J. Amer. Soc. Hort. Sci. 119(3):524–528. 1994.

Tolerance of Cherimoya (*Annona cherimola* Mill.) to Cold Storage

Rafael Alique, José P. Zamorano, Mª Luisa Calvo, Carmen Merodio, and José L. De la Plaza
U.E.I. Refrigeración Vegetales, Instituto del Frío, Consejo Superior de Investigaciones Científicas, Ciudad Universitaria, 28040 Madrid, Spain

Additional index words. respiration, ethylene, firmness, soluble sugars, malic acid, citric acid, chilling injury

Abstract. **'Fino de Jete' cherimoya fruit were stored at 20, 10, 8, or 6C, 80% relative humidity. Two rises of CO_2 production and an ethylene rise following the first peak of respiration were obtained in fruit held at 20C. The ripe stage coincided with the onset of the second respiratory rise. Soluble sugar and organic acid concentration were maximal, and flesh firmness was 18 N in ripe fruit. Lower temperature reduced respiration rate and ethylene production; however, some stimulation of ethylene synthesis was observed at 10C. Cherimoyas ripened to edible condition during 6 days at 10C, but fruit maintained at 8C for up to 12 days required transfer to 20C to ripen properly. Our results suggest that high increases in CO_2 are not sufficient to complete cherimoya fruit ripening without the concurrent rise in ethylene production. Citric acid accumulation, inhibition of ethylene synthesis, and reduced accumulation of sucrose were observed during storage at 6C. Removal to 20C after 12 days at 6C resulted in no ripening, almost complete inhibition of ethylene synthesis, and severe skin browning. Thus, 8C is the lowest tolerable temperature for prolonged cold storage of cherimoya 'Fino de Jete'. Fruit can be held at 8C for up to 12 days without damage from chilling injury.**

Cherimoya is native to tropical and subtropical South America. Recently, cherimoya fruit production has increased in Spain as a result of growing demand for fresh subtropical fruit. However, production for fresh-market consumption is limited because of rapid deterioration of the fruit due to skin browning, rapid softening, and sensitivity to fungal decay. Further, extended storage of cherimoya is limited by its high susceptibility to chilling injury (CI) (De la Plaza, 1980; Fuster and Prestamo, 1980; Lizana and Irarrazabal, 1984).

Biale and Barcus (1970) classified several annonas as climacteric fruit with a multiphasic increase in respiration. Two respiration peaks were detected 5 and 10 days after harvest in 'Chaffey' cherimoyas held at 20C by Kosiyachinda and Young (1975). Fruit softened and developed flavor and aroma early in the second respiratory rise and were at optimum edible condition by day 6. The onset of ethylene production occurred ≈4 days after harvest. Brown et al. (1988) observed two successive rises in respiration and an intermediate peak of ethylene production in 'Baldwin' and 'Deliciosa' fruit.

There are few published reports concerning the effect of temperature on postharvest changes in cherimoya fruit (Fuster and Prestamo, 1980; Lahoz et al., 1993; Lizana and Irarrazabal, 1984). Skin darkening has been observed as a symptom of CI in 'Fino de Jete' fruit by Fuster and Prestamo (1980) and in 'Concha Lisa' cherimoyas by Lizana and Irarrazabal (1984). Accumulation of reducing sugars and an increase in acidity for cherimoya 'Fino de Jete' stored at 9C were reported by De la Plaza (1980). Delayed ripening was observed during storage of cherimoya at 10C (Lahoz et al., 1993). None of these studies addressed the optimal temperature for cold storage of cherimoya. Our research was conducted to determine the lowest tolerable temperature for prolonging the storage life of cherimoya fruit. Respiration rate, ethylene production, changes in flesh and skin firmness, organic acid, and soluble sugar content were examined in cherimoya 'Fino de Jete' fruit during ripening at 20C and during cold storage at 10, 8, or 6C. Respiration rate, ethylene production, and evaluation of softening and eating quality were also determined in fruit on warming at 20C after 5 and 12 days of cold storage to assess CI symptoms.

Received for publication 4 May 1993. Accepted for publication 26 Oct. 1993. This research was carried out with the support of projects ALI88-0443 (Comisión Interministerial de Ciencia y Tecnología, Spain) and STD 0266 ES (JR) (Commission of the European Communities). We thank Delfina Carrero for her laboratory assistance during this study. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Material and Methods

Plant material. 'Fino de Jete' cherimoya fruit were harvested at Granada, Spain, in February (late season), shipped by truck, and received at the Instituto del Frío laboratory (Madrid) within 15 h. Mature-green fruit (light-green skin, carpels with shallow ridges) of uniform shape weighing 180 to 190 g were randomly divided into four groups of 85 fruit and stored at 20, 10, 8, and 6 ± 0.5C, 80% relative humidity (RH). Six groups of 10 fruit were weighed and placed into ventilated storage cabinets. Ten fruit from each cabinet were moved to 20C after 5 and 12 days of storage to determine CO_2 and ethylene production and evaluate skin and flesh color, texture, and flavor. The other 40 fruit were used for firmness analysis, soluble sugar, and organic acid determinations and evaluation of CI symptoms. The remaining 25 fruit from each temperature were used for CO_2 and ethylene production measurements.

Measurements of respiration and ethylene production rates. Carbon dioxide and ethylene production were determined twice daily for fruit held at 20C and once daily for fruit at the lower temperatures. Fruit (25) at each temperature were placed in 22-liter glass jars and flushed continuously with humidified air free of ethylene and CO_2. The air flow (≈5 liter·h^{-1}) was regulated by capillaries and needle valves. Effluent air samples taken with a 1-ml syringe were injected into a gas chromatograph (model 3700; Varian, Walnut Creek, Calif.) equipped with a six-way switching valve and Porapak-Q and molecular sieve columns (2 m × 3.2 mm) in series. Carbon dioxide and ethylene were detected by thermal conductivity and flame ionization detectors, respectively, with He as carrier gas (30 ml·min^{-1}). Quantification was by external standards, and results were expressed in mg CO_2/kg per h and µl ethylene/kg per h.

Weight loss and skin and flesh firmness. Weight loss was determined daily. Five fruit from each temperature were used to determine skin rupture force and its relationship to ripening and CI.

Skin rupture force was measured at two equatorial points using an Instron testing machine (model 1140; High Wycombe, U.K.) fitted with a 1-mm-diameter cylindrical, flat-surfaced plunger, with full-scale load set at 5 N and crosshead and chart speeds at 400 mm·min^{-1}. The same fruit were used for determining flesh firmness (N). The Instron was fitted with an 8-mm cylindrical, flat-surfaced plunger with a full-scale load of 100 N. Skin sections (≈1 cm in diameter) were removed from opposite sides before flesh firmness was determined. Skin and flesh firmness were determined daily for fruit held at 20C and each 3 days for fruit at the lower temperatures. Skin and flesh color, flesh texture, and flavor were subjectively evaluated.

Soluble sugar and organic acid determinations. Fruit used for firmness determinations were also used for chemical analyses. A 10-g sample of pulp (free of skin and seeds) was homogenized in 100 ml methanol with an Omnimixer (Waterbury, Conn.) at 7000 rpm for 5 min. The homogenate was refluxed at 50C for 15 min and then filtered under vacuum. The methanol was evaporated under vacuum in a rotary evaporator at 40C and the residue was resuspended in 50 ml Milli-Q water and passed through a methanol-activated Sep-Pak C_{18} minicolumn (Waters, Milford, Mass.). The eluate was filtered through a 0.45-µm Millipore filter and 20 µl was injected in the high-performance liquid chromatography equipment.

Soluble sugars were separated on a Sugar-Pak I (Waters) column (30 cm × 9.5 mm) at 92C with deionized water at 0.8 ml·min^{-1} and detected with a refractive index detector (refractometer R-401; Waters). Organic acids were separated on a 30-cm × 6.5-mm ION-300 (Interaction chemicals, Mountain View, Calif.) column at 45C using 0.01 N H_2SO_4 as a solvent (flow rate of 0.4 ml·min^{-1}) and detected by ultraviolet absorption at 214 nm (detector model 441; Waters). Quantitative assessment in both cases was based on external standards. Soluble sugar concentrations are expressed as percentage of fresh weight (FW) and those of organic acids as mg·g^{-1} FW (n = 3). Values were corrected at each temperature for weight loss.

Results and Discussion

Respiration rate at 20C showed two marked respiratory rises, the first occurring 1 day after harvest and the second 3 days later. Ethylene production increased markedly after 2.3 days, reaching a maximum at 3.5 days from harvest, when the respiration rate was almost stabilized (data not shown).

The time of the onset of the first respiration rise and ethylene production is similar to those reported by Kosiyachinda and Young (1975) for late-season cherimoya, but differs from the time reported by the same authors for early season fruit, which occurred later. The respiration rate pattern and the maxima obtained during our experiment are similar to those observed by Kosiyachinda and Young (1975) and Brown et al. (1988) for 'Chaffey', 'Baldwin', and 'Deliciosa' fruit, respectively. It seems that the respiration and ethylene production patterns are very similar for most cherimoya cultivars reviewed by Palma et al. (1993), which showed two respiration rises and an ethylene production peak following the first rise.

Flesh firmness declined rapidly (Fig. 1A), coincident with the first CO_2 peak, to reach a value of ≈18 N on day 3. Subjective assessment showed that cherimoya softening was initiated around the receptacle. Changes in skin softening were less pronounced (Fig. 1A). The firmness measurements confirmed that softening of cherimoya fruit begins during the onset of the first respiratory rise and then proceeds quickly during the increase in ethylene production (Palma et al., 1993).

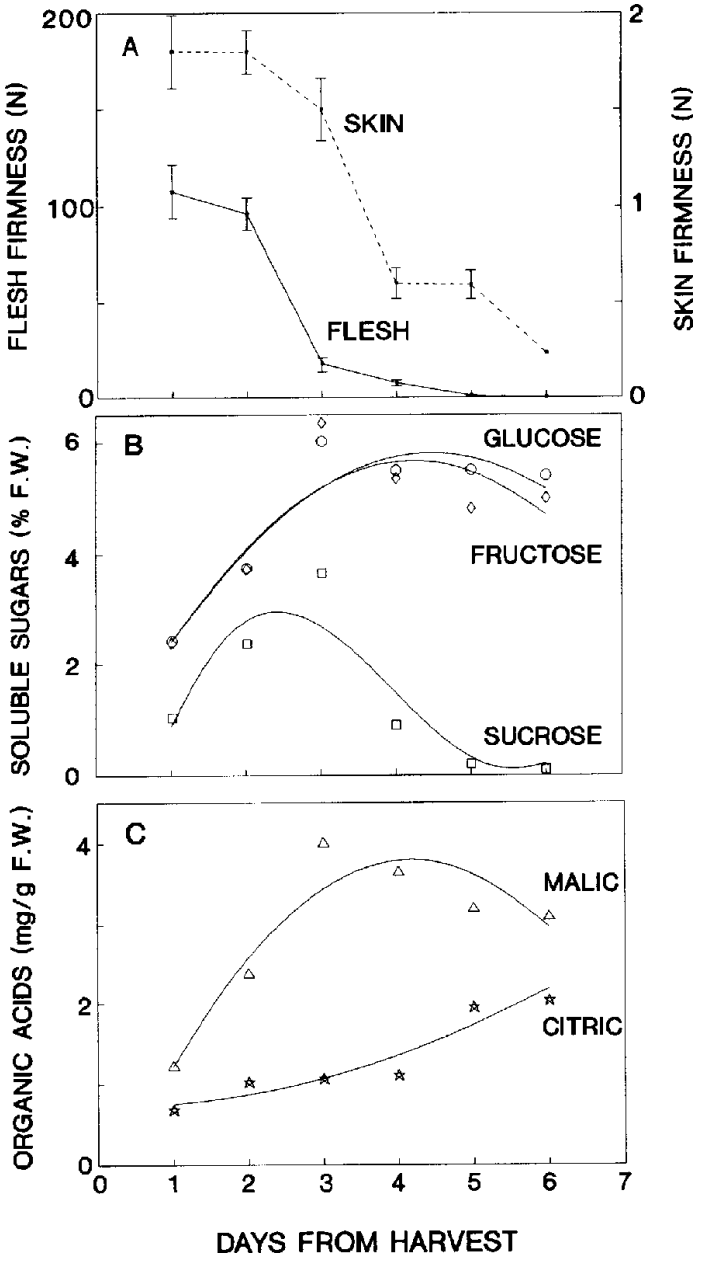

Fig. 1. Changes in flesh and skin firmness (**A**), soluble sugars (**B**), and organic acids (**C**) in cherimoya fruit stored at 20C. Each point represents a mean of five replicates for firmness and three measurements for soluble sugars and organic acids. Curves were fitted to the function $y = a + b \times x + c \times x^2$, except for sucrose, which fitted to the function $y = a + b \times x + c \times x^2 + d \times x^3$. Glucose (○), fructose (◊), sucrose (❑), malic acid (Δ), and citric acid (★).

The daily weight loss (data not shown) was higher after day 9 of storage at 10 and 6C, while at 8C it had stabilized by this time, a result suggesting that, although RH may have a stronger influence on cherimoya weight loss (Fuster and Prestamo, 1980), the effect of storage temperature is not negligible. After correcting the raw data for weight loss, regression models for sugars and organic acids content were fitted using a computer statistics program (Statgraphics, STSC, Rockville, Md.); model selection was based on R^2 values. Glucose, fructose, and citric acid responses to temperature and duration of storage fitted a second-degree polynomial regression of the form $y = a + b \times x + c \times x^2$. However, the best curve fit for sucrose content was obtained by a third-degree

polynomial regression of the form $y = a + b \times x + c \times x^2 + d \times x^3$, due to the drastic changes in its evolution observed at different temperatures. Malic acid content at 20C fitted to a second-degree polynomial regression but to a first-degree one at the lower temperatures (see below).

All of the sugars (Fig. 1B) and malic acid showed rapid increases after harvest, but citric acid increase was less pronounced (Fig. 1C). Similar results were obtained by Paull et al. (1983) for soursop (*Annona muricata* L.) and by Wills et al. (1984) for atemoya (*Annona atemoya*). The authors suggested that malic acid would be the major organic acid contributing to the increase in titratable acidity during the ripening of both fruit. According to our results, this also applies to cherimoya, since higher increases in malic acid with respect to citric acid were observed during ripening. If titratable acidity of cherimoya fruit is mainly dependent on malic acid content, the transient increase in titratable acidity observed by Lizana and Irarrazabal (1984) and Palma et al. (1993) during ripening of 'Concha Lisa' and 'Bronceada' fruit would be in accordance with our results for malic acid.

Bruinsma and Paull (1984) proposed that respiration of postharvest soursop fruit consists of a climacteric rise normally encountered in fruit with autocatalytic ethylene production, preceded by a harvest-induced, transient respiratory rise. These authors suggested that induction of this preclimacteric respiratory rise is effectuated by a temporary overshooting of the pathway of starch degradation or reduction in the level of a labile inhibitor of ethylene action. Our results suggest that a similar induction could be produced in cherimoya fruit, since the first respiratory rise paralleled the increases in soluble sugars and malic acid content (both possibly starch breakdown products), while ethylene production was very low. A temporary overshooting of the starch degradation pathway shortly after harvest was observed in green bananas (*Musa paradisiaca* L.) by McGlasson and Wills (1972).

Cherimoyas reached their optimal eating quality on day 3 at 20C. At this point, the skin was pale green with slight browning of the areoles and the flesh was creamy and white. In overripe or senescent fruit, skin browning was almost complete and began to appear in the flesh, especially around the receptacle. These fruit became watery and the flesh became translucent, having poor flavor with excessive sweetness. Translucent pulp was also observed in overripe atemoyas by Brown et al. (1988). The extensive cell-wall degradation and starch breakdown observed during cherimoya ripening (Lahoz et al., 1993) could contribute greatly to the occurrence of translucent flesh and excessive sweetness in overripe fruit.

Respiration rate was inversely related to temperature, with mean values of 100, 40, and 20 mg CO_2/kg per h at 10, 8, and 6C, respectively (data not shown). No marked variations were observed at any storage temperature. Lowering the temperature from 10 to 8C reduced respiration rate considerably more than from 8 to 6C. After 1.4 days at 10C, ethylene production increased dramatically with time, showing levels higher than the maximum at 20C at the end of storage, while at 8 and 6C remained below 20 and 1 $\mu l \cdot kg^{-1} \cdot h^{-1}$, respectively (data not shown).

Fruit softened earlier at higher temperatures and the soft stage occurred on day 6 and day 9 of storage at 10 and 8C, respectively (Fig. 2A). Fruit stored at 6C failed to attain the soft stage (18 N), even after 13 days of storage. These results suggest that softening of cherimoya fruit can be initiated even when ethylene production is very low, but that higher ethylene production may be necessary to induce adequately its complete ripening. This could be explained by the existence of multiple ethylene thresholds for different regulatory targets, as seen to occur for the transcriptional and

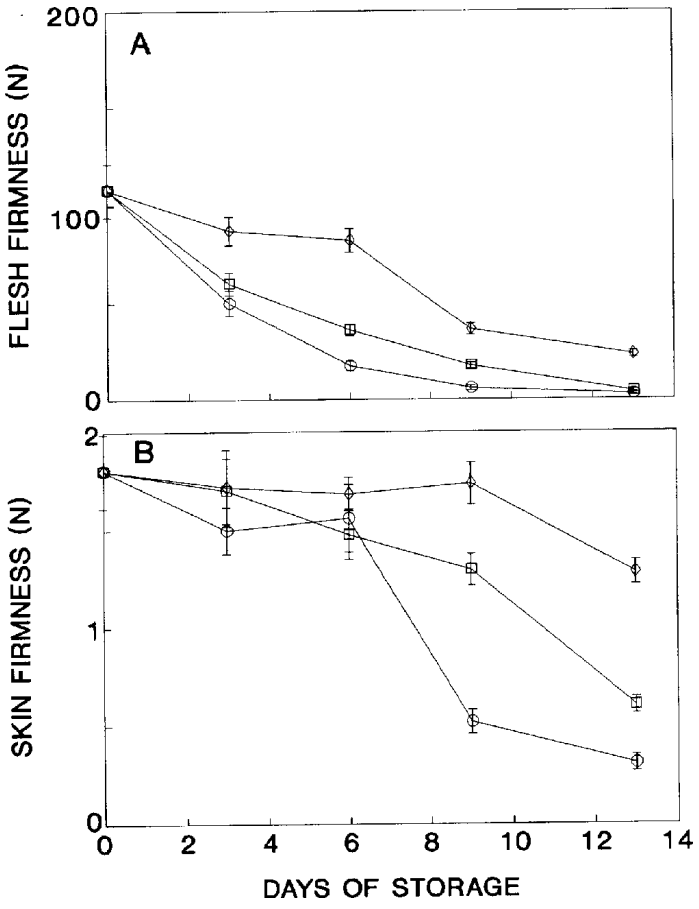

Fig. 2. Changes in firmness of cherimoya fruit stored at 10C (❍), 8C (❑), or 6C (◊). Flesh (**A**) measured as compression, skin (**B**) measured as rupture force. Each point represents a mean of five replicates ±SD (vertical bars).

posttranscriptional control of the expression of ripening related genes in tomato (*Lycopersicon esculentum* Mill.) and avocado (*Persea americana* Mill.) (Buse and Laties, 1993).

Skin rupture force decreased more slowly than flesh firmness at all temperatures (Fig. 2B). At 6C, skin rupture force decreased to only 94%, whereas flesh firmness decreased to 34% of the initial value after 9 days of storage. In contrast, at 10C, skin rupture force decreased to 28%, whereas flesh firmness decreased to 5% of the initial value after 9 days of storage. At 6C, skin softening could be a CI symptom, since skin rupture force decreased only after 9 days of storage, when fruit were entering into the irreversible stage of CI, as showed during transfer to 20C (see below).

The trends in glucose showed an increase from the beginning of storage at 10, 8, and 6C, with levels directly related to temperature (Fig. 3A). Fructose trends were similar to those of glucose, but showed slight decreases after 9 days at 8 and 6C (Fig. 3B). Therefore, low temperature delayed glucose and fructose accumulation but did not substantially affect the trends observed at 20C. Glucose and fructose accumulation during tomato ripening still took place when fruit were stored in a controlled atmosphere, a result suggesting that synthesis of these monosaccharides depended on enzymes already present in the fruit at the time of harvest (Goodenough et al., 1982). A similar event would explain the effect of low temperature on glucose and fructose accumulation in cherimoya.

The trends in sucrose content evolution at 10 and 8C were similar to those at 20C (Fig. 3C), but, at 6C, the pattern of sucrose evolution was drastically altered, with no variations until day 9,

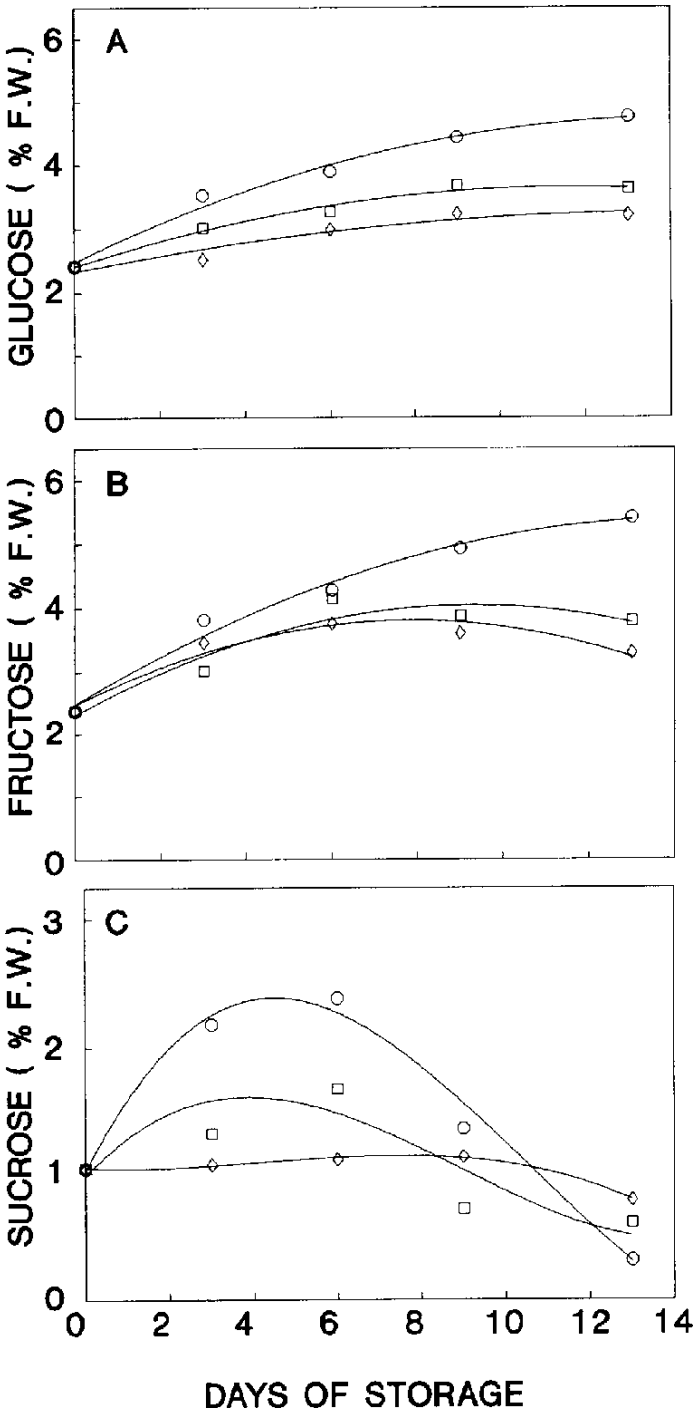

Fig. 3. Soluble sugars in cherimoya fruit stored at 10C (○), 8C (❑), or 6C (◊). Each point represents a mean of three measurements. Curves were fitted to the function $y = a + b \times x + c \times x^2$, except for sucrose, which fitted to the function $y = a + b \times x + c \times x^2 + d \times x^3$. Glucose (**A**), fructose (**B**), and sucrose (**C**).

when a very slight decrease was observed. It has been reported that sucrose in some plant tissues is hydrolyzed to reducing sugars by a low-temperature-induced invertase (Purvis and Rice, 1983). It could be speculated that induction of an invertase during storage of cherimoya fruit at 6C would reduce the harvest effect on sucrose accumulation (Bruinsma and Paull, 1984) leading to the observed glucose and fructose content, similar to those at 8C.

Low temperature delayed malic acid accumulation, with few differences between storage temperatures (Fig. 4A). The pattern of malic acid evolution at any temperature fits a single first-degree

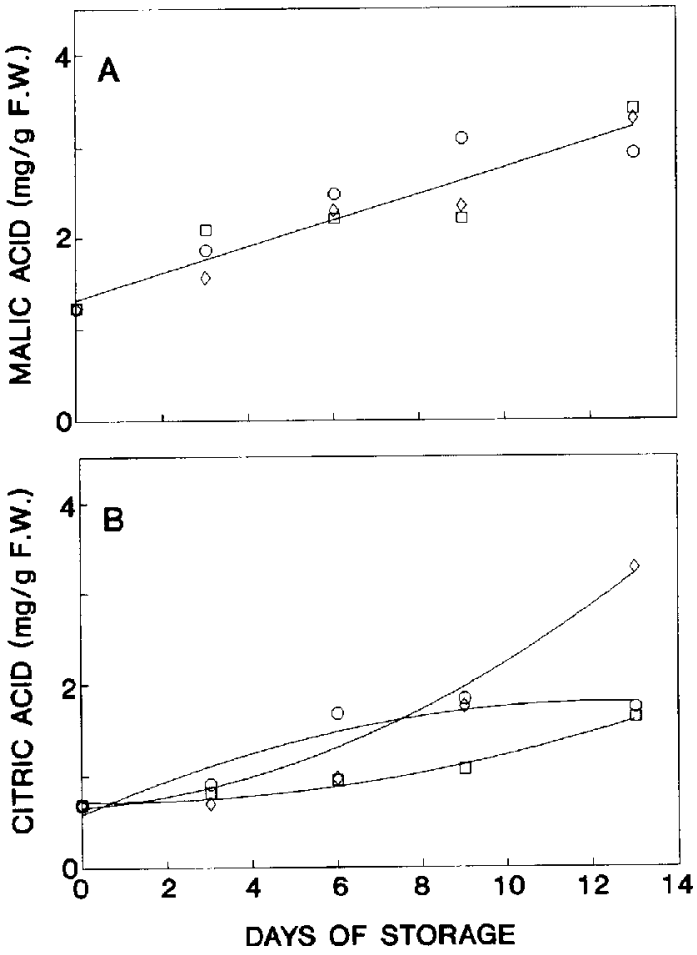

Fig. 4. Organic acids in cherimoya fruit stored at 10C (○), 8C (❑), or 6C (◊). Each point represents a mean of three measurements. Malic acid evolution at the three temperatures (**A**) was fitted to the following first degree polynomial function: $y = 1.3 + 0.1 \times x$, R^2=0.95. Curves for citric acid (**B**) were fitted to the function $y = a + b \times x + c \times x^2$.

polynomial function, showing fairly strong linearity (R^2 = 0.95). Citric acid content was lower at 8 than at 10C (Fig. 4B). However, at 6C, behavior was similar to that at 8C, except that the increasing trend was more marked, exceeding on day 13 the value obtained at 20C. Our results suggest that the effect of low temperature on organic acid synthesis is not explained only by a reduction in the maximal or limiting reaction rate of the different reactions involved and that citric acid content could be used as another possible indicator of CI in cherimoya. This suggestion is in line with the previous report of citric acid accumulation at chilling temperatures in tomato (Buescher, 1975).

Respiration increased after transfer to 20C, reaching values similar to those obtained for fruit held at 20C; prior storage temperature had no obvious effect after storage for 5 days (Table 1). Ethylene production increased in fruit transferred after 5 days of storage at 10, 8, or 6C, reaching maxima after 0.8, 1.8, and 2.5 days at 20C, respectively, and fruit were edible a few hours after these maxima. These results show that the time of the maxima in ethylene production after transfer to 20C may be used to distinguish between ripeness stages during storage. An abrupt increase in respiration rate after transfer to 20C was observed in fruit stored for 12 days. Ethylene evolution in these fruit showed clear differences between previous storage temperatures. Ethylene production decreased slowly in fruit stored at 10C after 0.8 days at 20C (at this time the fruit showed the first symptoms of senescence). At 8C

Table 1. Trends and days to reach the maxima in respiration and ethylene production rate of cherimoya fruit at 20C after storage for 5 and 12 days at three temperatures.

Storage		Trend[z]		Days at 20C to reach the maximum		Ripeness at
Temp (°C)	Days	Ethylene	Respiration	Ethylene	Respiration	20C
10	5	T	C	0.8	---	Ripe
8	5	T	C	1.8	---	Ripe
6	5	T	C	2.5	---	Ripe
10	12	D	T	---	0.9	Overripe
8	12	T	T	0.8	0.9	Ripe
6	12	S	T	---	0.9	Disrupted[y]

[z]T = transient increase; C = continuous increase; D = decrease; S = stabilized at low levels.
[y]Disrupted = fruit showing severe browning and off-flavors.

it increased, reaching a maximum 0.8 days after removal to 20C, and at 6C it was almost undetectable after 1.5 days at 20C. In cherimoyas transferred from 8C, the edible stage was attained a few hours after the ethylene peak, but fruit stored at 6C lost the capacity to ripen properly and developed severe skin browning (Table 1). Ethylene production at 20C after cold storage seems to be a good index to assess CI of cherimoya fruit. On the other hand, the ethylene production at 20C of fruit stored at 10C suggests that the high level of ethylene observed during storage could be produced by reversible low-temperature stress, whose effect would exceed the reduction in the fruit metabolism due to low temperature. Finally, our results indicate that the ethylene peak could serve to accelerate and coordinate the ripening changes in cherimoya (Lahoz et al., 1993; Palma et al., 1993) as seen to occur in other annonas (Paull, 1982), and that high increases in CO_2 are not sufficient to complete cherimoya fruit ripening without the concurrent rise in ethylene production.

The above results support previous findings (Fuster and Prestamo, 1980; Lahoz et al., 1993) on the extreme susceptibility of cherimoya to CI. Fruit ripened during 10C storage but those stored at 8C needed to be transferred to 20C to reach a similar eating quality. Further, cherimoyas stored at 6C for >5 days lost their ability to ripen and had off-flavors and severe skin browning when transferred to 20C. Other CI symptoms observed in cherimoya during storage at 6C were inhibition of ethylene production, marked reduction of skin softening and accumulation of sucrose, and, particularly, citric acid accumulation.

In the light of our results, we propose 8C as the lowest tolerable temperature to store 'Fino de Jete' cherimoya fruit.

Literature Cited

Biale, J.B. and D.E. Barcus. 1970. Respiration patterns in tropical fruits of the Amazon Basin. Trop. Sci. 12:93–104.

Brown, B.I., L.S. Wong, A.P. George, and R.J. Nissen. 1988. Comparative studies on the postharvest physiology of fruit from different species of *Annona* (custard apple). J. Hort. Sci. 63:521–528.

Bruinsma, J. and R.E. Paull. 1984. Respiration during postharvest development of soursop fruit, *Annona muricata* L. Plant Physiol. 76:131–138.

Buescher R.W. 1975. Organic acid and sugar levels in tomato pericarp as influenced by storage at low temperature. HortScience 10:158–159.

Buse, E.L. and G.G. Laties. 1993. Ethylene-mediated posttranscriptional regulation in ripening avocado (*Persea americana*) mesocarp discs. Plant Physiol. 102:417–423.

De la Plaza, J.L. 1980. Controlled atmosphere storage of cherimoya. Proc. XVth Intl. Congr. Refrigeration, 23–29 Sept., Venice, Italy 3:701–712.

Fuster, C. and G. Prestamo. 1980. Variation of cherimoya (*Annona cherimolia*) texture during storage, as determined with an Instron food testing instrument. J. Food Sci. 45:142–145.

Goodenough, P.W., G.A. Tucker, D. Grierson, and T. Thomas. 1982. Changes in colour, polygalacturonase and organic acids during storage of tomatoes. Phytochemistry 21:281–284.

Kosiyachinda, S. and R.E. Young. 1975. Ethylene production in relation to the initiation of respiratory climacteric in fruit. Plant Cell Physiol. 16:595–602.

Lahoz, J.M., M. Gutiérrez, M.M. Sola, R. Salto, L. Pascual, M. Martínez-Cayuela, and A.M. Vargas. 1993. Ethylene in cherimoya fruit (*Annona cherimola* Mill.) under different storage conditions. J. Agr. Food Chem. 41:721–723.

Lizana, L.A. and M.J. Irarrazabal. 1984. Comportamiento en post-cosecha de chirimoya (*Annona cherimola* Mill.) sometida a bajas temperaturas y encerado. Proc. Trop. Region Amer. Soc. Hort. Sci. 28:63–70.

McGlasson, W.B. and R.B.H. Wills. 1972. Effects of oxygen and carbon dioxide on respiration, storage life, and organic acids of green bananas. Austral. J. Biol. Sci. 25:35–42.

Palma, T., J.M. Aguilera, and D.W. Stanley. 1993. A review of postharvest events in cherimoya. Postharvest Biol. Technol. 2:187–208.

Paull, R.E. 1982. Postharvest variation in composition of soursop (*Annona muricata* L.) fruit in relation to respiration and ethylene production. J. Amer. Soc. Hort. Sci. 107:582–585.

Paull, R.E., J. Deputy, and N.J. Chen. 1983. Changes in organic acids, sugars, and headspace volatiles during fruit ripening of soursop (*Annona muricata* L.). J. Amer. Soc. Hort. Sci. 108:931–934.

Purvis, A.C. and J.D. Rice. 1983. Low temperature induction of invertase activity in grapefruit flavedo tissue. Phytochemistry 22:831–834.

Wills, R.B.H. , A. Poi, H. Greenfield, and C.J. Rigney. 1984. Postharvest changes in fruit composition of *Annona atemoya* during ripening and effects of storage temperature on ripening. HortScience 19:96–97.