

# Changes in Sugars and Organic Acids in Cherimoya (*Annona cherimola* Mill.) Fruit under Controlled-Atmosphere Storage

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Cherimoya fruits were stored in air and controlled-atmosphere conditions of 3% O<sub>2</sub> in combination with 0% CO<sub>2</sub>, 3% CO<sub>2</sub>, and 6% CO<sub>2</sub> at 9 °C to study their effects on respiration, ethylene production, flesh firmness, and sucrose, glucose, fructose, malic acid, citric acid, and fumaric acid contents. The largest diminutions in respiration, sugars, and acids were caused by low O<sub>2</sub>, those in ethylene and softening being caused by high CO<sub>2</sub>. The combinations of high CO<sub>2</sub>/low O<sub>2</sub> had an additive reducing effect on ethylene, softening, and malic acid and did not significantly affect sugars and citric acid. Both high-CO<sub>2</sub> atmospheres delayed the soft stage (about 18 N) by 5 and 14 days beyond 3% O<sub>2</sub> + 0% CO<sub>2</sub> and air storage, respectively. This allowed sufficient accumulation of sugars and acids to reach an acceptable quality. Thus, 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub> atmospheres would extend the storage life of cherimoya at 9 °C by 2 weeks over that of fruits under air storage.

## INTRODUCTION

Cherimoya is one of the few species of the Annonaceae family with a great interest for fresh market consumption. In Spain and in several countries of South America (especially Chile) its production has largely increased due to the high demand for exotic fruits. However, this commodity is not well-known by potential consumers of North America and northern Europe because of its limited supply and high cost, due to the requirement of fast (air) transportation.

Cherimoya fruit ripening is characterized by browning of the skin, a biphasic increase in respiration with an intermediate peak in ethylene (C<sub>2</sub>H<sub>4</sub>) production, and rapid softening of the pulp. The rapid ripening is concomitant with an increased sensitivity to fungal decay and lower tolerance to mechanical injury, resulting in a very limited marketing period (Lahoz et al., 1993; Palma et al., 1993). Due to its high susceptibility to chilling injury (CI), prolonged cold storage of cherimoya is doubtful. The lowest tolerable temperature appears to be 8 °C (Alique et al., 1993), but this temperature prolongs the ripening period only for 7-10 days. Thus, other supplementary technologies, such as controlled atmosphere (CA) (low oxygen and/or high carbon dioxide), should be considered to extend the storage life of cherimoya.

Exhaustive information of the effects of CA on the physiology and biochemistry of fruits has been reviewed by Kader (1986) and Wang (1990). The reduction in respiration rate has been assumed as the primary reason for the beneficial effects of CA on fruits and vegetables (Kidd and West, 1927; Young et al., 1962). In pears (*Pyrus communis* L.), it has been shown that the average respiration rate had a significant linear relationship with O<sub>2</sub> concentration but not with CO<sub>2</sub> levels during ripening at ambient temperatures (Blanpied and Hansen, 1968).

The effect of low oxygen on the capacity of plant tissues for ethylene biosynthesis also appears to be dependent on the O<sub>2</sub> concentration (Yang and Hoffman, 1984). Furthermore, Knee (1980) found in apples (*Malus domestica* L.) that the time of the onset in the ethylene production was inversely related to O<sub>2</sub> concentration, with the maximum directly related to O<sub>2</sub> level.

Carbon dioxide inhibits C<sub>2</sub>H<sub>4</sub> action competitively and helps to regulate its biosynthesis (Burg and Burg, 1967). Therefore, some of the benefits of storage in elevated CO<sub>2</sub> atmospheres arise when C<sub>2</sub>H<sub>4</sub> production or C<sub>2</sub>H<sub>4</sub>-mediated reactions are inhibited (Herner, 1987). According to the suggestion of Jeffery et al. (1984) of the occurrence of both ethylene-independent and ethylene-dependent processes during fruit ripening, some biochemical changes would be affected by high CO<sub>2</sub> while others would not. Several studies support this hypothesis. Thus, McGlasson and Wills (1972) reported that low oxygen (3%) limited the operation of the Krebs cycle in bananas (*Musa paradisiaca* L.), whereas high carbon dioxide (5%) showed no rate-limiting steps in this cycle. Synthesis of ethylene and polygalacturonase was retarded during storage of mature green tomatoes (*Lycopersicon esculentum* Mill.) in 6% O<sub>2</sub> + 6% CO<sub>2</sub> at 12 °C, while starch degradation and changes in tricarboxylic acid cycle enzymes proceeded with little inhibition (Jeffery et al., 1984). Blanpied and Hansen (1968) showed in pears that fruit firmness loss was linearly related to CO<sub>2</sub> concentration but not to O<sub>2</sub> levels. Arpaia et al. (1985) reported that elevated CO<sub>2</sub> had a greater effect on firmness retention than reduced O<sub>2</sub> in kiwifruit (*Actinidia deliciosa*).

In a recent review, Palma et al. (1993) found few publications concerning the physiology of cherimoya fruit, especially those regarding the responses to postharvest technologies (low temperature, controlled atmosphere, etc.). The only published study concerning the effects of CA on cherimoya (De La Plaza, 1979) showed stimulation of the respiration rate in Fino de Jete fruits stored in 2% O<sub>2</sub> + 10% CO<sub>2</sub> at 9 °C, while the increase was not significant in Campa fruits. This author found no differences among controlled atmosphere and air storages for the increases in reducing sugars and titratable acidity. The softening was retarded in both cultivars, and the storage period was prolonged for up to 1 week compared with air storage.

Since there is no precise information on the effects of CA in cherimoya fruit ripening, we studied the effects of different carbon dioxide concentrations in combination with low oxygen level (3%) on the physiological and biochemical processes during cold storage of Fino de Jete fruits. We report here respiration rate, ethylene production, flesh firmness, and soluble sugar and organic acid contents of cherimoya fruits at 9 °C and 98% relative

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humidity (RH) during storage in air (21% O<sub>2</sub> + 0% CO<sub>2</sub>) and under CA conditions of 3% O<sub>2</sub> + 0% CO<sub>2</sub>, 3% O<sub>2</sub> + 3% CO<sub>2</sub>, and 3% O<sub>2</sub> + 6% CO<sub>2</sub>.

## MATERIALS AND METHODS

**Plant Material.** Cherimoya fruits, cv. Fino de Jete, were harvested at Granada (Spain), shipped by truck, and received at the Instituto del Frio laboratory (Madrid) within 18 h. Mature green fruits (light yellowish green skin, carpels with shallow ridges) of uniform shape, weighing from 200 to 220 g, were randomly divided into eight groups of 35 fruits and placed in two sets of four 22-L glass jars at 9 ± 0.5 °C in cold rooms equipped with thermocouples. The treatments used were (1) 21% O<sub>2</sub> + 0% CO<sub>2</sub> (air), (2) 3% O<sub>2</sub> + 0% CO<sub>2</sub>, (3) 3% O<sub>2</sub> + 3% CO<sub>2</sub>, and (4) 3% O<sub>2</sub> + 6% CO<sub>2</sub>. Two jars for each atmosphere were maintained under a continuous stream of either air or the appropriate gas mixture (provided in cylinders by SEO, Madrid, Spain), with a flow rate of about 6 L/h regulated by capillary tubes and needle valves. Ethylene was removed from the entering streams by first passing the gas through a KMnO<sub>4</sub>-impregnated product. Relative humidity in the jars was maintained at near-saturation by passing the ethylene-free gas stream through humidifiers. For the atmospheres with 0% CO<sub>2</sub> (air and 3% O<sub>2</sub> + 0% CO<sub>2</sub>) a 10% KOH solution was used in the humidifiers. The gas mixtures were monitored by gas chromatography every 2 days to assure that the actual compositions were within 10% of the intended mixtures. Weight losses were determined daily.

**Measurement of Respiration and Ethylene Production Rates.** CO<sub>2</sub> production and ethylene production were determined daily. Effluent gas samples taken with a 1-mL syringe were injected into a Varian 3700 gas chromatograph (Walnut Creek, CA) equipped with a six-way switching valve and Porapak-Q and molecular sieve columns (2 m × 3.2 mm) located in series. After separation, using helium as the carrier gas (30 mL/min), CO<sub>2</sub> and C<sub>2</sub>H<sub>4</sub> were detected by thermal conductivity and flame ionization detectors, respectively. Quantification was by external standards, and results are expressed in mg of CO<sub>2</sub>/kg·h and μL of C<sub>2</sub>H<sub>4</sub>/kg·h.

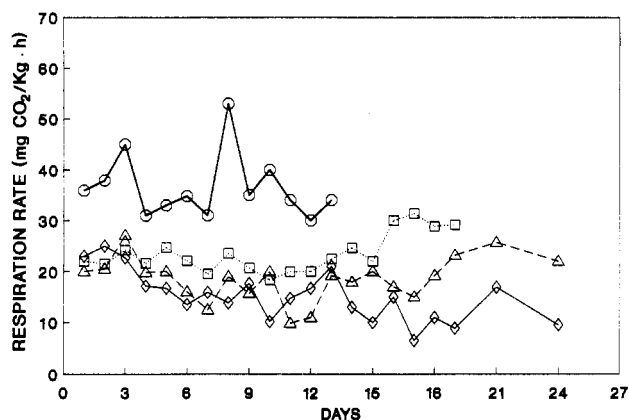
**Fruit Firmness and Quality Assessment.** Five fruits were periodically used for flesh firmness determination using an Instron 1140 testing machine (High Wycombe, U.K.) fitted with a 8 mm diameter cylindrical, flat-surfaced plunger, with full-scale load set at 200 newtons (N) and crosshead and chart speed at 400 mm/min. Skin sections (approximately 1 cm in diameter) were removed from opposite sides before firmness determinations. Flesh firmness was determined as the highest rupture force and expressed in newtons.

After firmness measurements, fruit quality was assessed by the research team in informal tests through flesh color, and texture and flavor of the pulp. The fruit was considered to reach optimal edible condition when the flesh was creamy and glossy white, with a custard-like consistency, and had developed proper sweetness and light sourness. In overripe fruits, browning was almost complete in the skin, and the pulp became watery and translucent, with excessive sweetness and poor sourness.

**Reagents.** All organic solvents were of HPLC grade. Laboratory-deionized water was further purified with a Milli-Q system (Millipore, Milford, MA). Sulfuric acid employed in the HPLC separation was purchased from Merck (Darmstadt, Germany). All of the mobile phases were degassed prior to use. The reference samples of organic acids and sugars were of analytical grade from Sigma-Aldrich Quimica S.A. (Madrid, Spain).

**Sample Preparation for HPLC.** Fruits from the firmness determinations were also used for chemical analyses. A 10-g sample of pulp (free of skin and seeds) was homogenized in 100 mL of methanol with an Omnimixer at 7000 rpm for 5 min. The homogenate was refluxed at 50 °C for 15 min and then filtered under vacuum. The methanol was evaporated under vacuum in a rotary evaporator at 40 °C and the residue resuspended in 50 mL of Milli-Q water and passed through a methanol-activated Sep-Pak C<sub>18</sub> minicolumn (Waters, Milford, MA). The eluate was filtered through a 0.45-μm filter and used for HPLC analyses.

**High-Performance Liquid Chromatography (HPLC) Equipment and Conditions.** Sample injection volumes were 20 μL. Soluble sugars were separated on a Sugar-Pak I (Waters) column (9.5 mm i.d. × 30 cm) at 92 °C with deionized water at



**Figure 1.** Respiration rate of cherimoya fruit stored at 9 °C in air (O), 3% O<sub>2</sub> + 0% CO<sub>2</sub> (□), 3% O<sub>2</sub> + 3% CO<sub>2</sub> (Δ), or 3% O<sub>2</sub> + 6% CO<sub>2</sub> (◇).

0.8 mL/min and detected with a refractive index detector (Waters refractometer R-401). Organic acids were separated on a 6.5 mm i.d. × 30 cm ION-300 (Interaction Chemicals, Mountain View, CA) column at 45 °C, using 0.01 N H<sub>2</sub>SO<sub>4</sub> as a solvent (flow rate of 0.4 mL/min) and detected by ultraviolet absorption at 214 nm (Waters detector, Model 441). Quantitative assessment in both cases was based upon external standards. Soluble sugar concentrations are expressed as percentage of fresh weight (FW) and those of organic acids as mg/g FW or, for fumaric acid, as mg/100g FW. Values were corrected to account for weight losses. Three replicates were conducted on each sample.

**Statistical Analysis.** Data were analyzed by an analysis of variance (ANOVA) at  $P = 0.05$ .

## RESULTS AND DISCUSSION

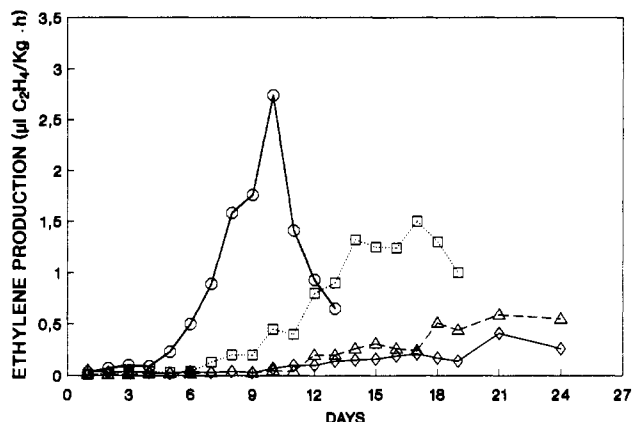
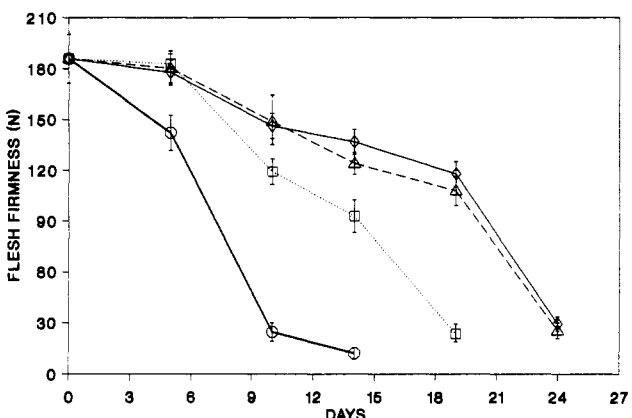
The respiration rate at 9 °C in air (Figure 1) showed two peaks for CO<sub>2</sub> production, occurring on days 3 and 8 of storage, respectively. An abrupt increase in CO<sub>2</sub> production has been suggested as an effect of harvest (Phan, 1987). This effect has been observed in soursop (*Annona muricata* L.) by Bruinsma and Paull (1984), suggesting that the first CO<sub>2</sub> peak is independent of the ripening process.

A reduced respiration rate of about 23 mg of CO<sub>2</sub>/kg·h was observed until after 15 days in 3% O<sub>2</sub> + 0% CO<sub>2</sub> condition, when a slow increase was observed (Figure 1). Until day 15 no difference was observed due to the combination of reduced O<sub>2</sub>/elevated CO<sub>2</sub> with respect to reduced oxygen only. After this date, the respiration rate was inversely related to CO<sub>2</sub> level, but no marked differences were found between the two elevated CO<sub>2</sub> atmospheres. These results do not agree with those obtained by De La Plaza (1979), who observed an increase in respiration rate induced by high CO<sub>2</sub> level in Fino de Jete and Campa fruits in 2% O<sub>2</sub> + 10% CO<sub>2</sub> at 9 °C. Differences among the methods used for gas measurements during air and CA storage and poor accuracy of these methods and of the system for maintenance of the controlled atmospheres could explain such disagreement. It is clear that the actual O<sub>2</sub> and CO<sub>2</sub> levels during CA storage must be considered, since Kader (1986) suggested that O<sub>2</sub> at about 2% and CO<sub>2</sub> above 10% (depending on the commodity, temperature, and storage duration) have additive effects on respiration rate and on the shift from aerobic to anaerobic respiration, increasing the total CO<sub>2</sub> production. Moreover, our results suggest that the respiration rate of cherimoya under CA conditions is mainly dependent of oxygen level and that the only noticeable effect of elevated carbon dioxide is a slight suppression. Similar results have been reported in bananas (McGlasson and Wills, 1972).

**Table 1. Soluble Sugar and Organic Acid Content<sup>a</sup> (Mean ± SD) in Cherimoya Fruit Stored in Air (21% O<sub>2</sub> + 0% CO<sub>2</sub>) at 9 °C**

day	sucrose	glucose	fructose	malic	citric	fumaric
0	1.22 ± 0.04	1.82 ± 0.04	2.38 ± 0.05	1.21 ± 0.04	0.70 ± 0.02	21.70 ± 2.15
5	2.27 ± 0.06	3.18 ± 0.07	3.88 ± 0.08	1.66 ± 0.06	0.67 ± 0.03	41.17 ± 3.52
10	1.64 ± 0.05	4.91 ± 0.09	5.13 ± 0.09	3.17 ± 0.07	1.38 ± 0.06	150.10 ± 8.91
14	0.54 ± 0.03	5.23 ± 0.06	6.68 ± 0.07	2.77 ± 0.06	1.71 ± 0.05	57.68 ± 3.69

<sup>a</sup> Sugar content expressed as % FW, malic and citric acid as mg/g FW, and fumaric acid as mg/100 g FW.

**Figure 2.** Ethylene production by cherimoya fruit stored at 9 °C in air (O), 3% O<sub>2</sub> + 0% CO<sub>2</sub> (□), 3% O<sub>2</sub> + 3% CO<sub>2</sub> (Δ), or 3% O<sub>2</sub> + 6% CO<sub>2</sub> (◇).**Figure 3.** Evolution of texture (flesh firmness) in cherimoya fruit stored at 9 °C in air (O), 3% O<sub>2</sub> + 0% CO<sub>2</sub> (□), 3% O<sub>2</sub> + 3% CO<sub>2</sub> (Δ), or 3% O<sub>2</sub> + 6% CO<sub>2</sub> (◇). Results are mean ± SD of five individual fruits. Determinations were made in triplicate.

Ethylene production at 9 °C in air (Figure 2) remained at about 0.1 µL/kg·h until day 4, showing a peak (2.8 µL of C<sub>2</sub>H<sub>4</sub>/kg·h) on day 10, 2 days after the second respiration rise. Reduced ethylene production was observed under 3% O<sub>2</sub> + 0% CO<sub>2</sub> condition, increasing slowly after day 6 and reaching a plateau on day 14. It has been shown that oxygen is required for ethylene synthesis (Yang, 1985) and increases the binding affinity of ethylene to its receptor (Burg and Burg, 1967). Ethylene production was drastically reduced by elevated CO<sub>2</sub>/low O<sub>2</sub>, with values below 0.6 µL/kg·h during all of the storage duration in both 3 and 6% CO<sub>2</sub> atmospheres. Reduction in ethylene production by high CO<sub>2</sub> has been reported in avocados (*Persea americana* Mill.), apples, pears, and tomatoes due to antagonism between CO<sub>2</sub> and ethylene synthesis (Burg and Burg, 1967).

Fruit softened from the beginning of storage in air (Figure 3), when ethylene production was below 0.1 µL/kg·h, and the respiration rate was at the onset of the first CO<sub>2</sub> peak (Figures 1 and 2), suggesting that cherimoya fruit softening is not directly induced by ethylene. Similar results were obtained for cherimoya fruit by Lahoz et al.

**Table 2. F Value and Significance Level<sup>a</sup> for the Effects of O<sub>2</sub> Concentration (21 and 3%) and Time on Soluble Sugars and Organic Acids of Cherimoya Fruit Stored at 9 °C**

factor	sucrose	glucose	fructose	malic	citric	fumaric
O <sub>2</sub>	43*	44*	35*	430*	50*	28*
days (D)	321*	554*	1027*	166*	591*	301*
O <sub>2</sub> × D	47*	22*	7*	110*	13*	25*

<sup>a</sup> An asterisk indicates significance at the 0.05 level.

(1993). The soft stage (about 18 N) was reached on day 10, near the ethylene maximum and immediately after the second respiration peak.

Low oxygen delayed fruit softening, reaching values near the soft stage 9 days later in 3% O<sub>2</sub> + 0% CO<sub>2</sub> than in air storage (Figure 3). The rate of fruit softening has been shown to be reduced by low oxygen in tomatoes (Kim and Hall, 1976) and apples (Knee, 1980). The combination of elevated CO<sub>2</sub>/low O<sub>2</sub> delayed cherimoya softening, with no significant differences between 3 and 6% CO<sub>2</sub> conditions (Figure 3). De La Plaza (1979) observed a delay in cherimoya softening stored at 9 °C in 2% O<sub>2</sub> + 10% CO<sub>2</sub> compared to air storage. A greater effect of high CO<sub>2</sub> on firmness retention with respect to reduced O<sub>2</sub> has been observed in pears by Blanpied and Hansen (1968) and in kiwifruit by Arpaia et al. (1985). The soft stage occurred on day 24 in 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub> conditions, the ethylene production rate being approximately 0.5 and 0.3 µL of C<sub>2</sub>H<sub>4</sub>/kg·h, respectively. These results suggest that low ethylene production is sufficient for cherimoya fruit to reach the soft stage during prolonged CA storage.

Sucrose content increased drastically on day 5 at 9 °C in air, decreasing afterward (Table 1). Rapid sucrose accumulation after picking has been reported in several *Annona* species (Broughton and Guat, 1979; Paull et al., 1983; Wills et al., 1984). Bruinsma and Paull (1984) suggested rapid accumulation of sucrose and malic acid and the first respiratory rise as effects of harvesting. The drastic decrease observed after this date was coincident with an increase in respiration rate (Figure 1 and Table 1).

Statistical analysis showed a significant effect at *P* = 0.05 of O<sub>2</sub> level and O<sub>2</sub> level–time interaction on sucrose content (Table 2). Delayed sucrose accumulation and reduced hydrolysis after the maximum was observed in 3% O<sub>2</sub> + 0% CO<sub>2</sub> (Table 3).

Sucrose evolution was not affected (*P* = 0.05) by the CO<sub>2</sub> level in both atmospheres with 3% O<sub>2</sub> (Table 4). However, lower sucrose accumulation was observed under these conditions, likely due to the effect of the CO<sub>2</sub> level–time interaction. Thus, elevated CO<sub>2</sub> may reduce the harvesting effect on sucrose content during CA storage of cherimoya fruit. Reduction of the loss of sucrose under CA storage has been reported in a number of crops (Wang, 1990).

The ANOVA showed high *F* values for the effects of the O<sub>2</sub>– and CO<sub>2</sub>–time interactions on sucrose content, as compared to those for the O<sub>2</sub> and CO<sub>2</sub> effects, indicating a modification of the pattern in sucrose evolution.

**Table 3. Soluble Sugar Content<sup>a</sup> in Cherimoya Fruit Stored under Three Controlled Atmospheres at 9 °C**

% O <sub>2</sub> -CO <sub>2</sub>	day	sucrose	glucose	fructose
3-0	0	1.22 ± 0.04	1.82 ± 0.04	2.38 ± 0.05
	5	2.03 ± 0.05	3.02 ± 0.06	3.42 ± 0.07
	10	2.46 ± 0.05	3.68 ± 0.07	4.95 ± 0.07
	14	1.02 ± 0.04	5.27 ± 0.07	6.00 ± 0.08
	19	0.05 ± 0.01	5.67 ± 0.09	6.79 ± 0.09
3-3	0	1.22 ± 0.04	1.82 ± 0.04	2.38 ± 0.05
	5	1.19 ± 0.03	3.25 ± 0.06	3.99 ± 0.07
	10	1.69 ± 0.05	3.64 ± 0.07	4.56 ± 0.06
	14	1.59 ± 0.05	4.95 ± 0.07	5.55 ± 0.08
	19	1.35 ± 0.03	5.30 ± 0.08	6.09 ± 0.07
	24	1.25 ± 0.04	5.27 ± 0.08	6.08 ± 0.09
3-6	0	1.22 ± 0.04	1.82 ± 0.04	2.38 ± 0.05
	5	1.31 ± 0.03	3.17 ± 0.07	3.97 ± 0.05
	10	1.80 ± 0.05	4.04 ± 0.06	4.98 ± 0.09
	14	1.49 ± 0.04	4.99 ± 0.08	5.59 ± 0.06
	19	1.17 ± 0.03	5.30 ± 0.06	6.26 ± 0.08
	24	1.19 ± 0.04	5.28 ± 0.09	6.12 ± 0.07

<sup>a</sup> % FW ± SD.

**Table 4. F Value and Significance Level<sup>a</sup> for the Effects of CO<sub>2</sub> Concentration (0, 3, and 6%) in Combination with 3% O<sub>2</sub> on Soluble Sugars and Organic Acids of Cherimoya Fruit Stored at 9 °C**

factor	sucrose	glucose	fructose	malic	citric	fumaric
CO <sub>2</sub>	1 <sup>NS</sup>	1 <sup>NS</sup>	2 <sup>NS</sup>	18*	3 <sup>NS</sup>	110*
days (D)	261*	373*	1535*	140*	333*	320*
CO <sub>2</sub> × D	114*	2 <sup>NS</sup>	12*	3 <sup>NS</sup>	50*	33*

<sup>a</sup> An asterisk indicates significance at the 0.05 level, <sup>NS</sup> nonsignificance.

Rapid glucose accumulation was observed in air at 9 °C until day 10 of storage, while fructose accumulation occurred later during storage, paralleled by a concomitant increase in the rate of sucrose hydrolysis (Table 1).

Glucose and fructose evolution was significantly affected ( $P = 0.05$ ) by O<sub>2</sub> level (Table 2). Delayed glucose and fructose accumulation was observed in the 3% O<sub>2</sub> + 0% CO<sub>2</sub> atmosphere (Table 3); however, a nonsignificant effect at  $P = 0.05$  of CO<sub>2</sub> level was observed (Table 4). The delay in fructose accumulation observed in 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub> atmospheres should be ascribed to the CO<sub>2</sub> level-time interaction effect. Goodenough and Thomas (1981) showed that CA (5% O<sub>2</sub> + 5% CO<sub>2</sub>) did not restrict changes in starch degradation and sugar accumulation in tomato fruit. De La Plaza (1979) observed no effect of CA storage on the accumulation of reducing sugars in cherimoya. These results suggest that different ripening events, as soluble sugar accumulation and fruit softening, respond differently to CA treatment.

Malic acid content reached a maximum on day 10 at 9 °C in air (Table 1), coincident with the soft stage (18 N), but under the three CA conditions it continued to increase with storage duration, reaching lower values (Table 5). Statistical analysis confirmed the influence of O<sub>2</sub> level and O<sub>2</sub> level-time interaction on malic acid content (Table 2); thus, a drastic reduction in malic acid content was observed in 3% O<sub>2</sub> + 0% CO<sub>2</sub> condition (Table 5). The combination of elevated CO<sub>2</sub>/low O<sub>2</sub> had an additive effect on the reduced malic content, with no significant differences at  $P = 0.05$  between 3 and 6% CO<sub>2</sub> atmospheres (data not shown). Shipway and Bramlage (1973) found that CO<sub>2</sub> level above 3% stimulated malate oxidation in apple mitochondria. This stimulation appeared to be saturated at low CO<sub>2</sub> concentration, there being no significant differences among the effects of 6, 12, or 18% CO<sub>2</sub>.

**Table 5. Organic Acid Content<sup>a</sup> in Cherimoya Fruit Stored under Three Controlled Atmospheres at 9 °C**

% O <sub>2</sub> -% CO <sub>2</sub>	day	malic	citric	fumaric
3-0	0	1.21 ± 0.04	0.70 ± 0.03	21.70 ± 2.15
	5	1.19 ± 0.03	0.90 ± 0.04	33.40 ± 2.47
	10	1.16 ± 0.04	1.22 ± 0.05	97.07 ± 5.87
	14	1.87 ± 0.05	1.39 ± 0.04	50.37 ± 2.85
	19	2.07 ± 0.04	1.63 ± 0.04	50.67 ± 2.76
3-3	0	1.21 ± 0.04	0.70 ± 0.03	21.70 ± 2.15
	5	0.99 ± 0.03	1.10 ± 0.03	60.73 ± 3.14
	10	1.03 ± 0.03	1.23 ± 0.04	85.63 ± 3.99
	14	1.48 ± 0.04	1.26 ± 0.05	96.61 ± 5.99
	19	1.68 ± 0.05	1.41 ± 0.06	110.8 ± 7.95
	24	1.85 ± 0.05	1.55 ± 0.05	111.8 ± 8.31
3-6	0	1.21 ± 0.04	0.70 ± 0.03	21.70 ± 2.15
	5	1.16 ± 0.03	1.11 ± 0.03	82.30 ± 4.27
	10	1.09 ± 0.04	1.13 ± 0.05	86.77 ± 4.11
	14	1.51 ± 0.04	1.20 ± 0.04	91.07 ± 6.48
	19	1.70 ± 0.05	1.40 ± 0.03	98.17 ± 6.79
	24	1.75 ± 0.04	1.45 ± 0.04	109.1 ± 7.89

<sup>a</sup> Values are expressed as either mg/g FW ± SD (malic and citric acid) or mg/100 g FW ± SD (fumaric acid).

Citric acid increased in all conditions (Tables 1 and 5). Drastic increases were observed after 10 days in air and in 3% O<sub>2</sub> + 0% CO<sub>2</sub>, reaching similar values at the end of storage. Citric acid accumulation was significantly influenced ( $P = 0.05$ ) by oxygen concentration (Table 2). Thus, reduced citric acid accumulation was observed in 3% O<sub>2</sub> + 0% CO<sub>2</sub>. Similar results were reported in bananas stored in 3% O<sub>2</sub> + 0% CO<sub>2</sub> by McGlasson and Wills (1972), who found significant decreases in malate and citrate compared with those in air storage. No significant differences ( $P = 0.05$ ) have been found in citric acid content between the two high-CO<sub>2</sub> conditions. Therefore, the modification in the citric acid evolution observed during CA storage with respect to air storage is explained by the high  $F$  values of the CO<sub>2</sub>-time interaction effect (Table 4). The combination of elevated CO<sub>2</sub>/low O<sub>2</sub> had no additive effects on the reduction in citric acid content.

Fumaric acid content rose to a maximum on day 10 in air and 3% O<sub>2</sub> + 0% CO<sub>2</sub> atmospheres; however, the maximum was lower in the latter condition (Tables 1 and 5). This increase was significantly affected ( $P = 0.05$ ) by O<sub>2</sub> level and O<sub>2</sub> level-time interaction (Table 2). Elevated CO<sub>2</sub> in combination with low O<sub>2</sub> significantly affects ( $P = 0.05$ ) fumaric content (Table 4), stimulating its accumulation. In apple mitochondria, Shipway and Bramlage (1973) found a reduction in the oxidation of fumaric acid by CO<sub>2</sub> levels above 3%.

The soft stage was retarded 9 days in 3% O<sub>2</sub> + 0% CO<sub>2</sub> atmosphere as compared with air; therefore, though soluble sugar and organic acid accumulation was also delayed, the fruit reached an optimal eating quality on day 19, similar to that achieved on day 10 in air. In cherimoyas stored in 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub> atmospheres a higher delay in fruit softening was observed, allowing soluble sugar content on day 24 to reach a level similar to that reached on day 19 in 3% O<sub>2</sub> + 0% CO<sub>2</sub> condition. The organic acid content was clearly lower in both elevated CO<sub>2</sub> atmospheres, resulting in a lower eating quality. However, an eating quality still acceptable for marketing was reached on day 24 in 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub>, with no practical differences among these conditions. Thus, storage of cherimoya Fino de Jete fruit at 9 °C may be prolonged for up to 14 days in 3% O<sub>2</sub> + 3% CO<sub>2</sub> and 3% O<sub>2</sub> + 6% CO<sub>2</sub> atmospheres, as compared to air storage.

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