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

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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 6°C, 80% relative humidity. Two rises of CO₂ production and an ethylene rise following the first peak of respiration were obtained in fruit held at 20°C. The ripening 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 10°C. Cherimoyas ripened to edible condition during 6 days at 10°C, but fruit maintained at 8°C for up to 12 days required transfer to 20°C to ripen properly. Our results suggest that high increases in CO₂ 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 6°C. Removal to 20°C after 12 days at 6°C resulted in no ripening, almost complete inhibition of ethylene synthesis, and severe skin browning. Thus, 8°C is the lowest tolerable temperature for prolonged cold storage of cherimoya ‘Fino de Jete’. Fruit can be held at 8°C 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 20°C 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 9°C were reported by De la Plaza (1980). Delayed ripening was observed during storage of cherimoya at 10°C (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 20°C and during cold storage at 10, 8, or 6°C. Respiration rate, ethylene production, and evaluation of softening and eating quality were also determined in fruit on warming at 20°C after 5 and 12 days of cold storage to assess CI symptoms.

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 ± 0,5°C, 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 20°C after 5 and 12 days of storage to determine CO₂ 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 CO₂ and ethylene production measurements.

Measurements of respiration and ethylene production rates. Carbon dioxide and ethylene production were determined twice daily for fruit held at 20°C 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 CO₂. 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 CO₂/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 C₁₈ 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 H₂SO₄ 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 CO₂ 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 produc-

Fig. 1. Changes in flesh and skin firmness (A), soluble sugars (B), and organic acids (C) in cherimoya fruit stored at 20°C. 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 $y = a + bx + cx^2 + dx^3$. Glucose (◊), fructose (□), sucrose (itious (§), malic acid (Δ), and citric acid (★).
ripe fruit. Occurrence of translucent flesh and excessive sweetness in over-
cell-wall degradation and starch breakdown observed during cheri-
served in overripe atemoyas by Brown et al. (1988). The extensive
became watery and the flesh became translucent, having poor
appear in the flesh, especially around the receptacle. These fruit
senescent fruit, skin browning was almost complete and began to
the areoles and the flesh was creamy and white. In overripe or
20C. At this point, the skin was pale green with slight browning of
explained by the existence of multiple ethylene thresholds for differ-
targets, as seen to occur for the transcriptional and
posttranscriptional control of the expression of ripening related
genes in tomato (Lycopersicon esculentum Mill.) and avocado
(Perssea americana Mill.) (Buse and Laties, 1993).
Skin rupture force decreased more slowly than flesh firmness at
temperatures. Malic acid content at 20C fitted to a second-degree
evolution was drastically altered, with no variations until day 9,
fruit with autocatalytic ethylene production, preceded by a harvest-induced, transient respiratory rise. These authors
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 cheri-
moya ripening (Lahoz et al., 1993) could contribute greatly to the
occurrence of translucent flesh and excessive sweetness in over-
rip fruit.
Respiration rate was inversely related to temperature, with
mean values of 100, 40, and 20 mg CO₂/kg per h at 10, 8, and 6C,
respectively (data not shown). No marked variations were ob-
served 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 dramati-
cally 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
µl·kg⁻¹·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 slow, but that higher ethylene production may be necessary
to induce adequately its complete ripening. This could be ex-
plained by the existence of multiple ethylene thresholds for differ-
ent regulatory targets, as seen to occur for the transcriptional and
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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 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...
it increased, reaching a maximum 0.8 days after removal to 20°C, and at 6°C it was almost undetectable after 1.5 days at 20°C. In cherimoyas transferred from 8°C, the edible stage was attained a few hours after the ethylene peak, but fruit stored at 6°C lost the capacity to ripen properly and developed severe skin browning (Table 1). Ethylene production at 20°C after cold storage seems to be a good index to assess CI of cherimoya fruit. On the other hand, the ethylene production at 20°C of fruit stored at 10°C 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 CO₂ 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 10°C storage but those stored at 8°C needed to be transferred to 20°C to reach a similar edible stage. Cherimoyas transferred from 8°C, the edible stage was attained a few hours after the ethylene peak, but fruit stored at 6°C lost their ability to ripen and had off-flavors and severe skin browning when transferred to 20°C. Other CI symptoms observed in cherimoya during storage at 6°C 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 8°C as the lowest tolerable temperature to store ‘Fino de Jete’ cherimoya fruit.

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