %					
	Short term			Long term			Short term			Long term		
	CO ₂ %			CO ₂ %			CO ₂ %			CO ₂ %		
	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %
1 % O ₂	3.02	3.20	3.03	5.03	4.28	4.08	22.9	22.7	21.8	20.8	20.5	20.4
2 % O ₂	2.95	3.07	3.14	4.93	4.80	4.58	21.9	22.2	22.4	21.6	21.0	20.3
4 % O ₂	2.81	2.93	3.14	4.10	3.80	4.00	21.2	21.6	22.4	21.8	22.0	21.2
21 % O ₂ + 0 % CO ₂	2.67			4.08			23.1			19.7		
LSD P = 0.05	0.30			0.29			NS			1.0		
CV %	8.00			5.00			5.0			4.0		

might be involved as can be seen in the case of long-term stored bananas. Some biochemical changes such as enzymatic breakdown of protopectin could be involved in softening of fruits¹⁷. Both O₂ and CO₂ levels, in the case of long term controlled atmosphere storage, showed an interaction in terms of firmness. This suggests that lower O₂ and increasing CO₂ levels in the storage atmosphere increased the fruit firmness and that the firmness was inter-related with the concentrations of both O₂ and CO₂. Although weight losses were high in bananas stored for three months, they were still firmer than the control when ripe. This might be due to increased compactness of peel with increased resistance to rupture of the peel surface. This compactness of peel was also observed by the taste panellists while peeling the bananas although they were softer to touch. While working on pomarac (*Eugenia* sp.) that the increase in fruit firmness during storage is not a normal behaviour of many fruits¹⁹. They attributed this increase to the drying and toughening of the skin as the fruit lost moisture. The current investigations support their interpretation.

Total soluble solids (TSS %): Bananas which were held in different controlled atmosphere conditions compared with those held as a control showed significantly (P = 0.05) similar TSS percentages in short term storage. However, bananas held in air as a control showed higher TSS contents than those held in controlled atmosphere conditions. The lower TSS contents (long term storage, Table-4) and higher off-odours and astringency (Table-6) scores were observed for bananas kept in 1 % O₂ with 5 to 9 % CO₂ and for those kept in 2 % O₂ with 9 % CO₂. This favours the assumption that these bananas might have switched from aerobic to anaerobic respiration during such a long period of storage. It might be possible that some fermentative decarboxylation took place under high CO₂ and low O₂ and glycolysis might have taken place resulting in the formation of acetaldehyde and ethanol. In this case sugars would have been metabolized faster than under aerobic conditions because anaerobic respiration requires more sugar to produce the same amount of energy as aerobic respiration.

Sensory evaluations: Bananas held in 4 % O₂ with 5 or 7 % CO₂ and those held in 2 % O₂ with 5 % CO₂ received higher flavour ratings than other bananas.

Bananas held in 1 % O₂ with 5 or 7 % CO₂ received lower flavour ratings than other bananas (Tables 5 and 6).

TABLE-5
EFFECT OF DIFFERENT CONCENTRATION OF GASES ON SENSORY EVALUATION
OF SHORT TERM STORAGE BANANAS (EXPERIMENT 2)

	Flavour			Sweet ness			Astringency			Acceptability		
	CO ₂ %			CO ₂ %			CO ₂ %			CO ₂ %		
	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %
1 % O ₂	3.4	3.0	2.9	3.6	3.4	3.4	1.3	1.7	1.9	3.6	3.5	3.4
2 % O ₂	3.6	3.5	3.3	3.3	3.4	3.3	1.2	1.5	1.6	3.7	3.3	3.2
4 % O ₂	3.8	3.7	3.4	3.9	3.8	3.3	1.1	1.2	1.5	3.8	3.5	3.2
21 % O ₂ + 0 % CO ₂	3.3			3.4			1.0			3.6		
LSD P = 0.05	0.5			NS			0.6			0.4		
CV %	11			12			10			10		

TABLE-6
EFFECT OF DIFFERENT CONCENTRATION OF GASES ON SENSORY EVALUATION
OF LONG TERM STORAGE BANANAS (EXPERIMENT 3)

	Flavour			Sweet ness			Astringency			Acceptability		
	CO ₂ %			CO ₂ %			CO ₂ %			CO ₂ %		
	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %	5 %	7 %	9 %
1 % O ₂	2.5	2.4	2.3	2.9	3.1	3.1	1.3	1.5	1.8	2.8	2.5	2.4
2 % O ₂	3.2	3.0	2.5	3.3	3.0	2.9	1.1	1.2	1.2	3.3	3.0	2.7
4 % O ₂	3.3	3.2	2.8	3.3	3.1	3.1	1.0	1.0	1.2	3.5	3.0	2.8
21 % O ₂ + 0 % CO ₂	3.2			3.2			2.0			3.4		
LSD P = 0.05	0.3			NS			0.1			0.4		
CV %	8.0			8.0			9.0			10.0		

Taste panellists could not detect any significant ($p = 0.05$) difference in sweetness of bananas held in different controlled atmosphere conditions (Tables 5 and 6). However bananas held in 4 % O₂ with 5 % CO₂ received the highest score for sweetness. Regarding sensory evaluation, 4 % O₂ with 5 % CO₂ emerged as the optimal controlled atmosphere condition for both short and long term storage of bananas. This suggests that bananas under these controlled atmosphere conditions remained in an ideal condition and completed their ripening processes normally when removed from controlled atmosphere storage and treated with exogenous ethylene. It was not possible to make direct comparisons between controlled atmosphere stored fruits and controls since they ripened at different times. But it can be at least inferred that controlled atmosphere stored fruits were not worse than air stored fruit at colour stage 6. Higher astringency in the lower O₂ levels (1 or 2 %) independently or combined with higher CO₂ (7 to 9 %) could be due to the restricted oxidation of phenolic compounds and accumulation of ethanol and acetaldehydes¹⁹. This process is called fermentative decarboxylation. Astringency in bananas is caused by tannins or phenolic substances¹⁸. Bananas ripened after being held in 4 % O₂ with 5 to 7 % CO₂ were highly acceptable to the panellists because of their flavour and lower

astringency and off-odours. Bananas of all controlled atmosphere conditions stored for three weeks were acceptable to the taste panellists. Taste panellists gave the highest marks to the fruit which had been stored in 2 or 4 % O₂ combined with 5 or 7 % CO₂. Off-odours and astringency were the highest in fruit stored at the lower O₂ level.

Conclusion

It is concluded that low O₂ and elevated CO₂ storage markedly enhanced the pre-climacteric life of bananas possibly by suppressing ethylene synthesis and respiratory metabolism. Bananas stored in controlled atmosphere retained their firmness and freshness better than those held in air so could be beneficial in reducing the mechanical damage during shipment. Under-peeled discolouration during storage at 14 °C could be alleviated using controlled atmosphere techniques. The residual effect of controlled atmosphere storage on delayed ripening is diminished when treated with ethylene. There was a little difference in quality of the ripe fruit after storage in a wide range of O₂ and CO₂ levels. Storage of pre-climacteric bananas in 4 % O₂ along with 5 % CO₂ is recommended.

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