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Effect of Reduced O_2 and Increased CO_2 (Controlled Atmosphere Storage) on the Ripening and Quality of Ethylene Treated Banana Fruit

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ABSTRACT

The objective of this research was to investigate whether ripening processes in banana fruits, which initiated artificially could be altered or continued in the controlled atmosphere storage. It was observed that reduced O_2 and increased CO_2 inhibited the action of ethylene such that the ripening processes of ethylene treated bananas were delayed. The ripening delaying effects of low O_2 were markedly greater than those of high level of CO_2. Controlled atmosphere storage produced firmer bananas which could be beneficial in avoiding mechanical damage during transportation and also extend shelf life. Bananas stored at 4 and 6% O_2 with 4 and 6% CO_2 extended their storage life by 12 to 16 days beyond that of the control with good eating quality. This factor has important commercial implications as ripe bananas could be reached to the ultimate consumers within extended period. It makes a useful contribution to the wholesale distribution of bananas. The storage of bananas after ethylene treatment at 2% O_2 extended the storage life up to 25 days beyond those of the control but there was some discoloration when they were transferred to normal air.

Key Words: Ethylene; Shelf life; CA storage; Banana

INTRODUCTION

Controlled atmosphere (CA) storage is the precise control of oxygen and carbon dioxide concentrations usually with a decrease in oxygen and an increase in the carbon dioxide to extend the storage life of produce (Wills *et al.,* 1981). A reduction in O_2 and an increase in CO_2 have been shown to reduce the respiration rate and extend the storage life of fruit, but there are limits to these effects (Mapson & Robinson, 1966; McGlasson & Wills, 1972). It has also been established that low O_2 levels affect the biochemical processes by depressing enzyme activities (Kanellis & Solomos, 1985). Ethylene has a link with respiratory activity of fruit tissues and also alters the onset of ripening (Burg & Burg, 1965).

Banana fruit is one of the most important fruit crops of the world and has a special place in human diet. It is also estimated that 100 million people subsist on banana as their main energy source (Rowe, 1981). Commercial ripening of bananas is initiated with exogenous ethylene treatment and these bananas can survive maximum of one week. In this short period these bananas can not reach the ultimate consumers so these become over ripe at the outlets and heavy losses occur.

CA storage conditions inhibit ethylene production and retards the rate of banana ripening (Quazi & Freebrain, 1971). However, care has to be taken because low oxygen can cause fermentation/ anaerobic respiration. Therefore, it is necessary to formulate different combinations of these two gases in order to establish the best combinations for delayed ripening of banana fruit, while allowing them to subsequently ripen to a good quality. Therefore, this research was carried out to find out the best combination of gases to extend the shelf life with good eating quality.

MATERIALS AND METHODS

This research was conducted in post-harvest laboratory of Cranfield University at Silso College UK in 1998. Pre-climacteric bananas were treated with 1000 ppm ethylene for 24 h then stored in 2, 4 and 6% O_2 with 4, 6 and 8% CO_2 and compared with the fruits stored at 21% O_2 + 0% CO_2 (control) at 16°C for ripening.

The closed system consisted of airtight plastic containers (Model C217, Mailbox International Ltd, Cheshire, UK) each of 75 litres 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 inlet tubes were connected to the channels of 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 (Balton 75-72). Controlled atmosphere combinations between O_2 and CO_2 were made according to the gas concentration needed. The gas output from the gas blender and controlled atmosphere storage containers was analysed regularly for O_2 and CO_2 levels using an Oxystat 2 Fruit Store Analyser fitted with an Infra Red Gas Analyser and a paramagnetic Oxygen Analyser (David Bishop Instrument Sussex, UK type 770). The actual gas mixtures during storage were as follows:

1. 1.7% O_2 + 4.2% CO_2
2. 1.8% O_2 + 6.0% CO_2
3. 1.8% O_2 + 8.1% CO_2
4. 3.9% O_2 + 4.2% CO_2
5. 3.9% O_2 + 6.1% CO_2
6. 3.9% O_2 + 8.2% CO_2
7. 6.0% O_2 + 4.2% CO_2
8. 6.0% O_2 + 6.1% CO_2
9. 6.0% O_2 + 7.9% CO_2
10. 21.5 %O_2 + 0.0 CO_2

The experiment was conducted with four replications. Fruits were analysed when they reached colour score 6.

Assessment of fruit ripening. Ripening of banana fruits was assessed according to peel colour changes compared with a colour chart as described by Stover and Simmonds (1987).

Assessment of fruit quality. The quality of ripe fruit was assessed by the following two methods:

Objective methods. The peel colour was measured by colorimeter (Minolta Model CR-200/CR-200b). Results were recorded in, a* and b* values. A positive a* value corresponding to the degree of redness while a negative value corresponds to the degree of greenness. A positive b* value represents the degree of yellowness and the negative one represents the blueness. Peel and pulp firmness was measured using an Instron Universal Testing Machine (model 2211) with an 8 mm cylindrical probe. Total soluble solids percentage was measured using a refractomter (Atago Co. Ltd, digital refractometer PR-1). Starch percentage was measured by using the technique recommended by Blankenship *et al.* (1993).

Individual fruit was weighed using a digital balance (precisa 6000 D) just before storage and then reweighed at score 6 (fully ripe) and then cumulative weight loss percentage was calculated as follows:

$$\text{Weight loss \%} = \frac{W_o - W_1}{W_o} \times 100$$

Where

W_0 = Original weight

W_1= Weight at sampling (when banana reached at colour score 6).

Weight loss percentage per day was calculated as follows:

$$\text{Weight loss percentage per day} = \frac{TWP}{SL}$$

Where

TWP= Total weight loss percentage at colour stage 6

SL= Storage life (total days when banana reached colour score 6 from pre-climacteric stage).

Pulp and peel were separated when banana reached colour stage 6. Peel and pulp were weighed individually and expressed as pulp peel ratio as follows:

$$Pulp\,/\,peel\ ratio = \frac{Pulp\ weight}{Peel\ weight}$$

Subjective assessments (sensory evaluation). The fruits were removed from storage when they were at colour score 6. A panel of eight assessors was selected from the college and the tests involved individual assessment in isolated tasting conditions under a standard light source. The judges were asked to assess pulp flavour, sweetness, off flavour, astringency and acceptance on five points scale as follows:

1 Low
2 Moderate
3 Moderate to high
4 Good /High
5 Very good /High

The scores marked by panellists were collected and an average was calculated for each parameter and sub-parameter. These averages were used for statistical analysis. Means of treatments were calculated and presented in the form of tables.

Statistical analysis. Data were processed and analysis of variance (ANOVA) was carried out based on completely randomised designs using MSTATC, a P.C. based programme. LSD at P= 0.05 was used to test for significant difference of results where applicable.

RESULTS AND DISCUSSION

Storage life (speed of ripening). Bananas stored at control (21% O_2 + 0% CO_2) ripened after nine days (Table I), whereas ripening was delayed in those stored at reduced O_2 and increased CO_2 storage. The delaying effect was evident at low O_2 levels but slightly more at high CO_2 levels. Bananas stored at 6% O_2 with 4, 6 and 8% CO_2 reached colour stage 6 at the same time but extended 12 days storage life as compared to those at control. Bananas stored at 4% O_2 with 6 and 8% CO_2 ripened at the same time but significantly later than those at 4% O_2 with 4% CO_2. These fruits (stored 4% O_2 with all combinations of CO_2) extended the storage life 16 days beyond that of the control. In contrast bananas stored at 2% O_2 with 4, 6 and 8% CO_2 ripened 19 to 25 days after control but they showed significant differences in their speed of ripening.

There are some ripening inhibitors, which inhibit the ethylene synthesis or ethylene action or lower the sensitivity of fruits to ethylene (Angelopoulos & Gavalas, 1991). Delayed ripening in ethylene treated bananas in reduced O_2 and increased CO_2 had the effect of an ethylene action inhibitor. The marked delay in the speed of ripening of fruit stored at low O_2 and the slight delay at high CO_2 indicates that low O_2 level had superseded increased CO_2 levels for the effect of ethylene action inhibition. Therefore, fruits stored in O_2 levels at 2 to 6% had extended storage life of 12 to 25 days more than those at control. In contrast all levels of CO_2 remained depressed at 6% O_2 while higher concentrations showed a slight response with 4% O_2 and

Table I. Effect of reduced O_2 and increased CO_2 on the speed of ripening and quality of banana fruit where ripening was initiated with 1000 ppm ethylene for 24 h followed by storage in different combinations of atmospheric gases at 16°C

Treatments	Storage life (day)	Weight loss %	Weight loss per day	Peel colour a* values	Peel colour b* values	Peel firmness (N)	Pulp firmness (N)	TSS (%)	Starch (%)
T1 (2% O_2+4% CO_2)	28.0	4.23	0.15	-6.00	+46.89	3.76	2.20	20.8	20
T2 (2% O_2+6% CO_2)	32.0	4.48	0.15	-6.36	+50.40	3.96	2.31	1.9	20
T3 (2% O_2+8% CO_2)	34.0	4.79	0.14	-6.77	+47.47	3.98	2.45	20.8	20
T4 (4% O_2+4% CO_2)	25.0	4.07	0.16	-4.32	+50.17	4.16	2.55	22.5	18
T5(4% O_2+6% CO_2)	25.5	4.15	0.16	-5.30	+49.95	3.92	2.33	21.8	19
T6 (4% O_2+8% CO_2)	25.5	4.11	0.16	-4.64	+49.94	3.72	2.14	21.6	19
T7 (6% O_2+4% CO_2)	21.0	3.80	0.18	-4.57	+48.25	3.37	2.04	21.0	14
T8 (6% O_2+6% CO_2)	21.0	3.78	0.18	-3.80	+48.52	3.39	2.15	21.3	15
T9 (6% O_2+8% CO_2)	21.0	3.66	0.17	-4.57	+48.16	3.48	2.18	21.0	16
T10 (21% O_2+0% CO_2)	9.0	2.21	0.24	-3.05	+49.77	3.02	1.99	22.4	10
LSD	0.34	0.09	0.003	0.14	0.80	0.18	0.09	0.34	0.41
CV	3.0%	4.9%	4.7%	6.2%	3.7%	10.5%	9.0%	3.4%	5.1%
Initial reading				-20.00	+33.47	40.0	18.1	3.4%	100%

Initial reading of different parameters at the start of experiment

strong response with 2% O_2. These observations have not previously been reported in the literature where there is interaction between O_2 and CO_2 on the speed of ripening of initiated climacteric fruit. Previous work has been on general storage of fruit probably to delay the initiation of ripening. The findings of Acedo and Bautista (1993) support the above results. They reported that fruit can be successfully stored under low O_2 conditions without raising the CO_2 in the atmosphere.

Weight loss percentage. The results are shown in Table I. Analyses of variance showed significant differences for weight loss percentage at the P=0.05 level. The total weight loss was greater in bananas which were stored at 2% O_2 with all combinations of CO_2 than those at other treatments. Bananas stored at control (21% O_2 + 0% CO_2) showed the highest weight loss per day, but their total weight loss during ripening was lowest. Fruits stored at higher levels of CO_2 showed lower weight loss than those at lower levels of CO_2 with each level of O_2. A positive correlation was found between total weight loss and the days taken to ripen because total weight loss increases with the length of storage.

Peel colour. The results are given in Table I. Analyses of variance showed significant differences for a* values (greenness) and non-significant differences for b* values (yellowness) at the P=0.05 level. Bananas stored at 2% O_2 were significantly greener than those at other treatments. Control bananas (21% O_2 + O% CO_2) were significantly less green than those of other treatments, while bananas ripened at higher levels of CO_2 were greener compared to those at lower levels of CO_2 with each level of O_2.

In terms of peel colour development greater a* values (greenness) in bananas which were stored at low O_2 levels indicated that decreased levels of O_2 and increased levels of CO_2 had the effect of inhibiting chlorophyll degradation. Therefore, processes related to chlorophyll degradation did not complete as fast as in higher levels of O_2. Knee (1980) reported that the chlorophyll breakdown was reduced clearly in reduced O_2.

Peel and Pulp firmness. The results are shown in Table I. The analyses of variance showed significant differences for peel firmness at the P=0.05 level. Bananas ripened at lower concentrations of O_2 were significantly firmer than those ripened at higher concentrations of O_2. Fruits ripened at control (21% O_2 + 0% CO_2) were significantly softer than those of other treatments followed by bananas ripened at 6% O_2 with 4, 6 and 8% CO_2. The results regarding pulp are also given in Table I. Statistical analysis showed significant differences for pulp firmness at the P= 0.05 level. The pulp firmness showed the same trend as in peel firmness. The pulp of control bananas was significantly softer than the pulp of other bananas at all other treatments. The overall situation showed similar results as in peel firmness with only minor differences.

The control of softening is important for the reduction of mechanical damage and in maintaining fruit quality. Bananas in CA storage showed that their ripening processes were dependent on the O_2 levels. Results showed similar total soluble solids to that in control but they were firmer than the control. This indicates that softening of bananas is not completely caused by starch hydrolysis. Therefore, in these cases some other mechanism must have been involved. This could be due to the reduced weight loss due to the reduced respiration or to lower enzyme activity. The same effect of low O_2 has also been found in tomatoes and apples (Kim & Hall, 1976; Knee, 1980) but they could not find the specific reason for this phenomenon.

It has previously been reported (Salunkhe *et al.,* 1985) that CA with high CO_2 inhibits the breakdown of pectic substances, retains fruit texture and remains firmer for a longer period. In the current research, this effect is most dominant at reduced O_2 levels but the effect of low O_2 cannot be separated from the effect of increased CO_2 (Weichmann, 1987). However, firmer ripe fruit is considered one of the benefits of the CA storage in order to reduce the mechanical damage, to avoid fungal infection and to increase the shelf life of ripe banana fruit.

Total soluble solids (TSS) and starch percentage. The results are presented in Table I. Analyses of variance showed significant differences at the P=0.05 level. The highest total soluble solids level was observed in bananas which were stored at 4% O_2 with 4% CO_2 which was statistically similar to those at 21% O_2 + 0% CO_2 (control). The overall situation showed that the highest level of total soluble solids was achieved by 4% O_2 with 4% CO_2 and the lowest with 2% O_2 with 6% CO_2. Statistical analysis also showed significant differences for starch percentage at the P= 0.05 level. The maximum starch percentage was observed in bananas which were ripened at 2% O_2 with 8, 6 and 4% CO_2. Control fruits showed significantly lower starch percentages than those in other treatments.

The significantly higher soluble solids in bananas at 6 and 4% O_2 than those at 2% O_2 indicated that processes regarding starch hydrolysis did not complete at 2% O_2 due to the inhibition effect of low O_2 against the processes of starch hydrolysis. The findings of Kanellis *et al.* (1989) support the results of this investigation. They reported that lower O_2 levels slowed the sugar accumulation and arrested the normal colour changes in bananas.

Sensory evaluation. The results are shown in Table II. Flavour, sweetness, astringency, off odours and acceptance showed significant results at the P=0.05 levels. Bananas ripened at control showed maximum flavour but they were statistically similar to those ripened at 4% O_2 + 6% CO_2 and 6% O_2 with 4, 6 and 8% CO_2. The lowest flavour was found in bananas at 2% O_2 with different levels of CO_2. The results of sweetness were similar to the results of flavour. Bananas ripened at lower level of O_2 (2%) had significantly greater astringency than those ripened at higher levels of O_2 (4 and 6%). CO_2 also produced significant effects in each treatment. Bananas stored at higher levels of CO_2 showed higher astringency than those at lower levels of CO_2. Off odours results were similar to those of astringency. Maximum off-odour was observed in bananas stored at 2% O_2 at all combinations of CO_2 and was lowest in bananas at control and at higher percentages of O_2. For acceptance, bananas stored at 4 and 6 O_2 with 4 and 6% CO_2 received statistically the same score as in the control.

The bananas stored in CA storage after ethylene treatment also showed similar eating quality except those which were stored at 2% O_2 with three combinations of CO_2. Maximum astringency and off flavour at 2% O_2 storage in fruits could be due to the lack of oxidation of phenolic compounds and the accumulation of ethanol and acetaldehyde (Ke & Kader, 1989). However, in spite of this, panellists gave good scores for acceptability. It is worth mentioning here that bananas which were ripened at 2% O_2 showed some discoloration or deterioration when removed to normal air. This might be due to the higher respiration rate of fruit in normal air such that the respiration of bananas at 2% O_2 remained low but increased rapidly when transferred to normal air. The enhanced entry of O_2 could oxidise the tannins, which then caused the discoloration of fruit. Therefore some disorders remained depressed during storage but appeared after a short period when removed to normal air. This could be a new disorder caused by low O_2. Further work is needed to explain the mechanism and provide further information.

Bananas stored in 4 and 6% O_2 with 4 and 6% CO_2 extended storage life by more than 12 to 16 days compared to those at control, with good eating quality. This factor could make a useful contribution to the wholesale distribution of bananas. This is because bananas are harvested at the pre-climacteric stage and shipped green to the distant wholesale market. Wholesale distributors initiate ripening of bananas with ethylene before sending them to retail outlets. These ethylene treated bananas can survive for a maximum of one week. Sometimes these bananas can not reach the ultimate consumers within the specific period so they become over ripe at the retail outlets and heavy losses occur. In earlier times, research workers tried to extend the pre-climacteric life, but unfortunately not much attention

Table II. Effect of reduced O_2 and increased CO_2 on the sensory evaluation parameters of banana fruit where ripening was initiated with 1000 ppm ethylene for 24 h followed by storage in different combinations of atmospheric gases at 16°C (where 5= maximum score (high or best), 1= minimum score (very low or nil)

Treatments	Flavour	Sweetness	Astringency	Off flavour	Acceptance
T1 (2% O_2+4% CO_2)	3.5	3.3	2.2	1.4	3.5
T2 (2% O_2+6% CO_2)	3.2	3.1	2.5	1.5	3.2
T3 (2% O_2+8% CO_2)	3.0	3.0	2.4	1.4	3.1
T4 (4% O_2+4% CO_2)	3.5	3.7	2.0	1.3	3.8
T5(4% O_2+6% CO_2)	4.0	3.8	2.0	1.4	4.0
T6 (4% O_2+8% CO_2)	3.7	3.8	2.0	1.5	4.0
T7 (6% O_2+4% CO_2)	4.1	4.0	1.3	1.0	4.1
T8 (6% O_2+6% CO_2)	4.0	4.0	1.5	1.0	4.1
T9 (6% O_2+8% CO_2)	4.0	4.0	1.8	1.1	3.9
T10 (21% O_2+0% CO_2)	4.1	4.1	1.0	1.0	4.1
LSD	0.2	0.17	0.18	0.06	0.15
CV	10.8%	9.1%	20.3%	11.5%	8.5%

has been paid to extending the climacteric life of banana fruit. The findings of the current investigation could be helpful in achieving this goal.

It would be beneficial for the wholesale distributors if they managed to initiate ripening at the production site or even during transport. Ripening could be initiated on the ship and they could save some packaging and handling charges, which are incurred between the production site and the wholesale market. By simplifying the transport route and eliminating the ripening room stage, they could also reduce the chances of mechanical injury during the transportation between production site and the wholesale market.

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