Role of Ethylene and Abscisic Acid in Physicochemical Modifications during Melon Ripening

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Hormonal metabolism associated with fruit ripening in two cantaloupe muskmelon cultivars, Talma and Manta, has been studied. The ethylene crisis began on day 33 after fruit set, reaching the maximum values of internal ethylene concentration and ethylene production rate on day 35 after fruit set. This was the optimum moment for consumption as shown by the higher content in soluble solids, ripening index, sensory analysis, and color parameter values. The *b* parameter and the *b*/*a* quotient values in peel were good indicators of the maturity stage, the optimum moment for harvesting being about day 33 after fruit set (when autocatalytic ethylene synthesis has begun), with values of 20 and 5 for the *b* parameter and *b*/*a* quotient, respectively. In both cultivars, free 1-aminocyclopropane-1-carboxylic acid (ACC) content increased until day 35 after fruit set and conjugated ACC increased in postclimacterium. The increase in both ACC-synthase and ACC-oxidase activities together could be responsible for the climacteric ethylene production. Significant differences in the abscisic acid evolution in Talma and Manta cultivars were reached, and also a possible stimulation of ethylene by this hormone could be established.

Keywords: *Cucumis melo; ethylene; abscisic acid; ACC-synthase; ACC-oxidase*

INTRODUCTION

Many diverse types of marketed melons differ in shape, external and internal color, surface netting, sweetness, flavor, and storability. However, morphological and physiological changes during fruit growth, maturation, and ripening of many melon cultivars have not been extensively studied, even in countries where these fruits are very popular and they are grown with commercial purposes. Many cultivars of netted summer melons (*Cucumis melo*, Reticulatus group) tend to have a rapid climacteric close to fruit maturity and abscission, with the interval between the preclimacteric minimum and the climacteric peak being between 24 and 48 h; fruits of other cultivars termed winter melons, such as Honeydew and Casaba cultivars (*Cucumis melo*, Inodorus group), which are nonnetted, do no abscise at maturity, and the climacteric may extend over several days or even may be absent (Kendall and Ng, 1988; Nukaya et al., 1986).

The role of other endogenous phytohormones, such as abscisic acid (ABA), in melon ripening is not yet clear, and contradictory results have been found. Thus, Zhang and Yang (1987) showed that endogenous ABA appeared first, and it accumulated during maturation and, after that, autocatalytic ethylene production was triggered. However, Larrigaudière et al. (1995) found that for melon fruits picked at different maturity stages, changes in ABA content were complementary to those of ethylene production, the maximum ABA levels being coincident with the maximum ethylene production for every maturity stage.

Therefore, further research is needed to clarify the relationship between chemical composition and hormonal changes that occur during melon maturation. The purpose of this work was the study of the evolution of the different chemical−physical parameters, which are quality indicators, during ripening of two melon cultivars, Talma and Manta, and its relationship with ABA and ethylene biosynthesis.

MATERIALS AND METHODS

Plant Material. For this assay, cantaloupe melons (*C. melo* L. Reticulatus) from Talma and Manta cultivars were used; 176 plants from each cultivar were grown in a multitunnel greenhouse. Pollination was made by hand, and female flowers were marked with the pollination date to know at the moment of sampling the number of days from the fruit set. To obtain fruits with homogeneous growing rate, four or five fruits were left for each plant.

Early in the morning five fruits from each cultivar were taken after 10, 14, 18, 22, 27, 31, 35, and 40 days from fruit set. In the laboratory, they were maintained at 20 °C for 5 h to allow the scar formation in the peduncle cut. Each fruit was then weighed, ethylene production rate measured, and color determined using the Hunter Lab system in a Minolta colorimeter: In the peel, four determinations were made in opposite faces of the fruit, and in the pulp, four determinations were made in the equatorial area in each fruit. After that, the pulp was cut into small pieces (0.2 cm × 0.5 cm), frozen in liquid N_2, and ground to obtain homogeneous samples of each fruit. These samples were then stored at −70 °C until analytical determinations were carried out.

Ethylene Analyses. The ethylene production rate of melon was determined by placing each fruit in a container hermeti-

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cally sealed with a rubber stopper. After 1 h, 1 mL of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a stainless steel column (3 m × 3.25 mm) containing activated alumina of 80/100 mesh. This was expressed as nanoliters given off per gram of tissue per hour (nL g^{-1} h^{-1}). Fruit internal ethylene concentration was monitored using an external collector gas apparatus (Ayub et al., 1995). Gas samples were taken early in the morning every 2 or 3 days with a syringe and analyzed by gas chromatography as described above. Ethylene was expressed as parts per million.

Total Soluble Solids Content (TSSC) and Titratable Acidity (TA) Determinations and Ripening Index (RI). TSSC in the pulp was determined in each fruit in triplicate using a P20 RL2 refractometer at 20 °C, and their concentration was designated in °Brix. TA was determined in triplicate by potentiometric titration with 0.1 N NaOH up to pH 8.1 using 1 mL of diluted juice in 25 mL of distilled H_2O. The results were expressed as grams of citric acid per 100 g of fresh weight. RI was determined as the relation TSSC/TA.

ACC Extraction and Quantification. Free ACC and conjugated ACC were extracted with trichloroacetic acid as described previously (Martínez et al., 1993). Conjugated ACC was hydrolyzed as stated by Hoffman et al. (1983), and ACC quantification was made according to the method of Lizada and Yang (1979). Two separate extractions were made from each sample, and for each extraction ACC was quantified in triplicate.

ACC-Synthase and ACC-Oxidase Activities. Both enzymes were extracted and assayed as described by Mansour et al. (1986). The enzymatic activity was given as nanomoles of ACC per milligram of protein per hour. The quantification of proteins was made according to the method of Bradford (1976). Two extractions were made for each fruit, and each one was assayed in triplicate.

ABA Quantification. ABA was extracted and quantified as previously reported (Martínez-Madrid et al., 1996) by an enzyme-linked immunosorbent assay (ELISA) using IgG monoclonal antibody. The ABA content was estimated by comparison with the standard curve prepared for each plate. Two extracts were made from each melon; for each extract four dilutions were prepared (which were quantified in duplicate), and at least three of them fell onto the standard curve. Results were expressed as picomoles of ABA per gram of fresh weight.

Sensory Analysis. A sensory analysis was carried out on all samples, with five trained judges, who judged fruit taste according to the following parametric scale: 1 = insipid; 2 = sweet; 3 = very sweet; and 4 = rare tastes. The results show the mean value of the five fruits analyzed in each tasting session.

Statistical Design. Experimental data are the mean ± SE of the determinations for each sample. A variance analysis using the Student *t* test was performed to determine whether differences between means of the cultivars were significant at the level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Weight. Figure 1 shows that fresh weight variation has a typical sigmoid curve with three clearly defined phases. The first one covers from fruit set until 10 days later, the second one until 21 days, and the third until 40 days, with daily increases in fresh weight of 19.9, 41.4, and 8.9 g for Talma and 22.5, 41.4, and 8.8 g for Manta, respectively. Both cultivars presented similar behaviors and a period of maximum growth between 10 and 21 days. Later, weight became practically stable, previous to the moment when the start of the ripening processes was appreciated, as will be seen later. Other melon cultivars, Honey Dew (Pratt et al., 1977; Bianco and Pratt, 1977), Honey Loupe, and Amarelo (Miccolis and Saltveit, 1991), showed sigmoid growth curves, although a bit displaced in time, due to the fact that stabilization started between 30 and 35 days from fruit set.

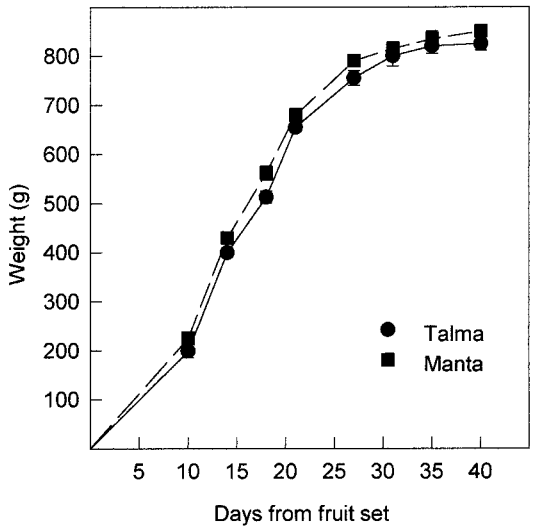

Figure 1. Fresh weight evolution during development of Talma (●) and Manta (■) melon cultivars. Data are the mean ± SE of five fruits.

Internal Fruit Ethylene and Ethylene Production Rate. Figure 2a shows the evolution of the internal ethylene given off by melon in the plant from the day 21 after fruit set. Autocatalytic ethylene synthesis started on day 33, and the maximum ethylene production occurred on day 35 for both cultivars, diminishing sharply later. This ethylene crisis led to the short ripening period in cantaloupe melon, which lasted ∼5 days. This fact made it difficult to choose the optimum moment for harvesting. Moreover, when the ethylene maximum was reached, an abscission area began to appear in the insertion of the peduncle with the fruit, which caused the detachment from the plant in a very short time. Ayub et al. (1996), using the same internal ethylene sampling system, found in cantaloupe melon a similar evolution and a very intense ethylene crisis on day 40 after fruit set. Lyons et al. (1962), with a different gas collector, determined ethylene concentration in the seed cavity of the cantaloupe melon, which was harvested in six ripening stages, and they found an ethylene concentration <0.3 ppm until day 37, which increased rapidly from this day on. Shellie and Saltveit (1993) found, in other melon varieties, very similar ethylene concentrations in the fruit internal cavity, during ripening in the plant and when split from it.

During 32 days from fruit set, the internal ethylene level was very low, <0.1 ppm. Due to this fact, we considered that in order to study ripening evolution, analytical determinations of parameters related to ripening must be initiated during the preclimacteric period and close to the moment of the beginning of autocatalytic ethylene synthesis. For this reason, these determinations were made on days 27, 31, 35, and 40 from fruit set.

Figure 2b shows ethylene production of those melons harvested on days 27, 31, 35, and 40. Behavior similar to that found when internal ethylene was determined can be observed. Ethylene production was very low on day 27 from fruit set, and it sharply increased from this day to reach the maximum production of 89 and 104 nL g^{-1} h^{-1} on day 35 from Talma and Manta cultivars, respectively, although these differences were not sig-

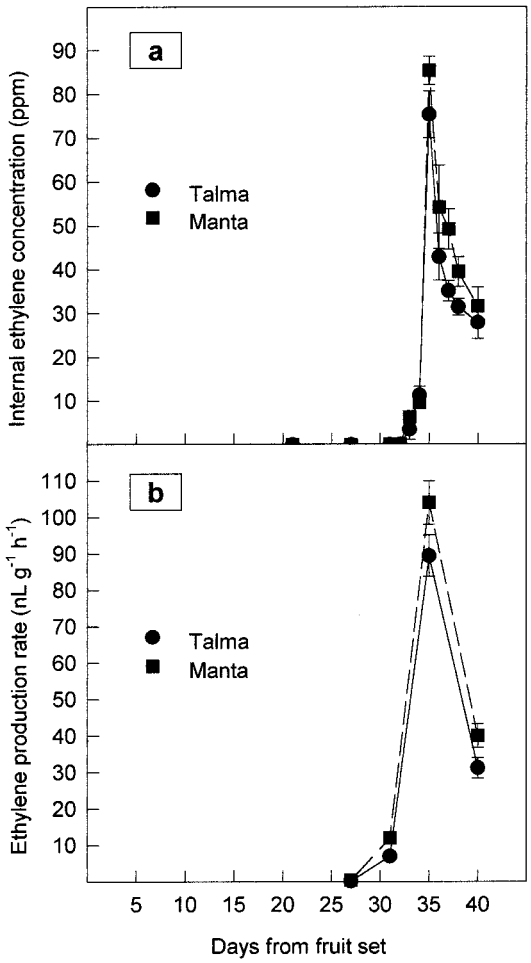

Figure 2. Internal ethylene concentration (a) and ethylene production rate (b) during ripening of Talma (●) and Manta (■) melon cultivars. Data are the mean ± SE of determinations made independently in five fruits.

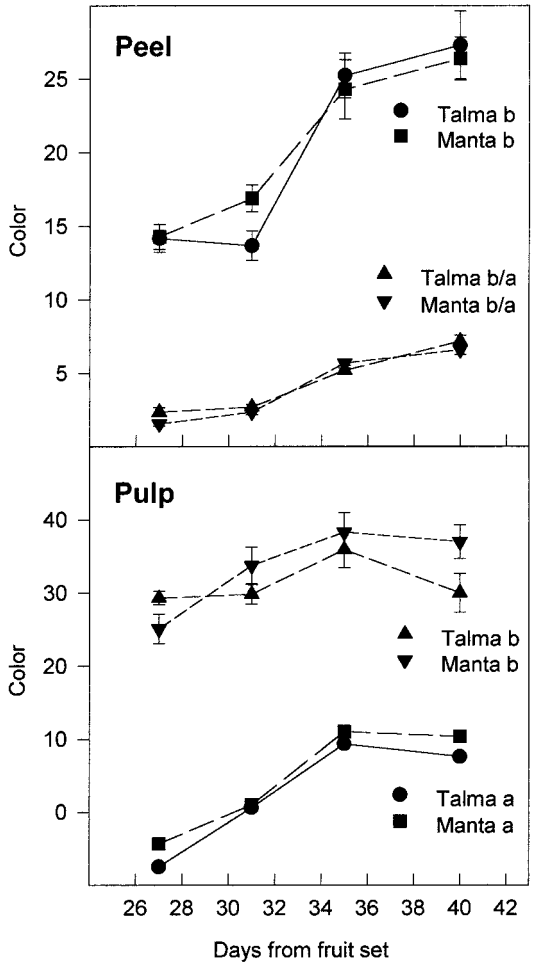

Figure 3. Peel and pulp color evolution during ripening of Manta and Talma melon cultivars. Data are the mean ± SE of four determinations made independently in each of the five fruits.

nificant at the level of $p \leq 0.05$. Production then diminished to values of ~40 nL g^{-1} h^{-1} 40 days after fruit set. Although both cultivars showed identical ethylene evolutions, the maximum ethylene peak was significantly higher for Manta cultivar, not only when internal ethylene concentration was measured but also when ethylene production rate was determined after harvesting. Similar results have been obtained in Alpha variety melons picked at different days before commercial harvesting, in which a pronounced ethylene crisis lasting ~5 days has been observed (Larrigaudière et al., 1995). Lyons et al. (1962) also found in cantaloupe melon a reduction in ethylene production after the maximum, although this was less pronounced. However, other melon varieties showed a stabilization of ethylene levels after reaching the maximum (Shellie and Saltveit, 1993). These differences could explain the rapid ripening and the short preservation period of cantaloupe melons in comparison with other varieties.

Color Parameter Evolution in Peel and Pulp. Figure 3 shows color evolution of the peel, which is represented by parameter *b* and the relation *b*/*a*. There is no representation of the evolution of parameters *a* and *L* because they changed very slightly, and consequently modifications in the ripening stage cannot be determined. Value of the parameter *b* increased in both cultivars from values close to 14 up to 26 and 27.03 for Manta and Talma, respectively. Both cultivars suffered a rapid increase from day 31 after fruit set, which indicated the beginning of significant changes related to photosynthetic pigments, with a chlorophyll degradation and carotenoid synthesis, showing an advance in the melon ripening process. Forbus and Senter (1989) and Ayub et al. (1995) also found a rapid increase of the *b* parameter from day 38 after fruit set in Charentais melon, reaching maxima values of 25. The relation *b*/*a* suffered a similar evolution, with an increase from day 31 to day 35 after fruit set, changing from 2.36 to 5.70 and from 2.70 to 5.21 in Manta and Talma, respectively. According to these results it could be established that in the peel the parameter *b* and the relation *b*/*a* could be used as indices of ripening stage. When *b* values are close to 20 and *b*/*a* values are close to 5, the optimum time for harvesting is quite near. This is due to the fact that climacterium has been initiated and the fruit will reach optimum sensory characteristics during the marketing period. These indices together with the senescence of the two leaves closest to the melon and the start of abscission at the peduncle area allow determination of the optimum moment for harvesting.

The parameter *a* in the pulp suffered a sharp increase between days 27 and 35 (Figure 3), with no significant differences between the cultivars. This increase is due to carotenoid synthesis, β-carotene in particular (Reid et al., 1970); the pulp reached the

Table 1. Chemical and Sensorial Attributes during Ripening of Talma and Manta Melon Cultivars

days after fruit set	TSSC		TA		RI		sensory analysis	
	Manta	Talma	Manta	Talma	Manta	Talma	Manta	Talma
27	6.19 ± 0.07	6.39 ± 0.36	0.12 ± 0.02	0.11 ± 0.03	51.4 ± 2.9	58.9 ± 2.7	1	1
31	6.99 ± 0.01	6.98 ± 0.31	0.10 ± 0.04	0.10 ± 0.02	69.9 ± 4.1	69.8 ± 3.5	1	1
35	12.21 ± 0.92	11.02 ± 0.96	0.09 ± 0.01	0.07 ± 0.01	135.6 ± 9.4	157.4 ± 9.2	3	3
40	13.77 ± 0.74	12.19 ± 0.57	0.07 ± 0.01	0.06 ± 0.01	196.7 ± 12.5	203.2 ± 13.5	4	4

maximum orange color, characteristic of this melon, at the same time of the ethylene crisis, and *a* diminished slightly in both cultivars in the last sampling. The evolution of parameter *a* appeared to indicate that carotenoid synthesis in these cultivars began before autocatalytic ethylene crisis, because it changed from negative values (−7.47 in Talma and −4.33 in Manta) to positive ones, thus indicating an increase in carotenoid content, between days 27 and 31 after fruit set. Reid et al. (1970) obtained similar results in cantaloupe PMR 45 and Ayub et al. (1996) in cantaloupe Charentais, with a carotenoid accumulation 10 days before climacteric ethylene production.

The value of parameter *b* in the pulp also rose, in a less pronounced way, until day 35, reaching values of 38.32 and 35.94 for Manta and Talma, respectively. When the fruits were overripe, those values decreased for Talma and more slightly for Manta cultivar (Figure 3). Parameter *L* in the pulp was ∼75 in both cultivars on day 27 after fruit set and decreased with maturity to 58.5 for Manta and 45.12 for Talma.

TSSC, TA, RI, and Sensory Characteristics. Total sugar levels remained low until day 27 for both cultivars. Once the maturity process started, sugar contents showed a sharp increase between day 31 and day 35 after fruit set with a similar growth rate in both cultivars, although the highest values were observed for Manta, which reached levels of TSSC of 13.77 °Brix on day 40, whereas in the Talma cultivar the TSSC was 12.19 (Table 1). In the case of cantaloupe melon, sugar content is a very important sensory attribute. For this reason it is interesting to remark that the values obtained in this work are higher than those found by other authors with the same melon varieties cultivated in different production areas (Guis et al., 1998; Larrigaudière et al., 1995). This fact highlights the good conditions of southeastern Spain for the production of this melon variety. The increase in the total sugar value is due to sucrose, which has a pronounced increase rate during ripening while glucose and fructose decrease (Guis et al., 1998).

TA presented little variation during ripening (Table 1), although it had a downward tendency. This circumstance made the increase in RI to be determined by the sugar level (Table 1), and for this reason their evolutions were similar. The increase rate for this parameter indicative of maturity was low between days 27 and 31, and it presented a great increase between the samplings on days 31 and 35, this increase being less pronounced at the end of the experience.

The increase in RI matches the results of sensory analysis (Table 1), which indicated a lack of taste due to nonacceptable maturity degrees in the first two samplings. The fruit reached the optimum maturity degree on day 35, with adequate taste and smell according to the taster panel. The assessment was less favorable on day 40, because the two melon cultivars were overripe and some rare tastes were detected.

Although there is a relationship between TSSC and melon quality according to the sensory analysis, a high TSSC alone does not adequately define good melon quality, because the aroma is a very important attribute in this fruit. However, the absence of high TSSC corresponds with poor quality (Bianco and Pratt, 1977; Miccolis and Saltveit, 1991).

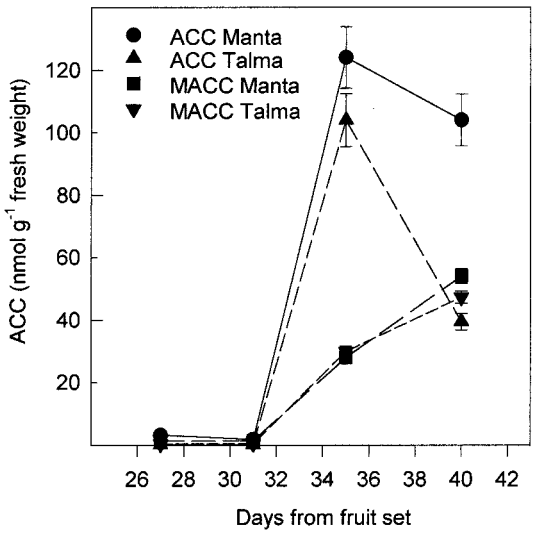

Figure 4. ACC and MACC pulp levels during ripening of Talma and Manta melon cultivars. Data are the mean ± SE of two extractions made independently in each of the five fruits, and each extract was quantified in triplicate.

The results showed that the optimum moment for consuming both cultivars is on day 35 from fruit set. However, due to the rapid ethylene crisis, if they are harvested at that moment, ripening would progress rapidly and the consumer would receive them in an overripe condition. On the contrary, if they are harvested on day 27, the climacterium would not be initiated and the constituents responsible for taste and smell would not change because ripening would not occur during postharvesting. According to these circumstances harvesting must take place on day 31 from fruit set, once autocatalytic ethylene synthesis has begun, because ripening will continue after harvesting; the fruit will reach its optimum organoleptic level for consumption in the next 3 or 4 days.

Free and Conjugated ACC. The free ACC content evolutions were similar in both cultivars (Figure 4), with low levels in samplings made on days 27 and 31 from fruit set. From this time, an increase on day 35 and a reduction on day 40 were found, especially in the Talma cultivar. As was observed in ethylene production, the Talma cultivar showed lower free ACC values than the Manta cultivar during the ripening phase. MACC levels also increased from day 31, although, in this case, the tendency was kept in the last sampling of the experiment (Figure 4). The evolution of malonyl ACC content was similar to that found by other researchers, who observed an increase in malonyl ACC levels in the postclimacteric stage (Guis et al., 1998).

ACC-Synthase and ACC-Oxidase Activities. These enzymatic activities (Figure 5) showed a slight increase

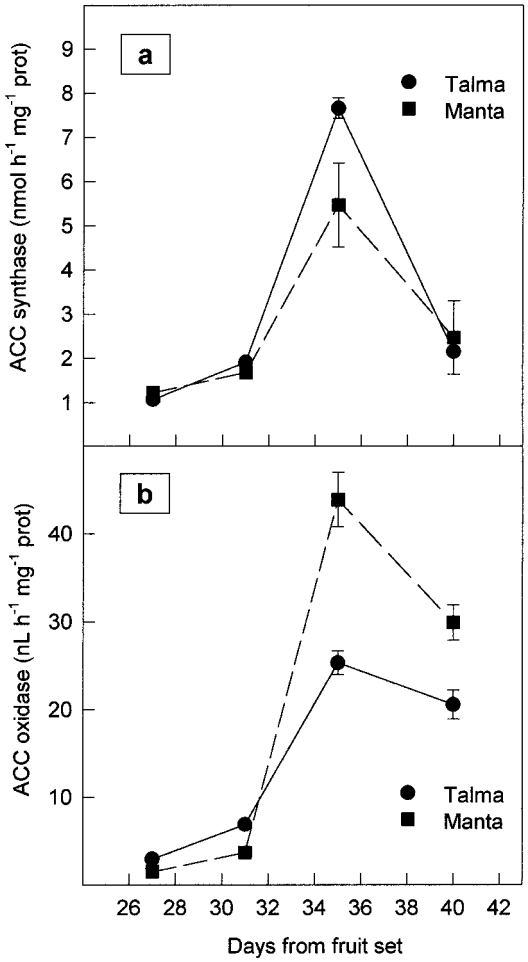

Figure 5. ACC-synthase (a) and ACC-oxidase (b) in the pulp of Talma (●) and Manta (■) melon cultivars during ripening. Data are the mean ± SE of two determinations made independently in each of the five fruits, and each one was quantified in triplicate.

between days 27 and 31. On day 35 there was a sharp increase in the activity of both enzymes, which decreased later. Although the evolutions of the enzymes involved in ethylene biosynthesis were very similar, there were some differences for both cultivars. Talma showed, at the moment of the ethylene crisis, a significantly higher ACC-synthase activity than Manta, contrary to the results obtained with ACC-oxidase, which was significantly higher for the Manta cultivar, whereas the maximum ethylene productions were similar in both melon cultivars. Therefore, it is possible that the increase in both ACC-oxidase and ACC-synthase activities, and not any of them individually, would be responsible for the climacteric ethylene production, as in other climacteric fruits such as apples (Mansour et al., 1986). In cantaloupe melon the study of ripening in the plant (Guis et al., 1998) and during postharvesting (Larrigaudière et al., 1995) also showed an increase in ACC levels and in the activity of the enzyme responsible for its transformation into ethylene when the maximum ethylene occurred.

ABA Levels. The content of this hormone decreased during ripening in both melon cultivars (Figure 6). It is important to mention that significant differences were found between Talma and Manta cultivars. The contents of the first one between days 27 and 31 from fruit set were 994 and 500 pmol g^{-1} of fresh weight, respectively,

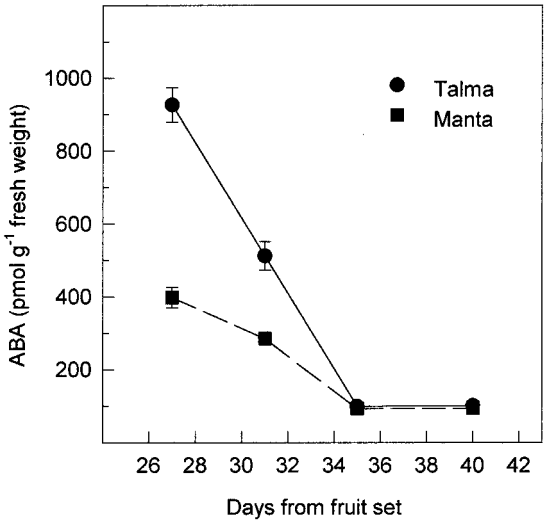

Figure 6. Abscisic acid levels in the pulp of Talma (●) and Manta (■) melon cultivars during ripening. Data are the mean ± SE of two determinations made independently in each of the five fruits.

and Manta cultivar reached only 400 and 250 pmol g^{-1} of fresh weight. From day 35 after fruit set (when the ethylene maximum was reached) ABA levels were low and they remained constant until the end of ripening. ABA is a hormone that can also function as a ripening regulator, because exogenous ABA application in some fruits stimulates ripening (Brady, 1987). However, its evolution during ripening can increase or decrease depending on the fruit (Serrano et al., 1995; Martínez-Madrid et al., 1996), and a clear relationship between the ripening process and ABA synthesis cannot be established. Our results in these melon cultivars are in good agreement with those found by Guis et al. (1998) and Zhang and Yang (1987) for cantaloupe Charentais melon, which suggests that ABA can be implied in stimulating the autocatalytic ethylene synthesis in melon. However, Larrigaudière et al. (1995) found, in cantaloupe melon, Alpha variety, that ABA showed, during postharvesting, a maximum which matched up with ethylene crisis. This difference in evolution could be explained if we consider that our fruits have been harvested on particular dates during their ripening in the plant and immediately processed. However, in the experiment of Laurrigadière et al. (1995), the melon ripening occurred after harvesting, and during postharvesting some modifications associated with harvesting stress can be presented.

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