Influence of Harvest Date within the Season and Cold Storage on Cherimoya Fruit Ripening

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Cherimoya ripening with and without prior storage at 8 °C was studied in fruit harvested on early- (EH), mid- (MH), and late-season (LH) dates. Most of the differences in the ripening behavior were observed between EH cherimoyas and fruit from the other two harvest dates. During ripening of nonstored fruit, the increases in ethylene production and respiration rates and in soluble sugars and organic acids contents were faster in EH than in the other fruits (which ripened 1 day later). These differences could be due to variations in the physiological stage at harvest as a result of the different heat units (degree/month) accumulated in the last month of fruit development. During ripening of cold-stored fruit the differences in the time to the onset of the increase in ethylene production and in the accumulation of malic and citric acid were minimized, especially after longer storage times, and the relationship of harvest date with the increases in respiration rate was lost. Glucose and fructose accumulation was reduced by prior cold storage, especially after longer storage duration and in MH and LH fruit, but sucrose hydrolysis was almost complete, as in nonstored fruit. It is suggested that glucose and fructose accumulation is more sensitive to low temperature than sucrose metabolism and that this differential sensitivity is more marked in MH and LH cherimoyas. The time to ripen was inversely related to prior cold storage duration and was dependent on harvest date: the later the harvest date, the longer storage time it took to shorten subsequent ripening.

Keywords: *Cherimoya; cold storage; harvest season; ripening*

INTRODUCTION

Cherimoya (*Annona cherimola* Mill.) is a fruit crop commercially cultivated in warm temperate areas of the Spanish Mediterranean coast (the largest producing area in the world), South America, and California (Merodio and De La Plaza, 1997). Cherimoya behaves like a climacteric fruit after harvest; it shows transient increases in respiration and ethylene production rates, which are connected with its ripening (Palma et al., 1993). Ripening of cherimoya is characterized by an increases in soluble solids content and acidity and softening of the pulp, giving the ripe fruit a pleasant blend of sweetness and mild sourness and a creamy texture.

The marketing potential of cherimoya is hampered by its high perishability (at 18−20 °C it will ripen in 3−6 days) and susceptibility to chilling injury (CI). The optimum temperature for prolonged cold storage of cherimoya, depending on the cultivar, ranges between 8 and 10 °C (Palma et al., 1993; Alique et al., 1994; Gutiérrez et al., 1994). It is likely that harvest date within the season and physiological maturity at harvest will influence the response of cherimoya to cold storage, as has been shown for several fruit crops (Saltveit and Morris, 1990). However, published studies concerning how harvest date affects the quality of cherimoya are very scarce. Kosiyachinda and Young (1975) found that cv. Chaffey cherimoya harvested late in the season ripened more rapidly and showed a shorter delay between the climacteric rises in respiration and internal ethylene concentration than early-season fruit, but no other data concerning the effect of harvest date was reported. Nomura et al. (1997) studied the relationship between developmental stage at harvest and the ripening of cv. Big Sister cherimoya. These authors used fruit grown in a greenhouse and therefore climatic conditions were quite different from those found in commercial orchards. Furthermore, a clear effect of harvest date on fruit ripening could not be elucidated because early-pollinated fruit did not ripen, indicating that they were not mature at harvest. On the other hand, neither Kosiyachinda and Young (1975) nor Nomura et al. (1997) conducted any research of the influence of harvest date in cold-stored cherimoya, although cold storage is the main postharvest technology used for preservation and transport of cherimoya fruit (Merodio and De La Plaza, 1997).

The differential effect of harvest season and date within the season has been shown in fruit crops that require multiple harvests, because their physiological maturity, ripening behavior, and response to cold storage have seasonal fluctuations. For example, despite similar grade and age, the lengths of the preclimacteric period (green life) of banana (*Musa* AAA) were different between fruit harvested at different times of the year (Marin et al., 1996). Similarly, Nomura et al. (1997) have shown that cherimoya age and weight are not good harvest indices, as maturity will depend on climatic conditions during growth. In purple passion fruit (*Passiflora edulia* Sims) yield and composition of juice are

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affected by harvest season, although it was concluded that juice quality was acceptable in both summer and winter (Saenz et al., 1998). Guavas (*Psidium guajava* L.) reached the ripe stage earlier in fruit from the spring−summer production season compared to autumn−winter fruit, but the latter had higher total soluble solids (TSS), titratable acidity (TA), and ascorbic acid content (Mercado et al., 1998).

The aim of the present work was to investigate the effect of harvest date within the season on cherimoya ripening with and without prior storage at 8 °C, the optimum temperature for cv. Fino de Jete fruits (Alique et al., 1994). The main objective was to study whether the response of physiologically mature cherimoya to cold storage is dependent on harvest date, as a first step to determine the possible relationship of harvest date within the season with optimum storage duration and fruit quality following storage.

MATERIALS AND METHODS

Plant Materials and Storage Conditions. Cherimoya fruits (*Annona cherimola* Mill. cv. Fino de Jete) were obtained from 25-year-old trees grown at Granada, Spain. Mature (skin becoming slightly pale green), unripe fruit were harvested on three dates: early-season (October 18; EH), mid-season (November 15; MH), and late-season (December 10; LH), corresponding to hand pollinations carried out on June 1, 10, and 24, respectively. Hand pollination of flowers with open petals (when the pistil is receptive to pollen) was carried out early in the morning in several areas of the field, and different areas were used in each pollination date. Fruits were received at the laboratory within 15−18 h after harvest, selected for freedom of defects, and randomly divided into six groups that were held at either 20 °C (three groups of 12 fruits, named nonstored fruit) or 8 °C (three groups of 21 fruits, named cold-stored fruit). Each group was placed within a 22-L glass jar and maintained under a continuous stream of air (provided in cylinders by Praxair, Madrid, Spain) with a flow rate of 5−7 L h^{-1}. Relative humidity in the jars was held at 95−98% by passing the gas stream through humidifiers. On the third, sixth, and ninth days of cold storage, a group of seven fruits was taken from each jar. One fruit from each group was used for firmness and chemical determinations, and each group of the remaining six fruits was transferred to 20 °C and placed within a jar as above and held at 20 °C. Three fruits (one from each jar) were used daily for analytical determinations and assessment of fruit ripeness and quality.

Development of a Heat Unit System. A heat unit system similar to that used to predict stages of plant development based on accumulated degrees per unit time was developed (Jenni et al., 1998). Daily ambient air temperature data were collected from a nearby meteorological station, and the average temperature was calculated for each month. A regression model for the evolution of the monthly mean temperature (expressed in degrees centigrade) with time (expressed in months), along the whole pollination−harvest date period (i.e., from the first pollination date−June 1−to the late-harvest date−December 10) was fitted using a computer statistics program (Statgraphics, STSC). Model selection was based on R^2 values, and the best curve fit was obtained by a fourth-degree polynomial regression. Accumulated heat units (degree/month) were then calculated by integrating the polynomial function either from pollination day to the corresponding harvest date (whole development period for each of the three harvests) or along the last month of development.

Measurement of Respiration and Ethylene Production Rates. CO_2 and C_2H_4 were determined in 1-mL samples of the effluent gas from each jar by gas chromatography (GC) as previously reported in Alique et al. (1994). Quantitative assessment was made by external standards, and results were expressed as milligrams of CO_2 per kilogram per hour (respiration rate) and microliters of C_2H_4 per kilogram per hour (ethylene production).

Table 1. Physiological (Fruit Weight and Age) and Chemical (Soluble Sugars and Organic Acids Content) Parameters in Cherimoya Fruit Harvested on Three Dates within the Season[a]

	harvest date		
parameter	early	mid	late
fruit age (days)	139	158	169
fruit wt (g)	580 ± 62 a	495 ± 43 b	475 ± 72 b
sucrose[b]	0.62 ± 0.11 a	0.29 ± 0.08 b	0.31 ± 0.02 b
glucose[b]	1.08 ± 0.12 a	0.81 ± 0.09 b	0.66 ± 0.11 b
fructose[b]	1.53 ± 0.16 a	1.19 ± 0.11 b	1.01 ± 0.16 b
citric acid[c]	61 ± 5 a	63 ± 11 a	57 ± 10 a
malic acid[c]	143 ± 16 a	145 ± 17 a	151 ± 20 a
fumaric acid[c]	1.37 ± 0.12 a	0.81 ± 0.08 b	0.98 ± 0.13 b

[a] Data are the means ± SD of the determinations carried out in 10 fruits. Means within a row not followed by the same letter are significantly different ($p \leq 0.05$). [b] Sucrose, glucose, and fructose contents are expressed as percent fresh weight (FW). [c] Citric, malic, and fumaric contents are expressed as mg/100 g (FW).

Fruit Firmness. Three fruits were periodically used for flesh firmness determination using an Instron 1140 testing machine. Two measurements were made in opposite sides of each fruit, after a skin section of ~1 cm in diameter had been removed. Flesh firmness was determined as the highest rupture force as described in Alique et al. (1994). Results were expressed in newtons.

TSS and TA. Samples of the pulp of each fruit used for firmness measurements were homogenized using an Osterizer, and TSS was determined twice per homogenate with a digital, temperature-compensated Atago dbx 30 refractometer. Results were expressed as percentage of fresh weight (FW). TA was determined twice per fruit homogenate using an automatic Mettler DL70 titrator, titrating to pH 8.1 with 0.1 N NaOH. Results were expressed as percentage of malic acid equivalent.

Soluble Sugars and Organic Acids Quantitation. Soluble sugars and organic acids were extracted from the pulp of the fruits used for firmness measurements and determined by high-performance liquid chromatography (HPLC) as previously reported (Alique and Oliveira, 1994). Two determinations were made on each fruit. Quantitative assessment was based on external standards, and results were expressed as percentage of FW (soluble sugars) or in milligrams per 100 g of FW (organic acids).

Assessment of Fruit Ripeness and Quality. Fruit ripeness and quality were assessed in the same fruits used for analytical determinations by three members of the research group, based on skin and flesh color and texture and flavor of the pulp. A cherimoya was considered to be ripe when the flesh was uniformly creamy and glossy white, with a custard-like consistency, and had developed proper sweetness and mild sourness, with no more than 10% browning of the skin area.

Statistical Analysis. Experimental data are the mean ± SD of the determinations made independently either in three groups of fruit contained in jars (gas measurements) or in three individual fruits (physical and chemical determinations). A variance analysis using the LSD test procedure was performed to determine if differences between means were significant at the $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Effect of Harvest Date on Fruit Characteristics at Harvest. Early-season fruit showed significantly ($p \leq 0.05$) higher weight and concentration of sucrose, glucose, fructose, and fumaric acid as compared to fruit harvested later (Table 1). No differences were observed in these parameters between MH and LH fruits. Harvest date had no significant effect in malic and citric acid contents.

Table 2. Accumulated Heat Units (Degree/Month) from Pollination to the Corresponding Harvest Date and during the Last Month before Harvest of Cherimoya Fruit Harvested on Three Dates within the Season[a]

harvest date	accumulated heat units (degree/months) during indicated period	
	pollination−harvest	last month
early	106.2	22.9
mid	115.5	18.5
late	117.8	16.8

[a] Flowers were hand pollinated on June 1, 10, and 24, and mature fruit harvested on October 18 (early), November 15 (mid), and December 10 (late), respectively.

Nomura et al. (1997) have shown that cherimoya fruit weight increases by growth, independent of pollination time. This growth is dependent on ambient temperatures, as Ibar (1986) pointed out that in the Spanish Mediterranean area temperatures in the range of 7−13 °C will reversibly stop cherimoya fruit development. Such temperatures were achieved only during November and December (see below) and therefore were withstood only by fruits harvested on the mid- and late-season dates. A faster development due to nonrestrictive temperatures in the field may also explain the finding that the earlier the pollination date, the younger the age at which fruit reached harvest maturity. Similar results have been reported by Gardiazabal and Rosenberg (1993) and support the view that days after pollination and/or fruit weight cannot be used as suitable maturity indices for harvesting cherimoyas (Nomura et al., 1997).

To better characterize the effect of preharvest temperature on fruit physiology, a heat unit system similar to that used to predict stages of plant development based on accumulated degrees per unit time was developed (Jenni et al., 1998). Monthly mean temperatures of the ambient air calculated from the daily temperature data were 22.4 ± 4.3 (June), 25.5 ± 4.5 (July), 26.9 ± 4.8 (August), 22.4 ± 5.2 (September), 18.6 ± 3.8 (October), 16.0 ± 2.9 (November), and 14.3 ± 3.7 °C (December). The relationship of this mean temperature (T_M) with the time from the first pollination (June 1) to the late-harvest day (December 10) was fitted to the following fourth-degree polynomial function: $T_M = 19.654 - 9.5792M + 3.6572M^2 - 0.4104M^3 + 0.0141M^4$, M being months after pollination. The correlation coefficient (R^2) was 0.94. Heat units (degree/month) accumulated either during the whole development period (from pollination day to the corresponding harvest day) or during the last month of development are shown in Table 2. Whatever the period considered for calculation of accumulated heat units, differences between EH fruit and fruit from the other harvest dates were higher than those between MH and LH fruit. Thus, accumulated heat units considering the pollination−harvest period for MH and LH fruit were, respectively, 9 and 11% higher than values for EH fruit, whereas heat units accumulated during the same period were only 2% higher in LH than in MH fruit. Considering only the last month of development, values for MH and LH fruit were 19 and 26% lower, respectively, than values obtained for EH fruit, whereas there was only a 9% decrease in the value between MH and LH fruit.

Most of the parameters measured at the time of harvest and during the postharvest period also showed larger differences between EH fruit and fruit from the other harvest dates and suggested that EH fruit had somehow a higher physiological activity. Therefore, this heat unit system, considering the last month of development, could be helpful in the prediction of maturity and ripening behavior of cherimoya: higher values of accumulated heat units could indicate a higher physiological activity of fruit, reflected in a faster ripening after harvest. However, it appears that this relationship may be applicable only in a range of accumulated heat units and that below this range no clear differences due to this factor should be expected. Further research is needed to validate this system, but this hypothesis is somehow similar to that of Nomura et al. (1997), who suggested that differences in cherimoya ripening may be attributed to differences in the heat accumulated during the two months (of the six months of development) corresponding to the resting stage prior to the final enlargement period. According to this hypothesis, a 6−7% difference in the heat accumulated during this period was enough to result in fruit with the lower accumulated heat being unable to ripen properly.

Fruits with better quality and slightly higher sugar content are obtained when the final period of cherimoya fruit growth, prior to harvest, is in a warmer month (Lizana and Reginato, 1990). Our results are similar; there was a direct relationship between heat units accumulated during the last month of development and TSS concentration (the sum of sucrose, glucose, and fructose contents), although differences between MH and LH fruit were not significant. Harvest season had no effect on malic and citric acid, the major organic acids contributing to TA in cherimoya (Palma et al., 1993; Alique et al., 1994). This is in accordance with the results of Pérez de Oteiza et al. (1998), who did not find a clear relationship between harvest date and TA in cv. Fino de Jete cherimoya.

Respiration Rate. Fruit from all of the harvest dates, either nonstored or previously cold-stored, showed a climacteric-like pattern of respiration at 20 °C (Figure 1). The biphasic respiration pattern found in cherimoya and in other *Annona* fruits (Palma et al., 1993) could not be confirmed during this study, a result similar to that reported by Sánchez et al. (1998a).

During ripening at 20 °C of nonstored fruit, maxima in respiration rate were significantly higher ($p \leq 0.05$) in EH fruit than in fruit from the other harvests, which showed no significant differences between them (Figure 1a). The time of the climacteric peak was not influenced by harvest date and occurred approximately on the third day in all fruit. Our results agree with those of Nomura et al. (1997), who found that the age of cv. Big Sister cherimoya did not influence the maximum respiration rate during ripening, but differ from those of Kosiyachinda and Young (1975). The latter authors found that the average time from harvest to the onset of the respiration rise was much longer in early-harvested (January) than in late-harvested cv. Chaffey cherimoyas. This disagreement may be due to the great differences in the climatic conditions during the development of California-grown cv. Chaffey and Spain-grown cv. Fino de Jete cherimoyas and in the postharvest physiology of the two cultivars.

It appears that EH fruit may have higher physiological activity, resulting in higher respiration rate, but that this was not due to differences in the maturity at harvest. Such differences should be reflected in the time of the climacteric maximum, as has been reported in

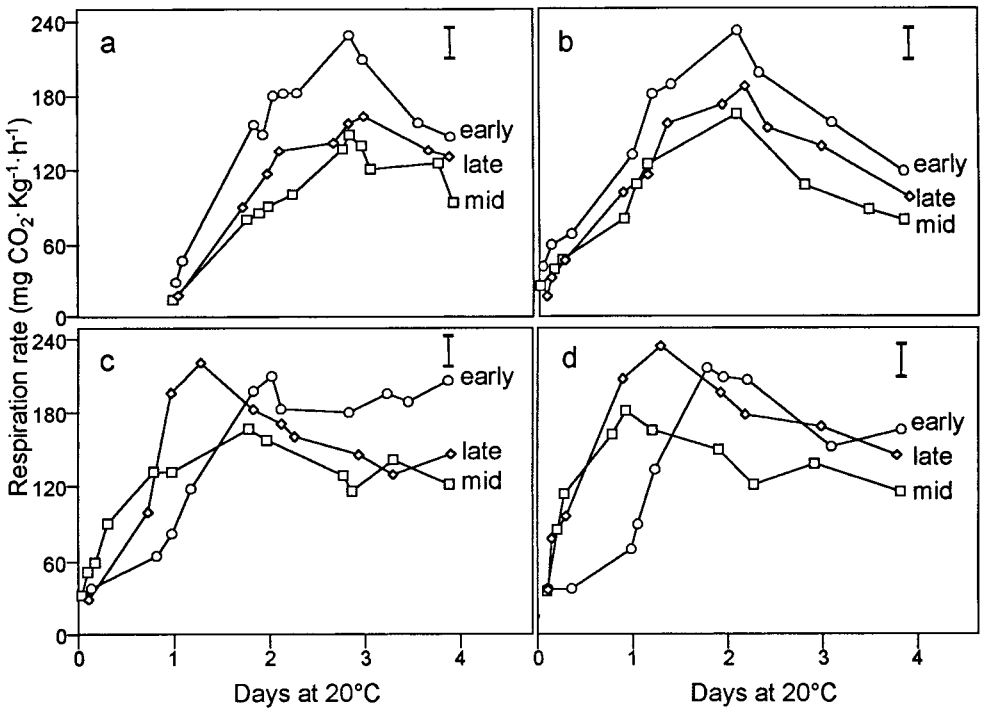

Figure 1. Respiration rate of cherimoya fruit harvested at three different dates within the season (early, ○; mid, □; late, ◇) during ripening at 20 °C without prior storage (a) and after storage at 8 °C for 3 (b), 6 (c), or 9 (d) days. Vertical bar represents the maximum standard deviation (SD) observed.

several climacteric fruit, including soursop, another *Annona* species (Worrel et al., 1994).

The interaction between harvest date and cold storage influenced the value and time of occurrence of maxima in respiration rate. EH fruit showed no clear variation in the values of the climacteric peak in respiration rate due to prior cold storage, but cherimoya from the other two harvest dates increased their climacteric maximum with the duration of storage (Figure 1b−d). This effect was more marked in LH fruit. The time to reach the climacteric maximum at 20 °C was shortened due to previous cold storage in fruit from the three harvest dates. However, the duration of cold storage had an effect on this hastening only in MH and LH fruits, which showed earlier climacteric peaks at longer storage times (Figure 1b−d).

Ethylene Production Rate. As pointed out by several authors (Palma et al., 1993; Sánchez et al., 1998a) the onset of the increase in ethylene production (OEP) of cherimoya fruit occurred after the climacteric rise in respiration (Figures 1 and 2). In nonstored fruit, the time of the OEP was directly related to the time of harvest: the earlier the harvest date within the season, the earlier the production of ethylene was detectable (Figure 2a). This result was opposite that found by Kosiyachinda and Young (1975), a difference that could be explained by reasons similar to those mentioned above. Nomura et al. (1997) reported that the number of days from harvest to the ethylene peak decreased with growth. However, considering only their results obtained with physiologically mature cherimoya (those from late-pollinated fruit harvested at 140, 150, and 160 days after pollination), it appears that the relationship between ethylene production and growth was not clear.

Differences in the time of the OEP between fruit from the three harvests had no clear relationship with the time of the climacteric peak in respiration (Figure 1a). Therefore, our results support the hypothesis that the rise in respiration of cherimoya fruit is not triggered by ethylene, although ethylene may have a role in regulating the climacteric period (Kosiyachinda and Young, 1975; Palma et al., 1993; Merodio and De La Plaza, 1997).

Ethylene production during ripening at 20 °C was promoted by prior cold storage. Thus, the time to the OEP was inversely related to the duration of previous cold storage, and differences due to harvest date gradually disappeared with previous storage time (Figure 2). Enhancement of ethylene production at 20 °C following cold storage was dependent on the interaction between harvest date and storage duration. MH and LH fruit reached higher ethylene production rates following longer times of storage. A similar trend was observed in EH fruit stored for up to 6 days at 8 °C (Figure 2b,c), but after 9 days of cold storage, levels of ethylene production at 20 °C were lower than in fruit transferred after 6 days (Figure 2d). Differences in the levels of ethylene at 20 °C between fruits from the three harvest dates were marked after 3 days of cold storage (Figure 2b). After 6 days at 8 °C, significant differences in the ethylene levels during subsequent ripening were found only between EH fruit and fruit from the other two harvest dates (Figure 2c). No marked difference was shown in the patterns of ethylene production of fruit transferred to 20 °C after 9 days of cold storage (Figure 2d).

Stimulation of the ethylene production of fruit following cold storage has been reported in several crops (Field, 1990), including cherimoya (Lahoz et al., 1993; Alique, 1995). This effect is likely related to accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor (Field, 1990 and unpublished data), and to higher accumulation of internal ethylene at lower temperatures (Lahoz et al., 1993). It appears that this stimulating effect is limited after 9 days of storage at 8 °C. As cherimoya fruit is increasing its ethylene produc-

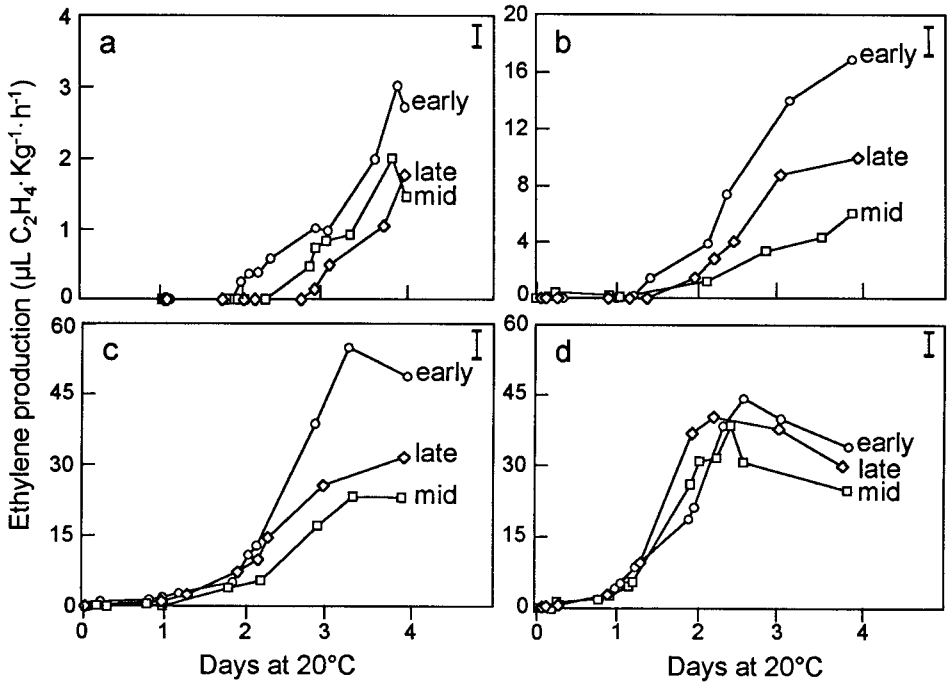

Figure 2. Ethylene production of cherimoya fruit harvested on three dates within the season (early, ○; mid, □; late, ◇) during ripening at 20 °C without prior storage (a) and after storage at 8 °C for 3 (b), 6 (c), or 9 (d) days. Vertical bar represents the maximum SD observed.

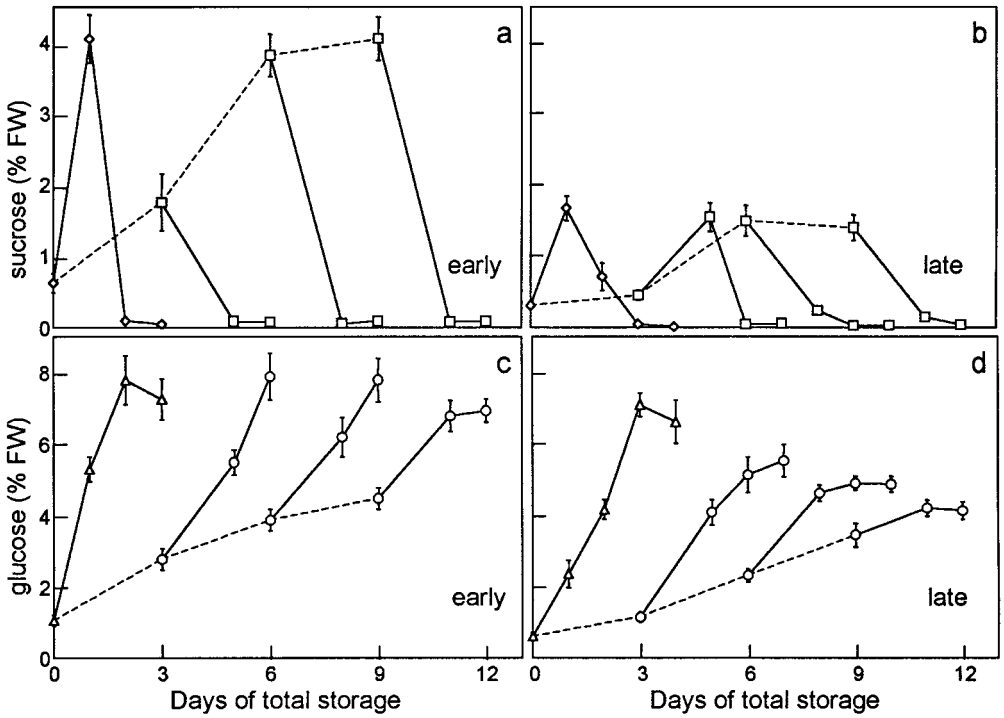

Figure 3. Changes in sucrose (◇, □) and glucose (△, ○) contents of cherimoya fruit during ripening at 20 °C (continuous line) without prior storage (◇, △) and after storage at 8 °C for 3, 6, or 9 days (□, ○). Broken lines represent the changes in sucrose and glucose contents during cold storage. Fruits were harvested at three dates: early (a, c), mid, and late (b, d). Data represent the mean ± SD of two determinations carried out in each of the three fruits analyzed.

tion during storage at this temperature, it is possible that accumulation of ACC and/or internal ethylene have already reached a maximum equilibrium on that date.

Soluble Sugars. Changes in fructose content in all conditions assayed were similar to those in glucose and therefore are not shown in Figure 3. Due to the high similarity in the evolution of the three sugars between MH and LH fruit, only changes in sucrose and glucose contents of LH fruit are represented (Figure 3b,d).

During ripening of nonstored fruit, maximum contents in the three soluble sugars were produced earlier in EH cherimoya than in those from the other two harvests, which showed no significant differences between them. The higher sucrose maximum and faster accumulation of the three soluble sugars in EH fruit may be indicative of higher starch content at harvest and/or faster starch hydrolysis. It has already been pointed out that cherimoyas harvested during warmer

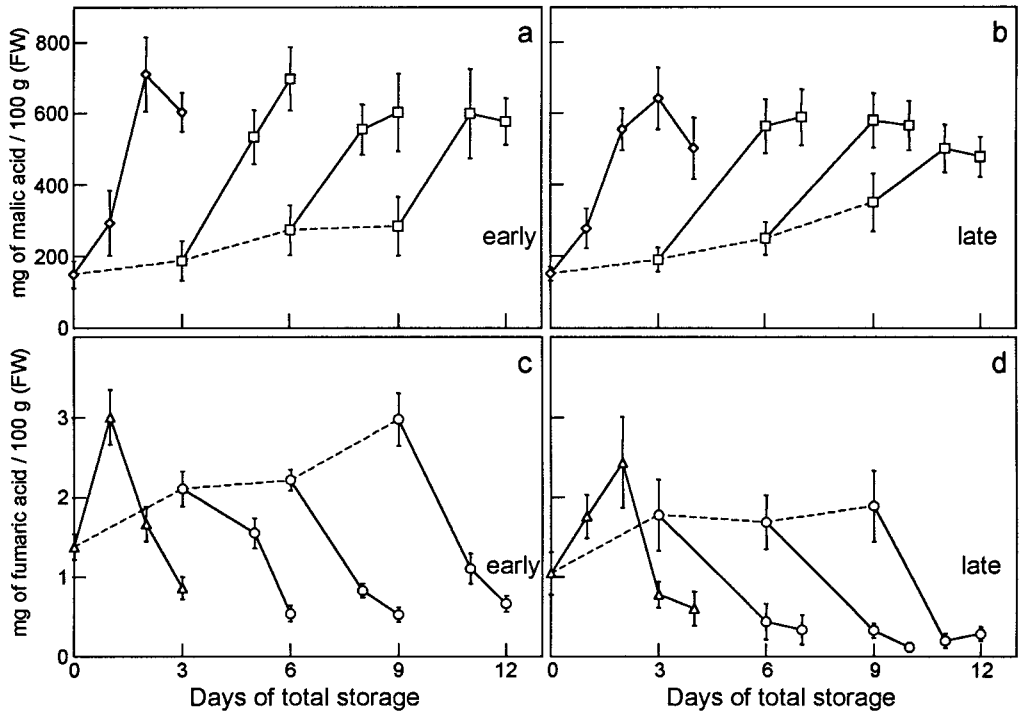

Figure 4. Changes in malic (◇, □) and fumaric (△, ○) acid contents of cherimoya fruit during ripening at 20 °C (continuous line) without prior storage (◇, △) and after storage at 8 °C for 3, 6, or 9 days (□, ○). Broken lines represent the changes in sucrose and glucose contents during cold storage. Fruits were harvested at three dates: early (a, c), mid, and late (b, d). Data represent the mean ± SD of two determinations carried out in each of the three fruits analyzed.

months, like EH fruit, had slightly greater sugar content when ripe (Lizana and Reginato, 1990).

Storage at 8 °C delayed accumulation of the three soluble sugars (Figure 3). For each harvest date, maximum sucrose levels in fruit at 8 °C were similar to those found at 20 °C (Figure 3a,b), but glucose and fructose levels were lower (Figure 3c,d). It has been shown in cherimoyas that levels of invertase, a sucrose-degrading enzyme producing glucose and fructose, are much lower at 8 °C than at 20 °C (Sánchez et al., 1998a,b). Therefore a lower rate of sucrose hydrolysis and glucose and fructose synthesis would be expected at 8 °C, although other enzymatic pathways involving these sugars could have a role on their levels.

All fruit showed almost complete sucrose hydrolysis after transfer to 20 °C, independent of harvest date and previous storage time (Figure 3a,b). Differences due to harvest date and storage duration were shown only after 3 days of cold storage: sucrose degradation was faster in EH than in MH and LH cherimoyas. The relative increases in glucose and fructose contents during ripening were reduced by prior storage as compared to increases during ripening of nonstored fruit, especially at longer times and in MH and LH fruit (Figure 3c,d). Thus, after 3 days at 20 °C, glucose content in nonstored EH and LH fruit showed about 7- and 11-fold increases, respectively. Considering the same period at 20 °C, prior storage for 3, 6, or 9 days at 8 °C produced increases in the glucose content of about 3-, 2-, and 2-fold, respectively, in EH fruit, and of about 4-, 2-, and 1.2-fold, respectively, in LH fruit. Similar results were obtained for fructose content.

It can be suggested that sucrose synthesis is greatly influenced by harvest date, resulting in a much higher sucrose content in EH than in the other fruits, and it would be just delayed and/or slightly inhibited by cold storage, therefore maintaining at 8 °C the differences due to harvest date. Glucose and fructose accumulation is less influenced by harvest date: maxima in fruit directly ripened at 20 °C, although reached later in MH and LH fruit, showed no differences due to harvest date. Prior cold storage would enhance this influence, especially after 6 days, resulting in a bigger reduction of the contents of both sugars in MH and LH fruits, as compared to EH fruits. It appears that cherimoyas harvested later within the season have a great capacity to accumulate glucose and fructose independently of sucrose hydrolysis. Thus, in MH and LH cherimoyas maximum sucrose content during ripening of nonstored fruit was about half that in EH fruit, but maximum contents of glucose and fructose were similar in all three fruit. This capacity is lost after longer cold storage duration, because although MH and LH fruit reached sucrose levels similar to those found in nonstored fruit, there was a progressive reduction of glucose and fructose contents with prior storage duration. This effect of cold storage is not due to an inhibition of sucrose hydrolysis, which proceeded normally during subsequent ripening.

Organic Acids. The evolution of the organic acids content during direct ripening, cold storage, and ripening after cold storage is shown in Figure 4. The changes in citric acid content (about half the malic acid concentration) are not shown due to their high similarity with those in malic acid (Figure 4a,b). A high similarity was also observed between the MH and LH fruit in changes in the content of the organic acids determined, and therefore these changes are shown only for LH cherimoyas (Figure 4b,d).

Organic acids content showed transient increases in fruit from all of the harvest dates when ripened directly at 20 °C (Figure 4). Maxima in the level of organic acids were reached 1 day earlier in EH than in MH and LH fruit. This again suggests a higher physiological activity

Table 3. Days To Ripen and Characterization of the Ripe Stage of Cherimoyas Harvested on Three Dates within the Season and Ripened at 20 °C without prior Storage (0 Days of Storage) and after 3, 6, or 9 Days of Storage at 8 °C[a]

parameter	days of storage	harvest date		
		early	mid	late
days to ripen	0	3	4	4
	3	3	4	4
	6	2	3	4
	9	1	2	3
firmness (N)	0	13 ± 3.2a	11 ± 2.5 a	12 ± 2.4 a
	3	11 ± 2.2 a	14 ± 3.7 a	15 ± 4.8 a
	6	12 ± 3.5 a	12 ± 2.4 a	14 ± 3.7 a
	9	8 ± 0.9 a	10 ± 1.0 a	12 ± 2.7 a
TSS[b] (% FW)	0	22.9 ± 1.1 a	20.1 ± 2.5 ab	19.5 ± 0.6 b
	3	22.8 ± 0.9 a	19.5 ± 0.7 b	19.8 ± 0.5 b
	6	23.1 ± 1.0 a	20.5 ± 0.7 b	18.5 ± 0.6 c
	9	22.6 ± 0.8 a	19.8 ± 0.5 b	18.1 ± 0.5 c
TA[b] (% FW)	0	0.21 ± 0.03 a	0.22 ± 0.06 ab	0.29 ± 0.03 b
	3	0.19 ± 0.02 a	0.21 ± 0.02 a	0.28 ± 0.03 b
	6	0.21 ± 0.02 a	0.23 ± 0.01 a	0.26 ± 0.01 b
	9	0.19 ± 0.03 a	0.23 ± 0.02 ab	0.25 ± 0.02 b

[a] Data are the means ± SD of two determinations carried out in each of three fruits. Means within a row not followed by the same letter are significantly different ($p \leq 0.05$). [b] TSS, total soluble solids; TA, titratable acidity.

of EH fruit as compared to fruit from the other harvests. However, differences in organic acids content due to the harvest date were not marked, in accordance with the results of Pérez de Oteiza et al. (1998).

Malic and citric acid contents were lower at 8 °C than in nonstored fruit, with no clear effect of the harvest date (Figure 4a,b). Fumaric acid rose to levels similar to those found in EH fruit directly ripened at 20 °C (Figure 4c), but the increase was lower in MH and LH fruit (Figure 4d). Malic and citric acid contents and their changes at 8 °C were similar to those reported by Alique et al. (1994). Because only fumaric acid showed clear changes during cold storage and the contribution of this acid to TA of cherimoya seems to be of minor importance (Palma et al., 1993; Alique et al., 1994), our results are in agreement with the slight changes in TA shown during cold storage of cv. Fino de Jete fruit reported by Alique (1995). During ripening following cold storage, malic and citric acid contents increased in all fruit, with no marked differences due to harvest date and previous storage duration (Figure 4a,b). Fumaric acid content decreased, reaching lower levels in MH and LH fruit, especially at longer storage duration (Figure 4c,d).

Fruit Quality at the Ripe Stage. The time to reach the ripe stage at 20 °C following cold storage was inversely related to the date of harvest and storage duration (Table 3). All fruit softened properly after storage and showed a white, creamy pulp with characteristic flavor and aroma. Only LH fruit ripened after 6 or 9 days of cold storage showed some pitting of the skin (brown spots with slight depressions), which has been described as a chilling injury (CI) symptom of cherimoya (Palma et al., 1993). We suggest that the appearance of skin pitting could be related to some preharvest factors, because LH fruit stored for >6 days also showed some black glomerulae in the pulp following storage. This symptom has been called "black pit" and is thought to be caused by mineral imbalances (Merodio and De La Plaza, 1997), although we could not confirm this hypothesis, as fruit from the different harvest dates had no significant differences in calcium, magnesium, or potassium contents (data not shown). Skin spotting, a CI symptom similar to pitting, is also related to preharvest factors because it can be found during the ripening of late-harvested cv. Concha Lisa and Bronceada cherimoyas, even with no previous cold storage (Palma et al., 1993).

TSS values at the ripe stage were lower at later harvest dates. Clear differences due to storage duration were found only in LH fruit, which showed lower TSS content with longer storage duration (Table 3). No significant differences were found in TA of ripe fruit due to harvest date or prior storage duration.

We have found that fruit ripening was promoted by prior cold storage and that this effect was more marked after longer storage duration and especially in earlier harvest dates. However, these observations cannot be explained by the influence of cold storage and harvest date on ethylene production (Table 3 and Figure 2). It has been suggested that ripening of *Annona* fruit could be promoted by starch breakdown (Bruinsma and Paull, 1984). Starch hydrolysis seems to be independent of the rise in ethylene during ripening of cherimoya (Lahoz et al., 1993) and is affected by cold storage to a lesser extent than acid metabolism and soluble sugars accumulation (Gutiérrez et al., 1994). Therefore, it is possible that differences in the rate of cherimoya ripening would be greatly influenced by the levels of starch-degrading enzymes and/or starch content at the time of harvest. Starch breakdown would still proceed during cold storage, resulting in a faster ripening after transfer to 20 °C as compared to nonstored fruit, but differences due to harvest date would be maintained.

The present work shows that ripening and response to cold storage of cherimoya are dependent on harvest date and that ultimately this influence could be related to the amount of heat received by fruit during the last month of development. Fruit receiving more heat during this period ripened more quickly, especially after longer cold storage duration. LH fruit, which received 26% less heat as compared to EH fruit, had a longer ripening, even after more prolonged cold storage, but showed lower TSS, glucose and fructose contents, and higher TA levels when ripe. To optimize its final quality, it may be advisable to minimize cold storage of cherimoyas harvested later in the season and/or when the last month of development has been relatively cold.

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