EFFECTS OF OXYGEN AND CARBON DIOXIDE ON RESPIRATION, STORAGE LIFE, AND ORGANIC ACIDS OF GREEN BANANAS

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Abstract

Green bananas were held in humidified gas streams comprising air (control); "high carbon dioxide" (A) (5% CO_2, 20% O_2, 75% N_2); "low oxygen" (B) (0% CO_2, 3%, O_2, 97% N_2); "high carbon dioxide–low oxygen" (C) (5% CO_2, 3% O_2, 92% N_2). Ripening in A, B, and C was delayed at least 2, 8, and 12 times respectively compared with air. These three gas streams also reduced the rates of oxygen uptake by the fruit but increased the total oxygen uptake over the period before the beginning of the respiratory climacteric.

In the first 4 days of treatment, A caused increases in pyruvate, oxaloacetate, 2-oxoglutarate, glyoxylate, glutamate, aspartate, citrate, and malate but not in succinate; B caused larger increases in the 2-oxo acids and decreases in the other acids; C caused smaller increases in pyruvate, 2-oxoglutarate, and glyoxylate, retarded the increase in oxaloacetate, and caused a further reduction in citrate, malate, and aspartate compared with B. The largest changes in the acids were found at 0–1 days.

Application of the crossover theorem of Chance to the data suggested that low oxygen limited the operation of the Krebs cycle between pyruvate and citrate, and 2-oxoglutarate and succinate. No control points for high carbon dioxide were apparent.

I. Introduction

Atmospheres containing higher carbon dioxide and lower oxygen concentrations than air can be used to delay the onset of ripening in banana fruit (Young, Romani, and Biale 1962; Mapson and Robinson 1966; Quazi and Freebairn 1970). Storage in such modified atmosphere is known as controlled atmosphere storage. The delay in ripening has been ascribed to reductions in respiration and ethylene production and to interactions between carbon dioxide, oxygen, and ethylene (Young, Romani, and Biale 1962; Mapson and Robinson 1966; Burg and Burg 1967; Quazi and Freebairn 1970). However, the mechanisms involved have not been resolved and little is known of the biochemical changes in bananas stored in controlled atmospheres. Studies with other plant tissues have shown that storage in controlled atmospheres can cause changes in the activity of specific enzymes and intermediates of the Krebs

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cycle (Hulme 1956; Dilley 1962; Young, Romani, and Biale 1962; Smith 1963; Kollas 1964; Williams and Patterson 1964; Frenkel 1966; Lebermann, Nelson, and Steinberg 1968).

In this work we examined the effects of increased carbon dioxide and reduced oxygen concentrations on oxygen uptake, time to ripen, and on changes in acids of the Krebs cycle and related compounds in green fruit. The analyses of the latter compounds were confined to the first 4 days of exposure of fruit to the atmospheres since preliminary experiments showed that most of the readjustments in respiration rates were completed within 1 day.

II. Materials and Methods

(a) *Source of Fruit*

Fruit of the Williams cultivar of the Intermediate Cavendish group were obtained from commercial plantations in New South Wales. In early experiments the fruit were dusted with sulphanilamide powder to minimize decay, while in later experiments they were dipped in an 0·1% w/v aqueous suspension of 2-(4′-thiazolyl)benzimidazole* containing 0·025% v/v wetting agent†.

(b) *Preparation of Gas Mixtures and Measurement of Respiration*

The following mixtures, containing four combinations of carbon dioxide and oxygen, were prepared by mixing humidified streams of air, carbon dioxide, and nitrogen metered by capillary type flowmeters:

Air (control)—0·03% CO_2, 21% O_2;

A, "high carbon dioxide"—5% CO_2, 20% O_2;

B, "low oxygen"—0% CO_2, 3% O_2;

C, "high carbon dioxide–low oxygen"—5% CO_2, 3% O_2.

Fruit used for the respiration measurements were enclosed singly in respiration jars while for the studies on acids, matched sets of six fruit were enclosed in jars. The ventilation rate was 1 litre/hour/fruit and all samples were held at 20°C.

The concentrations of carbon dioxide and oxygen were monitored respectively with an infrared gas analyser (model SB2, Grubb, Parsons, and Co. Ltd., England) and a paramagnetic oxygen analyser (D.C.L. Servomex Analyser, type 83, Servomex Controls Ltd., Crowborough, England). The latter instrument was also used for the respiration measurements.

(c) *Analytical Methods*

Each set of six fruit was frozen in liquid nitrogen and then powdered in a hammer mill at −20°C. The powdered tissue was stored at −80°C until extracted. The 2-oxo acids, pyruvate, oxaloacetate, 2-oxoglutarate, and glyoxylate, were determined by the method of Isherwood and Niavis (1956). This involved extracting samples with metaphosphoric acid at 0°C and separating the acids as the 2,4-dinitrophenylhydrazones by paper chromatography. The individual acids were measured colorimetrically using a Bausch and Lomb Spectronic 20. Samples (25 g) for the determination of non-volatile acids were killed in boiling 80% ethanol acidified with formic acid and extracted as described by McGlasson *et al.* (1971). The acidic fractions from these extracts, which included aspartic and glutamic acids, were separated by ion-exchange column chromatography and the amounts of each acid were measured by titration with 0·02N NaOH (Wills and McGlasson 1968).

* Marketed as Tecto 90^R by Merck, Sharpe, and Dohme (Australia) Pty. Ltd.

† Marketed as Citowett by B.A.S.F., Australia, Ltd.

III. Results

(a) *Respiration and Ripening*

The rates of oxygen uptake stabilized within 1 day after placing the fruit in the gas mixtures (Fig. 1) and remained steady until the onset of the climacteric rise.

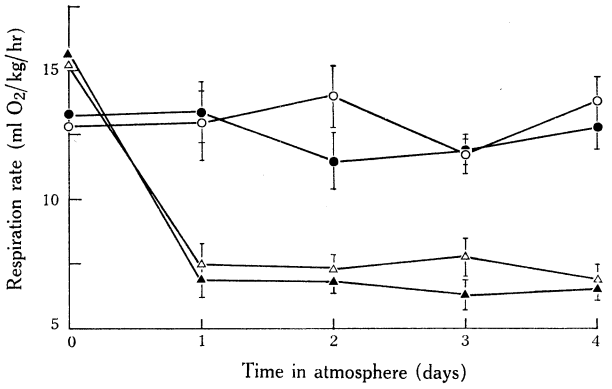

Fig. 1.—Rates of oxygen uptake by bulk samples of six fruits held in air (○), gas mixture A (●), gas mixture B (△), and gas mixture C (▲). The vertical bars represent estimates of the standard deviation of the population ($n' = 3$).

The time to ripen was increased by the modified atmospheres and the rates of oxygen uptake were considerably depressed (Table 1). In addition to the reduction in the

Table 1

Effects of modified atmospheres on respiration and storage life at 20°C*

The data are means for five single fruit except in gas mixture C for which the means for three fruits are shown. Total oxygen consumption in this mixture was calculated for 157 days

Gas mixture*	Time to climacteric (days)	Respiration rate (ml O_2/kg/hr)		Duration of climacteric rise (days)	Ratio of C to P	Total preclimacteric oxygen consumption (ml/kg)
		Preclimacteric (P)	Climacteric (C)			
Air	15·4	11·9	76·2	4	6·4	4,400
A	35·4	7·3	64·5	4	8·8	6,200
B	138	3·1	20·9	8	6·7	10,200
C	†	2·5	—	—	—	9,600

* See Section II.

† Individual fruits held for up to 182 days in this gas mixture did not commence to ripen until returned to air.

rates of oxygen uptake, the duration of the climacteric rise was extended by low oxygen. All fruit held in air, high carbon dioxide, and low oxygen ripened in the gas mixtures. No fruits ripened in the high carbon dioxide–low oxygen atmosphere, but fruit which were transferred to air at 116, 157, and 182 days ripened normally. The fruit transferred on day 116 began to ripen after 4 days and the other fruit began to

ripen immediately. Table 1 shows that total oxygen consumption by fruit before the onset of the climacteric was increased by the controlled atmospheres.

(*b*) *Effects of Controlled Atmospheres on Organic Acids*

Changes in the levels of nine acids during the first 4 days in the four atmospheres are shown in Figure 2 and Table 2. Low oxygen caused significant increases in pyruvate, oxaloacetate, 2-oxoglutarate, and glyoxylate compared with air and significant decreases in glutamate, aspartate, citrate, malate, and succinate. Maximum changes in the nine acids were found generally at 0–1 days.

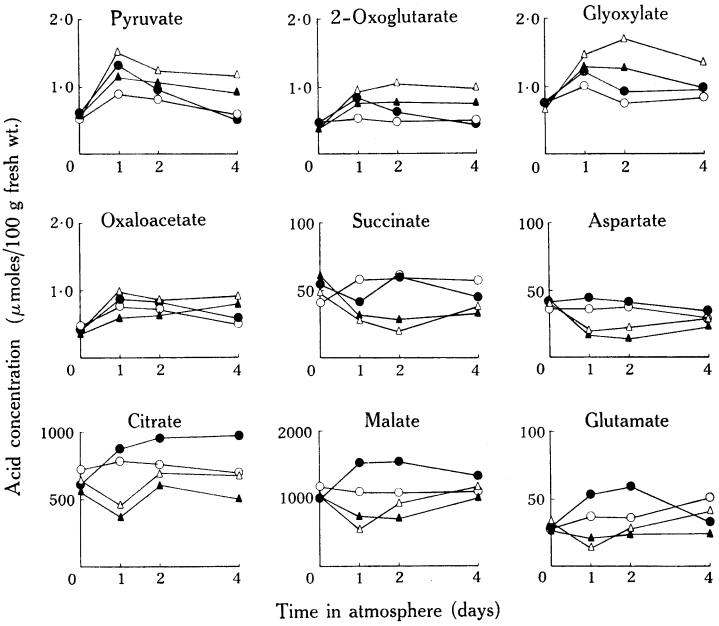

Fig. 2.—Changes in acids of the Krebs cycle and related compounds in bulk samples of six fruits ($n' = 3$) exposed to the controlled atmospheres listed in Figure 1. S.E. of values for days 1–4 (32 d.f.) are: pyruvate, ± 0·029; 2-oxoglutarate, ±0·018; glyoxylate, ±0·033; oxaloacetate, ±0·024; succinate, ±2·1; aspartate, ±2·0; citrate, ±30; malate, ±53; glutamate, ±1·9. Additional statistical information is given in Table 2.

The combination of high carbon dioxide with low oxygen also resulted in a significant increase in pyruvate, 2-oxoglutarate, and glyoxylate but the levels were significantly lower than in fruit held in low oxygen alone. Contrary to the effects on the other 2-oxo acids high carbon dioxide–low oxygen caused a slower build up of oxaloacetate than the other mixtures but by day 4 the level was significantly higher than in air or in high carbon dioxide, although lower than in low oxygen. The levels of citrate, malate, glutamate, and aspartate were further reduced by the combination of high carbon dioxide with low oxygen but the reduction in the level of succinate was less.

TABLE 2

CHANGES IN ACIDS OF THE KREBS CYCLE AND RELATED COMPOUNDS

Statistical information on data shown in Figure 2

Krebs cycle acid	Time in atmosphere (days)	Mean acid concentration (μmoles/100 g fresh wt.) 20% O_2	3% O_2	5% CO_2	0% CO_2	Overall (n' = 12)	Air	Mix. A (day 1–4, n' = 9)	Mix. B	Mix. C
			(n' = 6)							
Pyruvate	0	0·56	0·56	0·60	0·53	0·56				
	1	1·12	1·33	1·25	1·21	1·23				
	2	0·88	1·14	1·00	1·03	1·01	0·77	0·93	1·31	1·04
	4	0·55	1·04	0·71	0·88	0·79				
S.E.*			±0·021			±0·015		±0·017		
2-Oxoglutarate	0	0·47	0·37	0·42	0·42	0·42				
	1	0·69	0·84	0·79	0·73	0·76				
	2	0·54	0·90	0·68	0·75	0·72	0·50	0·62	0·98	0·75
	4	0·45	0·82	0·55	0·73	0·64				
S.E.*			±0·022			±0·016		±0·018		
Glyoxylate	0	0·77	0·70	0·76	0·71	0·73				
	1	1·05	1·37	1·26	1·16	1·21				
	2	0·83	1·49	1·09	1·23	1·16	0·82	1·03	1·50	1·17
	4	0·90	1·15	0·96	1·09	1·03				
S.E.*			±0·023			±0·017		±0·019		
Oxaloacetate	0	0·45	0·38	0·38	0·45	0·41				
	1	0·81	0·79	0·73	0·87	0·80				
	2	0·77	0·75	0·72	0·80	0·76	0·66	0·75	0·92	0·67
	4	0·53	0·85	0·68	0·70	0·69				
S.E.*			±0·017			±0·012		±0·014		
Succinate	0	47	55	57	45	51				
	1	49	30	36	42	39				
	2	59	24	44	39	42	58	48	28	31
	4	51	35	39	47	43				
S.E.*			±1·5			±1·0		±1·2		
Aspartate	0	39	42	42	38	40				
	1	40	17	29	28	29				
	2	39	18	27	29	28	34	39	23	17
	4	31	25	28	28	28				
S.E.*			±1·4			±1·0		±1·2		
Citrate	0	660	610	580	690	630				
	1	840	420	620	630	630				
	2	850	650	780	730	750	750	930	610	490
	4	820	590	730	680	710				
S.E.*			±15			±21		±17		
Malate	0	1090	1030	1010	1110	1060				
	1	1320	650	1130	840	980				
	2	1320	830	1140	1020	1080	1100	1980	090	820
	4	1230	1199	1190	1150	1170				
S.E.*			±37			±26		±36		
Glutamate	0	29	31	29	31	30				
	1	45	17	37	25	31				
	2	47	25	41	31	36	41	48	27	23
	4	41	32	28	46	37				
S.E.*			±1·3			±0·9		±1·1		

* 32 degrees of freedom.

High carbon dioxide caused significant increases in all of the acids except succinate. Maximum values were recorded at day 1 for pyruvate, oxaloacetate, 2-oxoglutarate, and glyoxylate, but by day 4 the levels had declined to those in air. The levels of citrate and malate reached maximum values on day 2 and at day 4 remained significantly higher than in fruit in any of the other mixtures. The pattern of changes in the levels of aspartate was similar to those of citrate and malate but glutamate, after reaching a maximum at day 2, decreased to a level well below that of the controls in air.

(c) *Effect of Time after Harvest on Organic Acids*

The fruit used in the analyses of acids reported above were held for 3 days in humidified air streams at 20°C to establish initial rates of respiration before the controlled atmosphere treatments were applied. The samples held continuously in air showed significant changes in the levels of pyruvate, oxalacetate, succinate, aspartate, and glutamate (Fig. 2). Most of these changes over the period which corresponded to days 3–7 after picking, probably represented metabolic readjustments following picking. An experiment was carried out to examine changes during the first 4 days after picking. The levels of 2-oxo acids were measured in samples immediately after picking and then daily until the fourth day after picking. The data (Table 3) confirmed that the amounts of the four acids increased with time after picking. Respiration rates fell steadily until about the third day after picking.

TABLE 3

CHANGES IN ORGANIC ACIDS AND RESPIRATION WITH TIME AFTER HARVEST

Krebs cycle acid	Acid concentration (μmoles/100 g fresh wt.) after:					S.E. of mean ($n' = 3$)
	0 days	1 day	2 days	3 days	4 days	
Pyruvate	0·34	0·36	0·42	0·41	0·60	±0·026
Oxaloacetate	0·16	0·21	0·33	0·45	0·61	±0·027
2-Oxoglutarate	0·21	0·27	0·34	0·42	0·54	±0·016
Glyoxylate	0·22	0·29	0·36	0·45	0·77	±0·025
Respiration* (mg CO_2/kg/hr)		64·3	34·2	25·9	30·0	±5·5

* Bulk samples of six fruits.

IV. DISCUSSION

It has been suggested (Mapson and Robinson 1966; Quazi and Freebairn 1970) that the delay in ripening of green bananas held in controlled atmospheres is due to the reduced respiration rate which either delays or prevents the build up of internal ethylene to concentrations which initiate ripening. Although rates of oxygen consumption were reduced, the delay in ripening in the different atmospheres was not directly related to absolute respiration as a higher total oxygen consumption occurred in these atmospheres before the onset of ripening (Table 1). The rapid onset of ripening when fruit stored for a long time in high carbon dioxide–low oxygen were returned to air suggests that the initial metabolic events, which precede the production

of ethylene required for ripening, took place while the fruits were exposed to the modified atmosphere, but that the final steps involving production of ethylene were inhibited.

The largest changes in organic acids were caused by low oxygen; the concentration of 2-oxo acids increased but that of the non-volatile acids decreased. The effects of high carbon dioxide were less consistent, resulting in at least a temporary increase in most of the acids, but its combination with low oxygen sometimes augmented the effects of low oxygen, while in other cases it reduced the changes.

The largest changes in the concentrations of individual acids were found after a 1-day exposure to the controlled atmospheres. To determine a metabolic role for these changes the Chance crossover theorem (Chance *et al.* 1958) was applied to the data for day 0 and day 1. This suggests that low oxygen limits the operation of the Krebs cycle at two steps (Fig. 3), the first between either oxaloacetate or pyruvate

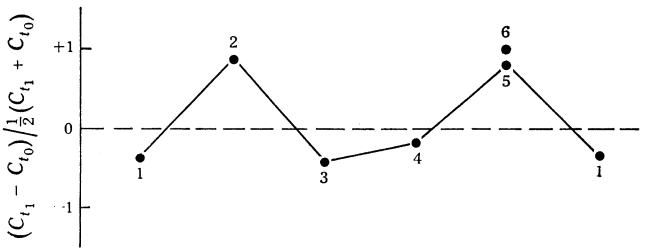

Fig. 3.—Crossover diagram. C_{t_0} and C_{t_1} refer to the concentrations of acids at days 0 and 1 respectively. The acids were: 1, citrate; 2, 2-oxoglutarate; 3, succinate; 4, malate; 5, oxaloacetate; and 6, pyruvate. Crossovers occurred between 2-oxoglutarate and succinate, and oxaloacetate (pyruvate) and citrate.

and citrate and the second between 2-oxoglutarate and succinate. The accumulation of glyoxylate and the reduction in malate in low oxygen would also be in agreement with a reduction in the activity of malate synthetase. Reactions involving coenzyme A are common to the three steps. The effect of low oxygen may thus be associated with an increase in the ratio of reduced to oxidized forms of this coenzyme. An increase in the ratio of reduced to oxidized pyridine nucleotides does not appear to be involved in the effects of low oxygen, otherwise there should have been an accumulation of citrate, malate, glutamate, and aspartate. The combination of high carbon dioxide with low oxygen does not change the location of the rate-limiting steps in the Krebs cycle. Evidently the further extension in the time to ripen achieved by the combination of high carbon dioxide with low oxygen results in regulation of other intermediates. The data for high carbon dioxide also showed no rate-limiting steps in the Krebs cycle.

V. Acknowledgments

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