Leaf:fruit ratio and irrigation supply affect seasonal changes in minerals, organic acids and sugars of mango fruit

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Abstract: To determine the effects of assimilate and water supply on the determination of mango fruit quality, the seasonal variations of minerals, acids and sugar concentrations were investigated over two successive years. To manipulate the assimilate supply, selected branches were girdled to provide ratios of 10, 25, 50 and 100 leaves per fruit. Irrigation was managed to provide two types of water supply treatments. Fruit growth rate was greater when increasing the leaf:fruit ratio. Structural dry matter content and total dry matter content of flesh were higher in fruit with higher leaf:fruit ratios. Treatments had no effect on the structural to total dry matter ratio of flesh. Potassium and magnesium to structural dry weight ratios were not affected by treatments, whereas the calcium to structural dry weight ratio was higher in the flesh of fruit grown under low leaf:fruit ratios. Low assimilate supply increased the ratios of malic and citric acid to structural dry weight. This treatment had little effect on acid concentrations. Glucose and fructose to structural dry weight ratios were higher when assimilate supply was lower. Low leaf:fruit ratios increased fructose concentration but not glucose concentration. Irrigation treatment strongly affected fructose concentration. Sucrose concentration, based either on structural dry matter or on fresh matter, was significantly increased by higher leaf-to-fruit ratios. When the fruit was close to maturity, levels of sucrose storage and starch breakdown were positively correlated with assimilate supply. Levels of starch breakdown were correlated with irrigation supply. The effects of these treatments on sugar concentrations may change fruit taste.

Keywords: Mangifera indica; mango; leaf:fruit ratio; irrigation; fruit composition; quality; structural material

INTRODUCTION
Fruit flesh taste is highly dependent on the balance between organic acids and soluble sugars. For mango, the predominant acids are citric and malic acids, and the major sugars are sucrose, fructose and glucose. The overall sweetness of fruit flesh depends, to a great extent, on the composition of its sugars. The sweet taste of mango fruit relies upon the storage of large amounts of sucrose and fructose. Indeed, fructose and sucrose are 2.3 and 1.4 times sweeter than glucose, respectively. Sweetness, however, is also affected by acid composition. Citric acid may decrease sucrose sweetness, while malic acid may decrease that of fructose. Mango fruit maturity decreases the acidity of flesh and increases its total soluble sugar concentrations. The loss of acidity during ripening is due to decreased citric acid content. The reduction in starch and the increase in sucrose concentration and, eventually, glucose and fructose during ripening, make the fruit sweeter. Fruit maturation, which is another feature of fruit quality, may be evaluated by the patterns of different biochemical compounds such as starch or sucrose. A consistent pattern of fruit maturation emerges from these studies whereby the drop in starch content is followed by a strong increase in sucrose and, consequently, sweetness.

Mineral ions are of prime importance in determining the fruit nutritional value. Potassium, calcium and magnesium are the major ones. In the tissue of many fruits, including mango, calcium is one of the minerals believed to be an important factor governing fruit storage quality. It has been reported to delay...
ripening and senescence, and to reduce storage disorders. Changes in mango fruit flesh composition associated with ripening have been the predominant subject of many studies dealing with quality. To our knowledge, only a few studies have evaluated the dynamics of mango fruit flesh composition during the growth and the maturation processes, which determine fruit quality at harvest.

Various orchard management practices affect fruit growth by changing the assimilate supply and water availability. Mango fruit size decreases with decreasing leaf:fruit ratios, probably due to the limited availability of assimilates. This effect results in both a decrease in dry matter and in water content of mango fruit. For peach trees, it has been reported that a low leaf:fruit ratio reduces fruit size and sweetness, and delays fruit maturity, as indicated by a late rise in the highest calcium concentrations, which positively affects fruit storage and reduced disorders.

Reducing irrigation decreases harvest weight but increases soluble sugar concentrations and acidity in pear, apple, citrus and kiwi fruits. Pear fruit tended to have lower calcium concentrations and more storage disorders when they were exposed to water stress early in the season. Water stress applied later in the season has less impact on fruit size and improved quality attributes either by advancing maturity in kiwi or by increasing sugar concentration and fruit storage quality in apple.

Biochemical compounds of fruit flesh linked to quality components were generally investigated in terms of concentration per unit of fresh weight. However, changes in fresh weight concentrations can be influenced by several factors such as dilution, the amount of cell wall material produced and the transport-metabolism processes of minerals, acids and sugars, which may exhibit different sensitivities to water and assimilate supply. Indeed, fresh flesh is composed of water and dry matter, the latter consisting of a structural component including cell walls, and a non-structural component consisting of soluble sugars, acids, minerals and starch.

To better understand which of these components are affected by water and assimilate supply, we analysed the changes in total dry matter content (total dry weight:total fresh weight), structural:total dry weight ratio (structural dry weight:total dry weight) and, for individual compounds, non-structural:structural dry weight ratios. In this study, the flesh structural dry weight was calculated as the difference between the total flesh dry weight and the sum of dry weights of all the non-structural compounds. The seasonal changes of the main quality components such as fruit weight, dry matter content of fruit flesh and the concentrations of minerals, acids and sugars were assessed in relation to assimilate and water supply over two successive years.

**MATERIALS AND METHODS**

**Experimental conditions and treatments**

This study was conducted on 11-year-old mango trees of cv. ‘Lirfa’, grafted on ‘Maison Rouge’, in Reunion Island (20°52′48″S, 55°31′48″E) during the 2000 and 2001 growing seasons. The 2000 experimental plot consisted of 10 rows, 7 m apart, each made up of nine trees, spaced 5 m apart and about 3 m high. The trees observed in 2001 were in an adjacent plot and were spaced 5 × 6 m and were around 3 m high. We observed the development of flower panicles each year at the orchard scale in order to assess the full bloom stage. Two successive and distinct flowering episodes generally occur in a mango orchard during the flowering period. These two episodes could be observed, to a varying degree, at the level of individual trees. For each flowering episode, the full bloom stage corresponded to the date when more than 50% of the panicles on all of the trees were open. The error margin due to the heterogeneity of flower panicles opening during a flowering episode is about 5 days.

During the 2000 growing season, all trees were well irrigated every 2 days at 100% replacement of evaporation. Data from eight linear variable displacement transducers (LVDT, Solartron, UK) showed that daytime shrinkage was less than 20 μm for branches with diameters of 3–4 cm, indicating that the trees were well watered. Six weeks after flowering, 250 branches were chosen on 23 trees. Branches were selected with different shoots of the current year and the previous one. Their position was randomly chosen at the top of the trees to reduce light variability at the leaf level, which could significantly alter carbon assimilation and fruit growth. The ‘Lirfa’ mango trees have a round shape. Twenty-five percent of the total branches chosen were positioned in each direction of the quadrant. From 10 to 15 branches per tree were used for this experiment, representing less than 10% of the total branches in the canopy. These selected branches were girdled and sometimes defruited and defoliated to establish ratios of 25, 50 and 100 leaves per fruit (i.e. 100 leaves for four, two and one fruit, respectively). Branches were girdled by removing a 10–15 mm wide strip of bark. To maintain constant leaf:fruit ratios within each treatment, all new emerging leaves were removed.

During the 2001 growing season, two irrigation treatments were applied: control irrigation (CI) and no irrigation (NI). CI trees were well irrigated, as was the case in 2000. In the NI treatment, irrigation was completely withheld one month after full bloom. These trees received only rainfall water, representing about 170 mm during the time of irrigation treatment...
application. This represented 24% of the total water received by CI trees. Trees were divided into six blocks of nine trees with neighbouring trees receiving the same irrigation treatment. Each treatment was applied to three blocks. Six weeks after flowering, carefully selected branches (see 2000 procedure) were girdled, sometimes defruited and defoliated to establish ratios of 10 and 100 leaves per fruit (ie 50 leaves for five fruits and 100 leaves for one fruit, respectively).

**Measurements of fruit composition**

During the 2000 growing season, six fruits from each treatment were harvested each week between 15 November 2000 and 4 January 2001. In the second year of the experiment, six fruits from each treatment were harvested every 15 days between 19 October 2001 and 21 January 2002. For each fruit, the fruit and the fresh flesh were weighed. The flesh of each fruit was sub-sampled, weighed and then dried at 75°C for 48 h. The corresponding dry weights were recorded to calculate total flesh dry weight. The remainder of the fruit was frozen at −20°C for future analysis. The structural dry weight was calculated as the difference between the total flesh dry weight and the sum of dry weights of non-structural compounds (minerals, acids, soluble sugars and starch).

For analyses of the 2000 growing season, the frozen flesh was defrosted, homogenized and centrifuged to extract the crude juice. Concentrations of calcium, magnesium and potassium were determined on the diluted mango juice using capillary ion analysis (CIA, Waters, Massachusetts, USA). The CIA conditions were: UV CAT2 electrolyte, silica capillary, 10 μA current and 20 kV voltage, hydrostatic injection, 10 nl sample injected, electro-osmotic flux migration and UV detection (185 nm, mercury lamp). Concentrations of organic acids were also determined with the Waters (Milford, MA, USA) CIA instrument based on the Laksaridou-Monnerville method, using diluted mango pulp. Briefly, the CIA conditions were: phosphate 7.5 mM/OFM-OH electrolyte, silica capillary 75 μm × 60 cm, 7 μA current and 17 kV voltage, hydrostatic injection, 10 nl sample injected, electro-osmotic flux migration and UV detection (185 nm, mercury lamp). Concentrations of sucrose, glucose and fructose were measured using a high-performance liquid chromatography (HPLC) system (DIONEX Co., Sunnyvale, CA, USA). The HPLC conditions were: CarbopacPA1 guard-column and column, 25 μl sample injected, isocratic elution by a linear gradient from 0.5 to 35 mM NaOH in 25 min, flow rate 2 ml min⁻¹, and conductimetric detection (type ED40 equipped with an automatic suppressor, ASRS cartridge). Peaks of organic acids were confirmed by comparison to a standard mixture.

For analyses of the 2001 growing season, the fresh pulp was defrosted and finely homogenized by a Polytron (PT1600E, Kinematica AG, Switzerland). The sugars, starch and minerals were analysed, just like in 2000. The concentrations of organic acids were measured by HPLC. The conditions were CarbopacPA1 guard-column, IonPacAS11 column, 25 μl sample injected, elution by a linear gradient from 0.5 to 35 mM NaOH in 25 min, flow rate 2 ml min⁻¹, and conductimetric detection (type ED40 equipped with an automatic suppressor, ASRS cartridge). Peaks of organic acids were confirmed by comparison to a standard mixture.

The concentration (in g kg⁻¹ fresh weight) of each biochemical or mineral compound was considered to be the product of three components obtained from the following equation:

\[
C_x = \frac{W_x}{FW} = \frac{SDW}{FW} \cdot \frac{W_x}{SDW} = \frac{DW}{FW} \cdot \frac{SDW}{FW} \cdot \frac{W_x}{SDW}
\]

in which \(C_x\) and \(W_x\) are the concentration and the weight of the compound \(x\), respectively, and FW, DW and SDW are the fresh, dry and structural dry weights of the flesh, respectively.

The structural dry matter content is defined as the ratio between the structural dry weight (SDW) and the total fresh weight of the flesh. The total dry matter content of flesh is the ratio between the dry and the fresh weights of the flesh. The structural to total dry matter ratio is the ratio between the SDW and the total dry weight of the flesh.

**Statistical analysis**

An analysis of variance was performed on observed fresh weights, total dry matter content, structural dry matter content, structural:total dry matter ratio and the non-structural components of the flesh from the two years of experiments, in order to study the effects of fruit age, assimilate supply, water supply and their two- or three-way interactions. In order to more accurately represent treatment effects during the season, an analysis of variance was carried out on each sampling date with leaf:fruit ratio (LF) and irrigation (I) as factors. All statistical analyses were computed on S-Plus software (MathSoft Inc., Cambridge, MA, USA).

**RESULTS**

Fresh weight of fruit flesh, structural dry matter content, total dry matter content and structural to total dry matter ratio of the flesh were all significantly influenced by fruit age (days after full bloom) in 2000 and 2001 (Table 1). Fresh weight of fruit flesh increased almost linearly until 100 days after full bloom and then tended to slow down, as shown for 2001 data (Fig 1(A)). The structural constituents of the flesh from the two years of experiments, in order to study the effects of fruit age, assimilate supply, water supply and their two- or three-way interactions. In order to more accurately represent treatment effects during the season, an analysis of variance was carried out on each sampling date with leaf:fruit ratio (LF) and irrigation (I) as factors. All statistical analyses were computed on S-Plus software (MathSoft Inc., Cambridge, MA, USA).
Seasonal variations of mango fruit flesh growth (A), structural dry matter content (B), total dry matter content (C) and structural to total dry matter ratio (D) of the fruit flesh during the 2001 experimental period. The labels 10 and 100 refer to leaf:fruit ratio. I and NI indicate the well-irrigated and non-irrigated treatments. Differences between the leaf:fruit ratio (LF) or between the irrigation (I) treatments were either significant at $p < 0.10$ (**), $p < 0.05$ (***), or non-significant (NS) on each sampling date. All curves are smoothed fits of the data points using the ‘loess’ method (Splus, statistical software, MathSoft Inc., Cambridge, MA, USA).

Table 1. Analysis of variance for mango fresh flesh weight (g), total dry matter content (%) and structural dry matter content of the flesh

<table>
<thead>
<tr>
<th>Year</th>
<th>Factors</th>
<th>Fresh flesh weight</th>
<th>Structural dry matter content</th>
<th>Total dry matter content</th>
<th>Structural:total dry matter ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Age (A)</td>
<td>67 257***</td>
<td>1562 $\times 10^{-6}$***</td>
<td>43.6***</td>
<td>0.0164***</td>
</tr>
<tr>
<td></td>
<td>Leaf:fruit ratio (LF)</td>
<td>129 047***</td>
<td>1426 $\times 10^{-6}$***</td>
<td>68.7***</td>
<td>0.0025NS</td>
</tr>
<tr>
<td></td>
<td>A $\times$ LF</td>
<td>6073***</td>
<td>224 $\times 10^{-6}$***</td>
<td>1.5NS</td>
<td>0.0053NS</td>
</tr>
<tr>
<td>2001</td>
<td>Age (A)</td>
<td>107 271***</td>
<td>1773 $\times 10^{-6}$***</td>
<td>229.9***</td>
<td>0.2697NS</td>
</tr>
<tr>
<td></td>
<td>Leaf:fruit ratio (LF)</td>
<td>230 180***</td>
<td>6531 $\times 10^{-6}$***</td>
<td>289.8***</td>
<td>0.0031NS</td>
</tr>
<tr>
<td></td>
<td>Irrigation (I)</td>
<td>3NS</td>
<td>15 $\times 10^{-6}$NS</td>
<td>19.5*</td>
<td>0.0007NS</td>
</tr>
<tr>
<td></td>
<td>A $\times$ LF</td>
<td>8523***</td>
<td>253 $\times 10^{-6}$NS</td>
<td>12.9**</td>
<td>0.0045NS</td>
</tr>
<tr>
<td></td>
<td>A $\times$ I</td>
<td>1276NS</td>
<td>409 $\times 10^{-6}$NS</td>
<td>6.1NS</td>
<td>0.0070NS</td>
</tr>
<tr>
<td></td>
<td>LF $\times$ I</td>
<td>3532NS</td>
<td>1303 $\times 10^{-6}$**</td>
<td>3.8NS</td>
<td>0.0452***</td>
</tr>
<tr>
<td></td>
<td>A $\times$ LF $\times$ I</td>
<td>2368NS</td>
<td>157 $\times 10^{-6}$NS</td>
<td>6.2NS</td>
<td>0.0013NS</td>
</tr>
</tbody>
</table>

NS, non-significant. * Significant at $p < 0.10$. ** $p < 0.05$. *** $p < 0.01$.

dry matter content decreased until 100 days after full bloom and then increased (Fig 1(B)). Total dry matter content increased during fruit development (Fig 1(C)). The structural:total dry matter ratio of the fruit flesh decreased rapidly until 120 days after full bloom and then remained nearly constant (Fig 1(D)). The effects of leaf:fruit ratio on the fresh weight, the structural dry matter content and the total dry matter content of the fruit flesh were significant in both years of the experiment (Table 1). Increasing the leaf:fruit ratio strongly increased fresh weight, structural dry matter content and total dry matter content of the flesh in 2001 (Figs 1(A–C)). At maturity, total dry matter content was about 20% higher in the 100-leaves-per-fruit treatment than in the 10-leaves-per-fruit treatment. In 2001, total dry matter content of the flesh was also affected by tree irrigation (Table 1). It seemed to increase under reduced irrigation (Fig 1(C)). Neither irrigation nor assimilate supply had a significant effect on the structural:total dry matter ratio of the flesh (Table 1), but their interaction did have
Table 2. Analysis of variance for mango flesh composition in minerals, organic acids and sugars (in g g\(^{-1}\) of structural dry weight).

<table>
<thead>
<tr>
<th>Year</th>
<th>Factors</th>
<th>K(^+)</th>
<th>Mg(^{2+})</th>
<th>Ca(^{2+})</th>
<th>Malic acid</th>
<th>Citric acid</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Age (A)</td>
<td>42 × 10(^{-6})*</td>
<td>10 × 10(^{-8})*</td>
<td>7 × 10(^{-7})**</td>
<td>0.026***</td>
<td>0.0126**</td>
<td>0.007**</td>
<td>0.018**</td>
<td>0.450***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf:fruit ratio (LF)</td>
<td>8 × 10(^{-6})NS</td>
<td>6 × 10(^{-6})NS</td>
<td>11 × 10(^{-7})**</td>
<td>0.013*</td>
<td>0.0029**</td>
<td>0.017**</td>
<td>0.027**</td>
<td>0.026NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × LF</td>
<td>33 × 10(^{-6})NS</td>
<td>5 × 10(^{-6})NS</td>
<td>1 × 10(^{-7})**</td>
<td>0.008**</td>
<td>0.0004NS</td>
<td>0.006NS</td>
<td>0.085NS</td>
<td>0.085NS</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Age (A)</td>
<td>236 × 10(^{-6})**</td>
<td>52 × 10(^{-8})**</td>
<td>32 × 10(^{-7})**</td>
<td>0.0129**</td>
<td>0.0343**</td>
<td>0.162**</td>
<td>0.601**</td>
<td>0.030NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf:fruit ratio (LF)</td>
<td>110 × 10(^{-6})NS</td>
<td>83 × 10(^{-8})**</td>
<td>168 × 10(^{-7})**</td>
<td>0.0142**</td>
<td>0.140**</td>
<td>0.248**</td>
<td>0.242**</td>
<td>0.064NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irrigation (I)</td>
<td>181 × 10(^{-6})NS</td>
<td>37 × 10(^{-8})**</td>
<td>18 × 10(^{-7})**</td>
<td>0.0007NS</td>
<td>0.034NS</td>
<td>0.004NS</td>
<td>0.004NS</td>
<td>0.004NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × LF</td>
<td>64 × 10(^{-6})NS</td>
<td>10 × 10(^{-8})**</td>
<td>13 × 10(^{-7})**</td>
<td>0.0030**</td>
<td>0.042**</td>
<td>0.010NS</td>
<td>0.017**</td>
<td>0.242**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × I</td>
<td>56 × 10(^{-6})NS</td>
<td>30 × 10(^{-8})**</td>
<td>5 × 10(^{-7})**</td>
<td>0.0009NS</td>
<td>0.009**</td>
<td>0.009**</td>
<td>0.015**</td>
<td>0.242**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LF × I</td>
<td>410 × 10(^{-6})**</td>
<td>57 × 10(^{-8})**</td>
<td>17 × 10(^{-7})**</td>
<td>0.0022NS</td>
<td>0.055**</td>
<td>0.010**</td>
<td>0.037**</td>
<td>0.383**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × LF × I</td>
<td>70 × 10(^{-6})NS</td>
<td>27 × 10(^{-8})**</td>
<td>6 × 10(^{-7})**</td>
<td>0.0014NS</td>
<td>0.019**</td>
<td>0.0010NS</td>
<td>0.019**</td>
<td>0.096NS</td>
<td></td>
</tr>
</tbody>
</table>

Mean square and F significance

NS, non-significant. * significant at p < 0.10. ** p < 0.05. *** p < 0.01.
0.015 and 0.030 g g⁻¹ (Fig 2(A)), whereas Mg²⁺ and Ca²⁺ to SDW ratios tended to decrease during fruit development (Figs 2(B, C)). The effect of leaf:fruit ratio was particularly pronounced on Ca²⁺ in the two years of the experiment (Table 2). The Ca²⁺ : SDW ratio was higher in fruit flesh with low leaf:fruit ratios. The effect of assimilate supply (leaf:fruit ratio) on Mg²⁺ was less pronounced. No treatment effect was found on the K⁺ : SDW ratio. However, there was an interaction effect between leaf:fruit ratio and tree irrigation.

The citric acid to SDW ratio increased until 90 days after full bloom to reach a maximum of about 0.35–0.50 g g⁻¹, depending on the treatment, and then gradually decreased to below 0.1 (Fig 3(A)). In contrast, the malic acid:SDW ratio decreased continuously from 0.5 to 0.1 g g⁻¹ to a minimum value of less than 0.037 at about 100 days after full bloom (Fig 3(B)). During the last month, there was a gradual and small increase. Malic and citric acids to SDW ratios were significantly affected by leaf:fruit ratio (Table 2). They were higher during most of the fruit development under conditions of low leaf:fruit ratios (Fig 3(A, B)), even if the difference between treatments decreased as fruit developed. Irrigation treatment did not affect acid concentration, even if there was an interaction effect between leaf:fruit ratio and tree irrigation (Table 2).

Soluble and insoluble sugars had different seasonal trends. Only the glucose:SDW ratio decreased during most of the fruit development (Fig 4(A)). The fructose:SDW ratio increased steadily during fruit development to reach peak values at fruit maturity of between 0.3 and 0.5 g g⁻¹, depending on the treatment (Fig 4(B)). The sucrose:SDW ratio remained low, around 0.25 g per g, until 100 days after full bloom. It then increased and rose to about 1.00 g g⁻¹ at the end.
of fruit growth (Fig 4(C)). Glucose and fructose to SDW ratios were significantly affected by assimilate supply during the two years of the experiment (Table 2). Both ratios were significantly increased by the lower leaf:fruit ratio (10 leaves per fruit) until 120 days after full bloom for glucose (Fig 4(A)), and throughout fruit development for fructose (Fig 4(B)). The sucrose:SDW ratio was higher in fruit with higher leaf:fruit ratios during the latter stage of fruit development (Fig 4(C)). Furthermore, an interaction between leaf:fruit ratio and tree irrigation was found on the three soluble sugars:SDW ratios (Table 2). The seasonal trend of the starch:SDW ratio could be separated into two phases (Fig 4(D)). During the first phase, the starch:SDW ratio increased to reach a maximum of about 1.0–1.2 g g\(^{-1}\), depending on the treatment. It then rapidly decreased to about 0.2 g g\(^{-1}\) towards the end of fruit development. From 130 days after full bloom onwards, starch seemed to be affected by leaf:fruit ratio (Table 2 and Fig 4(D)). As fruit reached maturity, the fruit flesh from the treatment with high assimilate supply had more sucrose and less starch than fruit grown at low leaf:fruit ratios. The NI treatment enhanced the difference, especially for starch. For the same leaf:fruit ratio, the starch:SDW ratio was higher in NI trees (Fig 4(D)). Moreover, starch breakdown occurred later in the fruit flesh when the treatment with less assimilate and water supply was applied.

The evolution of gustatory quality of mango fruit is depicted in Fig 5 by the changes in acids and soluble sugar concentrations in the fresh weight. The trends were not much different from those based on SDW. The effects of treatments on total dry matter content and on the compound x:SDW ratio resulted in changes in compound concentrations as shown in Fig 5. Citric acid concentration was not affected by irrigation treatment (Fig 5(A)). Leaf:fruit ratio significantly affected citric acid concentration, but differences between treatments were not particularly pronounced on Fig 5(A). Malic acid concentration in fruit flesh was greatly increased by the low leaf:fruit ratio, especially early in the season (Fig 5(B)). During the late harvest stages, malic acid concentration in fruit flesh was a little higher in the treatment with the high leaf:fruit ratio. Glucose concentration was negatively and then positively affected by an increase of leaf:fruit ratio during fruit development (Fig 5(C)).

![Figure 5](image-url)

*Figure 5.* Seasonal variations in citric (A) and malic (B) acids, glucose (C), fructose (D) and sucrose (E) concentrations, expressed in g per kg of fresh weight during the 2001 experimental period. Labels, statistics and curve smoothing are described in Fig 1.
Fructose concentration was increased by the shortage of both assimilate and irrigation supplies (Fig 5(D)). The leaf:fruit ratio considerably increased sucrose concentration in the fresh weight, whereas irrigation had no effect (Fig 5(E)).

**DISCUSSION**

**Fruit composition and seasonal changes**

The relative importance of minerals (ie K$^+$ > Ca$^{2+}$ > Mg$^{2+}$) in mango fruit flesh is similar to that found in apple$^{27}$ or in Asian pear.$^{24}$ This hierarchy was even reinforced during fruit development because of a seasonal increase of K$^+$ and a decrease of Ca$^{2+}$ and Mg$^{2+}$. The seasonal parabolic trends of citric acid concentration described in mango flesh are similar to those described for various species such as peach$^{17}$ and grapevine.$^{28}$ In various mango cultivars like ‘Nam Dok Mai’, ‘Langra’ or ‘Dashehari’, a rise in titratable acidity has been reported during the early period of fruit development, followed by a decline until the end of their growth.$^{9,29}$ This is in agreement with our report of a seasonal pattern of citric acid concentration (first increase, then decrease), the major organic acid in mango flesh. Malic acid concentration differs according to cultivars$^{30}$ but, in accordance with our present findings, various results have indicated that it decreases at the beginning of fruit growth and then increases slightly, particularly during ripening.$^{31}$ This agrees with a model of malic acid accumulation in peach fruit proposed by Lobit.$^{32}$ According to this model, at low pH values, like those observed in the early stage of mango development, a pH increase results in a decrease in malic acid concentration whereas, at higher pH values like those observed in the final stages of fruit development (in mango flesh, the pH increased from 3 to 4.5 during fruit growth, data not shown), a pH increase results in an increase in malic acid concentration. This behaviour is caused by the effect of pH on the transport of malic acid into the vacuole via the functioning of the proton pumps and the dissociation of malic acid, as proposed by Lobit.$^{32}$

Carbohydrate metabolism plays an important role during mango fruit development, particularly changes in starch content.$^{33}$ Fructose and glucose come from sucrose hydrolysis, glucose also being produced by starch hydrolysis. These hexoses fuel respiration and fruit growth. In our experiments, fructose represented about 20–30% of total sugars during mango fruit development and may be considered as a storage sugar. Moreover, fructose sweetness is more pronounced than other sugars and it may thus account for a significant part of the sweet taste of mango. From 140 days after full bloom onwards, our data suggest that the glucose:SDW ratio and glucose concentration become nearly constant, which is in agreement with published studies on reducing sugar concentrations during ripening.$^{34}$ During this period, the starch was mobilized to provide about 85% of the carbon required for sucrose accumulation, so the breakdown of starch during later stages of fruit development led mainly to an increase in the sucrose:SDW ratio rather than an increase in glucose.

**Assimilate and water supplies affect fruit quality**

In the present study, we demonstrate that increasing the number of leaves per fruit increased mango fruit growth rate and final fruit size at harvest, as reported earlier for other cultivars by Chacko et al$^{33}$ and Reddy and Singh.$^{35}$ Mango fruit flesh weight was not affected by a reduction of irrigation under the conditions of this study. These results contrasted with those reported in an irrigation management study on ‘Kensington’ fruit.$^{36}$ Although irrigation was completely withheld early in the period of fruit growth in our study, the presence of deep loamy soil, the extensive root systems with deep tap roots and the amount of rainfall (170 mm during this period) are sufficient to explain why mango trees did not experience medium or severe water stress under our experimental conditions.

In this study, high leaf:fruit ratios increased the total dry matter content of flesh fruit, as observed for ‘Kensington’ mango fruit by Simmons et al.$^{37}$ Our previous studies on mango fruit$^{14,37}$ showed that accumulation of both dry matter and water increase with leaf:fruit ratios, dry matter supply being more affected than water supply. Non-irrigated trees seemed to increase the total dry matter content of the flesh, which was also reported for mango by Diczkalns et al.$^{36}$ The structural:total dry matter ratio of the fruit flesh was not affected by the treatments. This demonstrates that treatments do not affect the amount of cell wall material produced. According to equation (1), we can therefore assume that assimilate and water supplies affected the concentration of a compound $x$ by changing either the total dry matter content, the compound $x$:SDW ratio, or these two components at once.

Cations were differently affected by leaf:fruit ratio. The K$^+$:SDW ratio was not affected by leaf:fruit ratio. This cation is transported to the fruit with assimilate through the phloem.$^{38}$ Thus, the leaf:fruit ratio possibly influences both the assimilate and K$^+$ translocations to the fruit in the same way. The published results on apple$^{18}$ and mango,$^{37}$ reporting that a higher leaf:fruit ratio increases fruit K$^+$ concentration, reflect, in fact, differences in total dry matter content of the flesh, as is the case in this study (data not shown). The poor effect of leaf:fruit ratio on the Mg$^{2+}$:SDW ratio can be explained by the same argument. Our results show that the Ca$^{2+}$:SDW ratio was up to 50% higher in treatments with 10 leaves per fruit compared to treatments with 100 leaves per fruit. Faster fruit growth rate associated with high leaf:fruit ratio and a decline in the surface area to volume ratio as fruit enlarges may reduce water loss per unit of fruit weight via transpiration and the rate of Ca$^{2+}$ accumulation$^{39}$ through the xylem. Moreover, diurnal fluctuations in fruit growth rate have been linked to bidirectional fluxes in apple fruit.$^{40}$ These
data reported a positive relationship between xylem sap inflow during the night and xylem sap outflow during the day. Jones et al.27 presented evidence that the outflowing sap contains much less Ca$^{2+}$ than the inflowing sap. For mango trees, Urban et al.41 showed that, by decreasing the leaf:fruit ratio from about 100 to 25, the leaf diffusive conductance to water vapour ratio increased by about 45%. Thus, in treatments with low leaf:fruit ratios, larger amounts of water may be lost by the fruits during the day, basically to fuel the higher transpiration rate of surrounding leaves. At night, however, larger quantities of water should enter these fruits via the xylem in order to compensate for these water losses. These additional inflows of sap also provide additional imports of Ca$^{2+}$ into the fruit and extra water for fruit growth.

Low assimilate supply increased the citric acid:SDW ratio. Citric acid is synthesized when fruit growth becomes slower and when energy demand is low.32 Thus, fruit flesh from lower leaf:fruit ratios may accumulate more of this organic acid. Citric acid concentration was unchanged regardless of leaf:fruit ratio treatment, due to the opposite effect of this factor on the dry matter content of the flesh. Low assimilate supply significantly increased the malic acid:SDW ratio and malic acid concentration, especially early in the season. This agrees with the observation made in peach of a negative relationship between assimilate supply and malate concentration in the flesh at the beginning of growth.17 At maturity, however, malic concentration was positively correlated with assimilate supply as observed in peach. Fruit flesh with the highest leaf:fruit ratios seemed to store more malic acid and cations. Lobit32 reported that malic acid concentration is linked to total cation concentrations in ripe fruit.

The glucose:SDW ratio was lower in larger fruit. During fruit development, the increase of total dry matter content of fruit flesh according to treatments results in a negative and then positive relationship between glucose concentration and assimilate supply. The beginning of starch hydrolysis at the latter stages of fruit development could also explain the high glucose concentration observed in larger fruit. In the case of a shortage of assimilate supply, the role of higher fructose concentrations during fruit development in the osmotic adjustment has not been studied but may be a strategy to contribute to sustaining growth during periods of low assimilate supply, as reported in apple42 during periods of water stress.

The rates of sucrose accumulation and starch breakdown seemed to be higher when assimilate supply increased, as was reported on peach fruit,15 and was delayed in the case of low assimilate and water supply. This could be explained by a strong relationship between sucrose and the rate of assimilate inflow and by an increase of enzyme activities related to sucrose accumulation. Hence, in the mesocarp of muskmelon fruit, sucrose phosphate synthase activity was higher in control fruit than in fruit developed with lower leaf area.17

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