Compositional Changes during the Fruit Development of Two Peach Cultivars Differing in Juice Acidity

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ABSTRACT. Changes in metabolites were studied during the fruit development of two greenhouse grown peach [*Prunus persica* (L.) Batsch] cultivars with low acidity ('Jalousia') or normal acidity ('Fantasia'). Both cultivars had the same sucrose concentration in fruit mesocarp at maturity. In the fruit juice, pH was higher and titratable acidity was lower for 'Jalousia' than for 'Fantasia' from 80 days after bloom to maturity. At four different times during fruit development, in vivo ¹³C NMR spectroscopy was used to measure the vacuolar pH of fruit mesocarp. At 55 days after bloom, the vacuolar pH of fruit mesocarp was not significantly different between 'Jalousia' and 'Fantasia', whereas the juice pH was different between cultivars. The three major organic acids in fruit mesocarp were malic, citric, and quinic acids for both cultivars. Citric acid concentrations were similar in both cultivars until ≈85 days after bloom and then became significantly higher in 'Fantasia'. A significantly higher concentration in malic acid in 'Fantasia' than in 'Jalousia' was observed from the end of the first growth phase to maturity. At maturity, 'Fantasia' fruit had two and five times more malic and citric acid, respectively, than 'Jalousia' fruit. The differences observed between 'Jalousia' and 'Fantasia' fruit for malic and citric acid concentrations accounted for the difference in titratable acidity. The differences in acid concentration appeared during the plateau between the two rapid growth phases of the fruit, i.e., far before the onset of maturation. The three major amino acids were asparagine, glutamic acid, and proline for both cultivars. Their concentration followed similar patterns in acid and low-acid fruit.

For most fruit, ripening corresponds to a number of coordinated biochemical and physiological processes that result in changes in color, texture, flavor, and aroma, making the fruit desirable for consumption. During peach fruit development, as in other fruit, sugar and organic acids are actively metabolized and, together with increased aroma, contribute to the fruit organoleptic quality. At maturity, in peach fruit, the major sugar is sucrose and the main organic acids are malic and citric acid (Brady, 1993; Byrne et al., 1991; Chapman et al., 1991; Harker and Maindonald, 1994; Ishida et al., 1971; Moriguchi et al., 1990; Robertson et al., 1989; Vizzotto et al., 1996).

Some variability in fruit pH and acidity exists in the peach

germplasm. Most European and American varieties are more acidic than the peento varieties introduced from China (Reimer, 1904; Yoshida, 1970). Although sugar and organic acid accumulation during fruit development have been described in a number of peach varieties with acid fruit, it has never been studied in low-acid fruit.

The objective of the present study was to compare how the compositional changes involving the concentration of certain soluble sugars, organic acids, and amino acids evolve during fruit development of two varieties differing in juice acidity, and how they relate to vacuolar pH. The determination of the timing and nature of the biochemical changes should give preliminary indications of the spatial origin of differences in acidity. The methods used and results obtained should be of wider interest as fruit low in acid have been identified in a number of species but the controlling mechanism remains unknown.

Materials and Methods

PLANT MATERIALS. Two peach (*Prunus persica* L. Batsch) cultivars—Jalousia and Fantasia—were used. 'Jalousia' is characterized by low fruit acidity, whereas 'Fantasia' is characterized by normal fruit acidity. One year after grafting on Rubira (*Prunus persica*) seedlings in winter, the plants were transferred to 75-L pots in a 1 peat : 1 sand : 1 soil mixture (by volume). The trees were

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kept outdoors during the two following growing seasons, with minimal pruning during the dormant season. Standard pest control measures for peach trees were used. At the end of the second winter, three trees of each cultivar were transferred to a greenhouse. Fruit were hand thinned in March 1996, ≈ 1 month after bloom to obtain a load of about one fruit for each 20 cm of shoot. These trees were in their third year at the time of the study.

FRUIT GROWTH. The diameters of 12 individual fruit of each variety were repeatedly measured weekly in the greenhouse, from ≈ 3 weeks after bloom to fruit maturity (≈ 18 weeks after bloom). Maturity corresponded to the beginning of softening. About every 3 weeks, five fruit were harvested from the three different trees of each cultivar (two fruit on the first two trees and one fruit on the third tree). These fruit were separated into epidermis plus mesocarp and stony endocarp, except for the first sampling date, and fresh and dry mass were determined before and after freeze drying.

JUICE pH AND ACIDITY MEASUREMENTS. Five fruit were harvested every two or three weeks. The excised fruit were kept in ice and brought to the laboratory within 15 min. After the fresh mass of each fruit was determined, a portion of the mesocarp (≈ 2 g except for the first sampling time, from the equatorial part of a fruit) was immediately frozen in liquid nitrogen in a 5-mL syringe containing a small paper filter at the needle. After thawing at 4°C , the juice was extracted by hand pressing. Two-hundred microliters of the juice was used for pH measurement with a LPH-330T pH meter (Tacussel, Villeurbanne, France) equipped with a microelectrode. Titratable acidity was determined on the same sample, diluted with distilled water (1 juice : 5 water, by volume), by microtitration with $0.1\text{ mol}\cdot\text{L}^{-1}$ NaOH to a pH of 8.3. This pH was chosen because it corresponds to the color change of phenolphthalein indicator (used in the INRA Bordeaux peach breeding program) at maturity.

MEASUREMENT OF VACUOLAR pH USING ^{13}C NMR SPECTROSCOPY. Measurement of vacuolar pH gives some indirect information on the functioning of tonoplastic proton pumps, which energize the vacuolar storage of metabolites (Martinoia and Rentsch, 1994). At four different times during fruit development, the vacuolar pH was measured on a mesocarp portion using ^{13}C NMR. The juice pH was measured on expressed liquid of another portion of the same fruit (or of the portion used for NMR measurement, for the first sampling time), as indicated above. Four fruit were used for each cultivar at each time. A Bruker (Wissenheim, France) NMR spectrometer (DPX 400) with a 9.4 T superconducting magnet (89-mm bore) operating in the pulsed Fourier transform mode at 100.61 MHz was used to characterize the various intracellular carbon compounds in peach fruit. A 25-mm-diameter broad band probe was used to record spectra. ^1H decoupled in vivo ^{13}C spectra from one portion of fruit were obtained with a 65° pulse angle, a recycle time of 1.95 s. Two levels of decoupling (WALTZ pulse sequence) were used: 2.5×10^{-6} W (64.1 dB) during the data acquisition (0.739 s) and 1 W (8 dB) during the delay period (1.21 s). Between 512 and 1024 free-induction decays (FID) were necessary to record in vivo ^{13}C NMR spectra (16.5 to 33 min total accumulation time). FIDs were recorded using 32,000 data points and zero filled to 64,000 prior Fourier transformation. A 15-Hz linebroadening was applied. A reference capillary containing silicon mastic (Gebescicone, GEB, Bobigny, France) was inserted inside the NMR tube. Chemical shifts were obtained by reference to silicon taken as 0.99 ppm by reference to tetramethylsilane positioned at $\delta = 0$. D_2O (5 mL) provided a lock signal during the acquisitions. The identification of each carbohydrate component was achieved by comparison with authentic samples from Sigma (St Quentin, France). Vacuolar pH values were estimated from the chemical shift of the intracellular citric or malic acid. Calibration

curves, relating chemical shifts to pH values, were established according to the method of Stidham et al. (1983). Malic and citric acid solutions ($0.1\text{ mol}\cdot\text{L}^{-1}$) were prepared, adjusted to the appropriate pH (from 2 to 7.5, 0.5 step) with $1\text{ mol}\cdot\text{L}^{-1}$ KOH, and introduced in a double insert 10 mm NMR tube, which contained in its central part D_2O and a silicon capillary. This method gives accurate intracellular pH values in the range pH 2.5–5.5 with malic or citric acid.

SUGAR, ORGANIC, AND AMINO ACID ANALYSIS. A mesocarp portion, from the equatorial part of the fruit harvested about every 3 weeks for fresh and dry mass determination, was used for sugar and acid analysis. For the first sampling time, 0.5 g of fruit mesocarp was extracted from each fruit. For the other sampling times, ≈ 1 to 1.5 g of fruit mesocarp were ground in liquid nitrogen and extracted for 30 min with 3 mL 80°C 80 ethanol : 20 water (by volume). The mixture was centrifuged ($3,800\text{ g}$ for 10 min), and the pellet was extracted again with 3 mL 80°C 50 ethanol : 50 water (by volume). After a second centrifugation, the two supernatants were combined, dried under vacuum, and used for analysis of soluble sugars and organic and amino acids.

Soluble sugars were purified using ion exchange resins and analyzed by high-performance liquid chromatography (HPLC) with a refractive index detector, as described by Moing et al. (1992).

Organic acids were analysed without sample purification, using anion exchange HPLC (IonPac AS-11 4 mm column and ATC precolumn from Dionex Corp., Sunnyvale, Calif.). Elution was done with degassed NaOH solutions containing 16% (by volume) methanol at a flow rate of $2\text{ mL}\cdot\text{min}^{-1}$ with the following NaOH gradient: 0 min to 5 min, 0.5 to $4.2\text{ mmol}\cdot\text{L}^{-1}$; 5 min to 15 min, 4.2 to $37.5\text{ mmol}\cdot\text{L}^{-1}$; and 17 min, $37.5\text{ mmol}\cdot\text{L}^{-1}$. Acid was detected with a PED2 conductivity detector from Dionex Corp. after anion chemical suppression with H_2SO_4 ($25\text{ mmol}\cdot\text{L}^{-1}$). Twenty-five microliters of the crude extract, diluted with water to avoid detector saturation, was injected.

Amino acids were analysed by HPLC, without sample purification, using the AccQ.Tag method from Waters (Milford, Mass.). Amino acids derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (Cohen and De Antonis, 1994; Cohen and Michaud, 1993) were detected by fluorescence (excitation at 250 nm and emission at 394 nm) with a FL 2000 dual monochromator fluorescence detector from Thermo Separation Products (San Jose, Calif.). The total amino acids is the sum of aspartic acid, glutamic acid, serine, asparagine, glycine, glutamine, histidine, threonine,

Fig. 1. Fruit growth as a function of days after bloom for 'Jalousia' and 'Fantasia' peach fruit. Mean of 12 fruit. Vertical bars represent SD of the means.

Fig. 2. pH and titratable acidity of fruit juice as a function of days after bloom for 'Jalousia' and 'Fantasia' peaches. Mean of five fruit. Vertical bars represent SD of the means.

arginine, alanine, γ -aminobutyric acid, proline, tyrosine, valine, methionine, isoleucine, leucine, lysine, and phenylalanine. The other amino acids were found as traces or not detected.

Sugar, organic, and amino acid quantification was carried out with Millenium software from Waters (Milford, Mass.). HPLC peaks were identified by cochromatography with known standards, their area calculated, and calibration was carried out with known quantities of sugar, organic, or amino acid from Sigma (St Quentin, France).

DATA ANALYSIS. To facilitate comparison between varieties, for each sampling time and variety, the time from bloom was calculated as the number of days after bloom (DAB). Data are presented as mean $\pm$ standard deviation of the mean. Comparison between 'Jalousia' and 'Fantasia' was done using *t* test ($P < 0.05$), except for pH where Tukey's test was used.

Table 1. Vacuolar or juice pH, in peach fruit mesocarp of 'Jalousia' and 'Fantasia' cultivated in a greenhouse. The vacuolar pH was determined using the NMR chemical shift of C3 of malate or C2–C4 of citrate, depending on the stage of development, as indicated in the NMR column. The values represent the mean of four fruit, except for vacuolar pH of 'Jalousia' at maturity where there were only two replications.

Stage (DAB ^a)	NMR	Jalousia			Fantasia		
		Vacuolar pH	Juice pH	(Δ pH) ^b	Vacuolar pH	Juice pH	(Δ pH)
34	C3 malate	2.85 c ^y	3.94 a	(1.09)	3.19 b	3.85 a	(0.64)
55	C3 malate	2.95 b	3.50 a	(0.55)	2.91 b	3.38 a	(0.47)
86	C2–C4 citrate	3.28 b	3.78 a	(0.50)	3.31 b	3.60 a	(0.29)
Mature	C2–C4 citrate	4.08 b	4.66 a	(0.58)	3.30 c	3.48 c	(0.18)

^aDAB = days after bloom.

^b Δ pH, indicated in parentheses = difference between vacuolar pH and juice pH.

^yFor each stage, pH values accompanied by the same letter are not significantly different according to Tukey's test ($P < 0.05$).

Results

FRUIT GROWTH. 'Fantasia' has round fruit. 'Jalousia' has flat fruit. Fruit matured at 125 and 118 d after bloom in the greenhouse for 'Fantasia' and 'Jalousia', respectively. Although the shape of the fruit differs between cultivars, they followed a similar pattern of growth, when expressed as changes in diameter (Fig. 1) or in dry mass (data not shown). At maturity, flesh dry mass was close to 20 g per fruit. Increase in diameter is a typical sigmoidal curve, characterized by two stages of rapid growth separated by a plateau (Fig. 1). The durations of the two stages were similar for both cultivars. The only significant difference at maturity was for kernel dry mass: 'Jalousia' was characterized by a smaller stony endocarp than 'Fantasia' (data not shown).

pH AND TITRATABLE ACIDITY. The fruit juice pH of both cultivars decreased from 4.5 at ≈ 20 DAB to 3.5 at 50 DAB (Fig. 2A). The juice pH of 'Jalousia' then increased until maturity while the pH of 'Fantasia' remained low and essentially unchanged. The juice titratable acidity of both cultivars was similar and followed the same pattern from 25 to ≈ 85 DAB. Then the juice titratable acidity of 'Jalousia' decreased, whereas that of 'Fantasia' increased sharply up to 410 meq·L⁻¹ at 110 DAB and then decreased (Fig. 2B). At maturity, the pH values of the fruit juices were 4.6 and 3.3 for 'Jalousia' and 'Fantasia', respectively (Fig. 2A). Titratable acidity was ≈ 220 meq·L⁻¹ higher in 'Fantasia' than in 'Jalousia' (Fig. 2B).

The method used to estimate intracellular pH relies on the fact that the chemical shifts (δ , resonance frequency relative to a standard) of ¹³C in many organic compounds depend on pH. Curves showing the pH dependence of methylene carbons (C-3) and (C-2 + C-4) and carbon atoms with the hydroxyl group (C-2) and (C-3) of malate and citric acids (0.5 mol·L⁻¹) signals were established (data not shown) according to the method of Stidham et al. (1983). At 34 DAB, the vacuolar pH of 'Fantasia' was significantly higher than that of 'Jalousia', whereas there was no pH difference for their juice, i.e., their cellular sap (Table 1). At 55 and 86 DAB, the vacuolar pH of 'Jalousia' was not significantly different from that of 'Fantasia'. Between 34 DAB (end of first growth phase) and maturity, the vacuolar pH increased from 2.85 to 4.08 in the 'Jalousia' fruit, and only from 3.19 to 3.30 in 'Fantasia' fruit. At maturity, the vacuolar pH of 'Jalousia' fruit was significantly higher than that of 'Fantasia' ($P < 0.05$). The pH of 'Jalousia' juice also declined from 55 DAB to maturity. Juice pH increased less in 'Fantasia' than in 'Jalousia' between 55 DAB and maturity. The Δ pH, calculated as the difference of juice and vacuolar pHs (Table 1), showed that, when mixed with cytoplasm and apoplasm, the vacuolar sap of 'Fantasia' had a higher capacity to lower juice pH, than that of 'Jalousia' for all stages investigated.

SUGAR, ORGANIC, AND AMINO ACID CONCENTRATION. Both cultivars were characterized by increased sucrose accumulation in fruit

Fig. 3. Soluble sugar concentrations, on a dry mass basis, in the mesocarp of 'Jalousia' and 'Fantasia' peaches. Mean of five fruit. Vertical bars represent sd of the means. (A) Changes in sucrose concentration. (B) Changes in hexose concentration. (C) Changes in sorbitol concentration.

mesocarp after 45 DAB (Fig. 3A). The mean sucrose concentration was significantly higher (*t* test, $P < 0.05$) in 'Jalousia' than in 'Fantasia' from ≈ 85 DAB to maturity. At maturity, it reached 620 and 490 $\text{mg}\cdot\text{g}^{-1}$ dry mass in 'Jalousia' and 'Fantasia', respectively. The highest hexose concentration was observed at ≈ 45 DAB for both cultivars (Fig. 3B). Then hexose concentration remained significantly higher in 'Fantasia' than in 'Jalousia', except at maturity. No significant difference in sucrose and hexose concentration was observed between 'Jalousia' and 'Fantasia' at maturity (Fig. 3). Sorbitol concentrations of both cultivars (Fig. 3C) were not significantly different for most sampling times.

The three major organic acids in fruit mesocarp were malic, citric, and quinic acids. In 'Fantasia', malic acid (Fig. 4A) accumulated from 25 to 65 DAB, then decreased to 115 DAB and then increased again at maturation. In 'Jalousia', malic acid concentra-

Fig. 4. Organic acid concentrations, on a dry mass basis, in the mesocarp of 'Jalousia' and 'Fantasia' peaches. Mean of five fruit. Vertical bars represent sd of the means. (A) Changes in malic acid concentration. (B) Changes in citric acid concentration. (C) Changes in quinic acid concentration.

tion decreased steadily from 25 DAB to maturity. 'Fantasia' had a significantly higher concentration of malic acid than 'Jalousia' from 65 DAB to maturity. At maturity, 'Fantasia' fruit had about two and five times more malic and citric acids, respectively, than 'Jalousia' fruit. Citric acid concentrations (Fig. 4B) were similar in both cultivars until after 85 DAB. At 115 DAB, citric acid concentration peaked up to 61 $\text{mg}\cdot\text{g}^{-1}$ dry mass in 'Fantasia', which was ≈ 8 times higher than in 'Jalousia' (7 $\text{mg}\cdot\text{g}^{-1}$ dry mass). The pH NMR data (Table 1) suggest that malic and citric acids were stored in the vacuole. The changes in quinic acid concentration (Fig. 4C) followed similar patterns in both cultivars, with the same maximum and minimum concentrations. At maturity, the quinic acid concentration was not significantly different between cultivars.

The major amino acids were asparagine, glutamic acid (Fig. 5) and proline. These three amino acids represented between 60%

Fig. 5. Changes in the concentration of the two major and the sum of all amino acids, on a dry mass basis, in the mesocarp of 'Jalousia' and 'Fantasia' peaches. Mean of four fruit. Vertical bars represent SD of the means. (A) Asparagine. (B) Glutamic acid. (C) Total amino acids.

and 90% of the total amino acids depending on the stages of development. Asparagine concentration (Fig. 5A) increased from 45 to 85 DAB and then decreased during maturation when sucrose accumulated (Fig. 3A) for both cultivars. Its maximum concentration was close to 25 mg·g⁻¹ dry mass. The maximum concentration of glutamic acid (Fig. 5B) was observed at 25 DAB. Its concentration decreased between 25 and 45 DAB for both cultivars, and remained <0.9 mg·g⁻¹ dry mass thereafter. The concentration of proline (data not shown) showed the same pattern as that of glutamic acid. The concentration of γ -aminobutyric acid (data not shown), which might be involved in pH homeostasis, was <0.1 mg·g⁻¹ dry mass for both cultivars at all stages. Few differences were observed between the two cultivars for the concentration of each of the other amino acids (data not shown). Minimum and maximum concentrations were similar and the changes followed similar patterns. The sum of all amino acids (Fig. 5C) followed a

pattern similar to that of asparagine because this amino acid represents between 34% and 91% of total amino acids, depending on the stage of development. At maturity the total amino acids represented <1% of dry mass.

Discussion

The measurement of juice pH and titratable acidity confirmed that 'Jalousia' fruit have low acidity at maturity. The fruit growth pattern of the low acid cultivar was similar to that of the acid cultivar. Growth curves and sucrose accumulation of both cultivars are in agreement with Tukey (1933) and Pavel and DeJong (1993). However, the metabolite analyses showed that there were significant differences in metabolites before the onset of ripening.

During the second part of the first stage of rapid fruit growth between 21 and 55 DAB of both cultivars, mesocarp cells expand actively (Masia et al., 1992; Zanchin et al., 1994) possibly with high metabolic rates. Sucrose and sorbitol, which are the main sources of carbon for peach fruit (Moing et al., 1992), were not accumulated in either cultivar as reported by Moriguchi et al. (1990). During the same period, the total concentration of free amino acids decreased dramatically in both cultivars, suggesting a strong need of amino acids for protein biosynthesis. This period was also characterized by a general acidification of the fruit for both cultivars, but the metabolism began to differ between 'Jalousia' and 'Fantasia'. In 'Fantasia', malic acid was actively synthesized and accumulated in the vacuolar compartment, whereas its concentration decreased in 'Jalousia'.

The second stage of fruit development, between 55 and 85 DAB, was characterized by a slow growth rate indicating that cell expansion had slowed. In both cultivars, sucrose was slowly accumulated and its concentration was close to that of cultivar Majestic (Chapman et al. 1991). The total amino acid concentration and particularly asparagine increased in both cultivars, indicating that the need for protein synthesis had greatly diminished.

During the third stage (between 85 and 110 DAB) which was characterized by a rapid growth rate, differences between cultivars in term of acidity greatly increased. Both peach cultivars continued to accumulate sucrose. 'Fantasia' accumulated citric acid on a large scale in agreement with data on 'Majestic' peach (Chapman et al., 1991), whereas 'Jalousia' produced less citric acid or its citric acid may have been metabolized. The decrease in quinic and malic acid concentration during the third stage certainly resulted in the low fruit acidity characteristic of 'Jalousia'. In contrast, the increase in citric acid concentration in 'Fantasia' maintained an acidic vacuolar pH and juice pH, and doubled the juice titratable acidity. Amino acid concentration decreased in both cultivars, which may indicate a resumption of protein biosynthesis.

There are few studies on fruit malate metabolism except in grape (*Vitis vinifera* L.) berries (Hawker, 1969; Kanellis and Roubelakis-Angelakis, 1993; Ruffner, 1982) and tomato (*Lycopersicon esculentum* Mill., Knee and Finger, 1992). In reference to these studies and studies on other tissues, three hypotheses can be drawn to explain the difference in organic acid concentration between the cultivars. They concern malate and citrate synthesis, catabolism, and compartmentation. These three hypotheses are not independent.

The key enzyme of the synthesis pathway of malate and citrate is phosphoenolpyruvate carboxylase (E.C. 4.1.1.31, PEPC, O'Leary, 1982). However, there has been little study of PEPC in fruit (Blanke and Lenz, 1989; Law and Plaxton, 1995; Notton and Blanke, 1993). PEPC activity is regulated by phosphorylation by a PEPC kinase (Chollet et al., 1996). Therefore the difference in malate accumulation observed between peach genotypes could

result from differences in PEPC or PEPC kinase concentration and/or activity.

To allow malate accumulation in mesocarp cells, malate and citrate have to be stored in the vacuole (Yamaki, 1984). Therefore, low-activity malate or citrate transporters (Rentsch and Martinoia, 1991) or proton pumps energizing the anion transport (Martinoia and Rentsch, 1994) are relevant hypotheses for 'Jalousia'. As the vacuolar pH of 'Jalousia' fruit was lower than or equal to that of 'Fantasia' at all stages except at maturity, the proton pumps seem to be functioning in the low acidity genotype. If malate storage in the vacuolar compartment is limited in low acidity peaches, then a small increase in malate concentration in the cytosol may strongly down regulate PEPC activity, and therefore limit malate accumulation. Moreover, malate in cytosol could be catabolized by the enzymes involved in malate remobilization (NADP-dependent malic enzyme and malate dehydrogenase, Ruffner, 1982; Taureilles-Saurel et al., 1995) before maturation. These hypotheses remain to be tested.

Asparagine is the major amino acid in peach flesh as in other Rosaceae fruit like *Prunus mume* Sieb. et Zucc. (Kakiuchi et al., 1985), apricot (*Prunus armeniaca* L., Nigam and Sharma, 1988), and apple (*Malus domestica* Borkh, Blanco Gomis et al., 1990). Asparagine concentration does not seem to be modified in the flesh of the low acidity genotype. γ -Aminobutyric acid was present in very low concentration in the two peach cultivars differing in acidity; therefore, its role in pH homeostasis, as studied by Carroll et al. (1994) in *Daucus carota* L. and Crawford et al. (1994) in *Asparagus sprengeri* Regel, seems improbable in peach mesocarp cells.

Conclusions

In the peach fruit, the changes in the concentration of the major metabolites are developmentally regulated. However, during fruit development, malate and citrate accumulation in the mesocarp strongly depends on the cultivar. In 'Jalousia' low acid fruit, malate and citrate accumulation is markedly reduced, and amino acid concentration is not modified. This reduction in organic acid accumulation, which results in fruit with lower acidity and higher juice pH, occurs far before reaching ripening.

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