DOI:10.1021/jf101216t

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Chlorophyll Fluorescence, a Nondestructive Method To Assess Maturity of Mango Fruits (Cv. 'Cogshall') without Growth Conditions Bias

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The quality of ripe mango fruits depends on maturity stage at harvest, which is usually assessed by visible criteria or from estimates of the age of fruit. The present study deals with the potential of chlorophyll fluorescence as a nondestructive method to assess the degree of fruit maturity regardless of fruit growing conditions. Chlorophyll fluorescence parameters were measured along with respiration rates of fruits still attached to the tree. At the same harvest stage, based on the fruit age or the thermal time sum (degree-days) method, physical and biochemical measurements related to fruit maturity and quality were made. Shaded fruits had a significantly greener flesh color, as well as a lower fruit density and flesh dry matter content, than well-exposed fruits, showing that fruits at the top of the canopy were more mature than fruits within the canopy, which were still in a growth phase. Additionally, chlorophyll fluorescence parameters, F_o, F_m, and F_v, were significantly lower for fruits taken from the top of the canopy than for those from within the canopy. The unique relationship observed between chlorophyll fluorescence parameters and fruit maturity, estimated by internal carbon dioxide content, on fruit still attached to trees is independent of growing conditions, such as the position of the fruit in the canopy and carbohydrate supply. The chlorophyll fluorescence method evaluates maturity much more accurately than the degree-day method and, moreover, nondestructively provides values for individual fruits before harvest.

KEYWORDS: Carbon dioxide; chlorophyll; fluorescence; *Mangifera indica*; nondestructive method; quality

INTRODUCTION

There is an increasing demand for high-quality mango fruits on the international market. To ensure the supply of high-quality fruits, it is important to control maturity at harvest precisely because the quality of ripe mangoes depends on the maturity stage at harvest (*1*,*2*). When fruits are harvested too early, they do not ripen evenly. They present excessive shrinkage and low levels of sweetness and may not develop full flavor and aroma. On the other hand, fruits harvested at a late maturity stage result in reduced shelf life with greater susceptibility to disease. Besides, fruit maturity at the tree level is heterogeneous due to variations in flowering time between branches on the same tree as well as to variability in environmental conditions of the fruit-bearing branches (*3*).

The ideal method to evaluate fruit maturity has to be simple, precise, quick, reliable, and nondestructive. If possible, maturity should be measured on individual fruits on the tree.

Fruit age from full bloom or from fruit set represents one of the most commonly used methods for estimating fruit maturity, but it was shown that the same mango cultivar grown in different countries may mature at different times due to the influence of environmental conditions on mango growth and maturation (*2*). Because temperature strongly influences fruit growth and maturation rates, the thermal time sum, expressed in degree-days, is often used as an indicator to harvest fruit. The sum of degree days required to reach maturity has been estimated for mango cultivars, such as 'Carabao' or 'Cogshall' (*4*), and then applied to various orchards or sites. However, air temperature and temperature at the level of the fruit may vary according to the position of the fruit-bearing branch or the fruit itself in the canopy (*5*). The use of a mean value of degree-days leads to overestimation of the sum of degree-days required to reach maturity of shaded fruits. Moreover, the position of the fruit-bearing branch has an impact on the fruit growth rate by affecting carbon availability (*3*). This can be an additional source of error when the stage at which to harvest fruit is estimated.

Evaluating maturity often involves some physical or chemical characteristics. Maturity standards for mango are based on skin

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 Published on Web 06/15/2010 © 2010 American Chemical Society

color, shape, global and shoulder size, firmness, and specific gravity (*6*). However, visible criteria cannot systematically be used as indicators of maturity because mangoes are generally harvested at a green-mature stage, before any visible color changes occur, and then put to ripen after harvest. Moreover, this criterion depends on preharvest factors, such as variety, position in the tree, and leaf-to-fruit ratio (*3*). Some chemical parameters such as total soluble solids, soluble sugars, starch, acidity, and content in phenolic compounds, carotenoids, and dry matter have also been used to evaluate maturity of mango (*7*), but these indicators are destructive, and they can be employed only on a reduced number of fruits in a batch after harvest, whereas thermal time sum provides only an average estimate of the degree of maturity.

Nondestructive techniques to evaluate quality have been reported for mango. The electronic nose is a basic simulation of the human olfactory system used to monitor mango fruit maturity by analyzing headspace volatiles (*8*). Ultrasonic technology was proposed to assess mango maturity and estimate shelf life. This method is based on the relationships found between ultrasonic wave measurements and quality indices such as sugar content, acidity, and firmness (*9*). However, these nondestructive techniques are applied only to fruits after harvest. Jha et al. (*10*) proposed a technique using a hand-held colorimeter to predict maturity from calibration curves between maturity level and peel color values. Other nondestructive techniques include a portable near-infrared spectroscopy instrument (*11*), which provides maturity estimates in the field.

Optical methods were reported to be particularly appropriate to answer the problem of spatial and temporal crop heterogeneity, especially of fruit maturity of temperate fruits (*12*). The color of the skin and flesh of apricot cultivars can be used for rapid screening of carotenoid content. Optical sensors measuring fruit chlorophyll fluorescence were developed to assess winegrape phenolic maturity (*12*). Chlorophyll fluorescence was also reported to be a nondestructive technique available in postharvest to study ripening and senescence of green tissues in several chlorophyll-containing tropical fruits (*13*, *14*). Chlorophyll fluorescence is a simple technique that can be used relatively quickly, even under field conditions, with a portable apparatus. Moreover, chlorophyll fluorescence has the advantage over other techniques of detecting changes at the cellular level well before the appearance of visible ones. Chlorophyll fluorescence represents a small portion of energy re-irradiated after excitation of chlorophyll molecules by light absorption and not used by the photochemical reaction of photosynthesis (*15*). When protein−chlorophyll complexes associated with the thylakoid membrane are damaged by some kind of stress or natural physiological changes (ripening, senescence, etc.), the chlorophyll fluorescence is altered.

Chlorophyll fluorescence has been mainly used in postharvest studies, for instance, for evaluating chilling injury due to low temperature in mango (*14*) and physiological disorders related to low oxygen or high carbon dioxide stress in stored apples (*16*). The decline in chlorophyll fluorescence was observed during the ripening of many fruits such as papaya (*13*) and mango (*14*). For mango and banana, the decrease in minimal fluorescence, referred to as F_o, during ripening can reflect directly the loss of chlorophyll (*14*). The maximal fluorescence intensity, referred to as F_m, was observed as the chlorophyll fluorescence parameter , which was the best correlated with fruit firmness during storage for papaya (*13*) and apple (*17*). However, for many fruits, both F_o and F_m parameters correlated with the color changes associated with ripening. Such color changes are associated with chlorophyll degradation and, at the same time, carotenoid accumulation (*18*, *19*). A recent study, conducted during the preharvest period, showed that chlorophyll fluorescence measurements are well-suited for noninvasively assessing the degree of ripeness in grape berries (*20*). The authors examined levels of chlorophyll fluorescence with growth parameters and soluble sugar contents during the ripening process in the vineyard. The best relationships were always obtained between F_o and sugar concentrations.

The specific objective of this study is to evaluate chlorophyll fluorescence measurements as a method to assess the maturity of individual mango fruits before harvest regardless of fruit growth conditions (fruit position in the canopy and carbon supply). To our knowledge, this investigation has not been carried out before. By comparing physical and chemical characteristics related to fruit quality and maturity measured at harvest and after ripening on fruits with the same age from two positions in the canopy, we demonstrate that this nondestructive method is more precise than the degree-day method. Moreover, by establishing a unique relationship between chlorophyll fluorescence and maturity for fruits attached to tree independently of growing conditions, it can be stated that this method can be used on individual fruits and before harvest.

MATERIALS AND METHODS

Plant and Fruit Material. This study was conducted on 14-year-old (in 2004) mango trees (*Mangifera indica* L. cv. 'Cogshall'), grafted on 'Maison Rouge', in Réunion Island (20° 52′ 48″ S, 55° 31′ 48″ E) during the 2004, 2005, and 2006 growing seasons. Trees were well irrigated, spaced 5 × 6 m, and around 3 m high.

Full flowering (days after full bloom (DAFB) = 0) corresponds to the date when more than 50% of the panicles of all trees were open (August 10, 2004, August 8, 2005, and August 15, 2006). The error margin on the date of full flowering, due to the heterogeneity of flower panicles opening during a flowering episode, is about 5 days. Every year, 8 weeks after full flowering, about 10−15 branches per tree were chosen on 5 trees and equally divided into two (2004 and 2006) and three (2005) groups depending of the number of treatments applied each year. Branches were composed of shoots belonging to the last three flushes. At 60 DAFB, to isolate the branch carrying the fruit from the rest of the tree, these selected branches were girdled by removing a 10−15 mm wide band of bark and sometimes defruited and defoliated to establish a fixed leaf-to-fruit ratio treatment. At this period of mango fruit development of cv. 'Cogshall', the cell division phase and fruit set are completed (*4*). To maintain a constant leaf-to-fruit ratio within each treatment, all new emerging leaves were removed. In 2004 and 2006, the leaf-to-fruit ratio treatment applied was 100 leaves per fruit, corresponding to the nonlimiting condition of carbohydrates supply for fruit growth. For these two growing seasons a fruit-bearing branch and the fruit itself were chosen either at the top of the trees for the well-exposed treatment or within the canopy for the shaded one. In 2005, a fruit-bearing branch and the fruit itself were chosen at the top of the tree canopy and three leaf-to-fruit ratios were established: 10, 25, and 100, with 50 leaves and 5 fruits, 100 leaves and 4 fruits, and 100 leaves and 1 fruit, respectively.

During the three growing seasons studied, a "nondestructive" monitoring was performed on four to seven fruits from each treatment at 4 day and then 2 day intervals as the measurement day approached the day of fruit fall.

Because a general metabolic change occurs at 126−133 DAFB under nonlimited conditions of assimilate supply (*2*), corresponding to fruit maturation in 'Cogshall' mango, we decided to harvest the fruit at 120 DAFB. This harvest date was chosen to harvest fruits before they reached complete maturity and to obtain ripened fruit with a good edible quality after storage. During the 2004 growing season, 30 fruits from each treatment (at the top or within the canopy) were harvested on December 8, 2004, at 120 DAFB. Some of these fruits were analyzed the day of harvest, and the others were stored at 20 °C and 82−85% relative humidity to ripen. When fruits were ripe, fruit quality analyses were performed.

Measurements of Fruit Quality and Maturity. In 2004, for each harvested fruit (30 fruits), the fruit fresh mass was assessed. Density was calculated using Archimedes' principle, by measuring the fruit fresh mass in the air and its upward force when the fruit is immersed in water (the fruit

is placed in an immersed basket hanging from a balance). The fruit density (fd, dimensionless) is deduced as $fd = m/|m - r|$, where m is the fruit fresh mass (kg) and r is the upward force (kg). The day of harvest, all of the analyses (respiration rate, peel and flesh color, total soluble solids, titratable acidity, firmness measurements, and biochemical compounds) were performed on six fruits, whereas the other fruits were placed in the climate chamber for ripening.

The peel and flesh colors of each fruit were assessed with a Minolta Chroma meter CR300 (Konica Minolta, Osaka, Japan). The CIELAB coordinates (L^*, a^*, b^*) were measured on the fruit surface and on the internal side of the flesh after having cut off the two cheeks of the fruit to calculate hue angle ($H^\circ = \tan^{-1}(b^*/a^*)$ in degrees). b^* represents the yellow/blue and a^* the red/green color coordinates.

Firmness was measured using a TAXT2 analyzer (Stable Micro Systems, Godalming, U.K.) and expressed as the rupture force to apply to a 5 mm diameter probe on the lateral face of a cheek (expressed in newtons).

The flesh of the six fruits was subsampled, 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 flesh was homogenized using a Grindomix blender (Retsch, Haan, Germany) and a Polytron PT1600E (Kinematica AG, Lucerne, Switzerland), prior to freezing at −20 °C for future analysis.

Total soluble solids were determined using a refractometer ATC-1E (Atago, Tokyo, Japan).

Three grams of ground flesh was homogenized in 30 mL of distilled water. The titratable acidity, expressed as milliequivalents of acid per 100 g of fresh matter, was measured by titration with a 0.1 N NaOH solution up to a pH 8.1 end point, using an automated titrimeter (Schott, Mainz, Germany).

Concentrations of sucrose, glucose, and fructose were measured using a high-performance liquid chromatography (HPLC) system (Dionex Co., Sunnyvale, CA) (*4*). Starch was hydrolyzed with amyloglucosidase (Novo Nordisk Bioindustries Ltd., Bagsværd, Denmark), and its concentration was determined by colorimetric dosage of glucose using a glucose analysis kit (Boehringer Mannheim Corp., New York).

The concentrations of organic acids were measured using a HPLC system (Dionex Co.) under the same conditions (*4*).

Fruit respiration rates (expressed in millimoles of CO_2/kg/h) were measured using a closed system method the day of harvest for the 6 fruits analyzed at harvest and daily for the other 24 fruits stored at 20 °C to ripen, in order to follow the climacteric rise and to ensure that ripe fruits had the same physiological age for analysis. 'Cogshall' mangoes were ripe 3 days after the respiration rate had reached its highest value (*21*). On each measuring day, fruits from each treatment were placed in individual 18 L airtight jars at 20 °C. Carbon dioxide and oxygen changes inside airtight containers were measured every 20 min for 3 h by gas chromatography, with an Agilent M200 apparatus (SRA, Marcy l'Etoile, France), equipped with two manifolds and two columns. The columns used were a PoraPLOT Q (length = 8 m, diameter = 0.32 mm), thermostated at 55 °C and a MS-5A (length = 4 m, diameter = 0.32 mm) thermostated at 60 °C, with helium (entrance pressure = 1.58×10^5 Pa, corresponding to air flow of 1 mL/min at exit port) and argon (entrance pressure = 1.38×10^5 Pa, corresponding to air flow of 2 mL/min at exit port) as carrier gases, respectively. Both of them were fitted with thermal conductivity detectors.

Internal concentrations of carbon dioxide were measured nondestructively each year on 9−14 fruits attached to the tree. A gas collection apparatus was made with a small water glass. A hole has been made at the bottom of the glass and then closed with a septum to take the gas sample. The open side of the gas collection apparatus was held in place on the fruit surface with a ring of putty as a sealant to prevent leaks, 30 h before a gas sample was taken, so that the gas concentration in the apparatus was in equilibrium with the internal concentration of gas within the fruit at the time of measurement. Gas samples were taken early in the morning to minimize the effect of temperature on the fruit respiration rate, by inserting a needle in the gas collection apparatus through the septum. The other extremity of the needle was inserted into a glass Venoject blood-collecting tube (Terumo Corp., Tokyo, Japan) in which there was a vacuum. The vacuum being broken, gas in the collector migrated to the Venoject blood-collecting tube. After 2 min, to equalize pressures in the gas collection apparatus and the Venoject tube, the Venoject tube and then the needle were removed. Carbon dioxide and oxygen concentrations inside the Venoject tube were measured by gas chromatography.

Measurements of Chlorophyll Fluorescence Parameters. Chlorophyll fluorescence was measured on the fruit surface near the apex zone where the first changes in peel color appear. Measurements were made on a peel area of 7 mm diameter of fruits attached to the tree at 4 day and then 2 day intervals as the measurement day approached the day of fruit fall for the "nondestructive" monitoring and the day of harvest for the 30 fruits studied in 2004. The pulse amplitude-modulated fluorometer FMS2 (Hansatech, King's Lynn, U.K.) was used to measure the minimal and maximal chlorophyll fluorescence (F_o and F_m, respectively), variable chlorophyll fluorescence ($F_v = F_m - F_o$), and maximum photochemical quantum yield of photosystem II equal to the ratio between variable and maximal chlorophyll fluorescence (F_v/F_m ratio). Before chlorophyll fluorescence measurements were made, the measured spot of the peel was dark-adapted for 2 h by using a cover, which was retained when the fiber optics was positioned under the spot for the measurement of chlorophyll fluorescence. F_o was probed with a measuring beam at a light intensity of < 0.05 μmol/m/s, generated by a 594 nm light-emitting diode (LED). F_m was elicited by a 0.8 s pulse of saturating light (18000 μmol/m^2/s). F_o and F_m were detected by a PIN diode equipped with a long pass filter ($\lambda > 700$ nm).

Statistical Analysis. Analysis of variance was performed to assess the effect of the light environment on all of the data presented in the tables (chlorophyll fluorescence parameters, fruit density, hue angle of the peel and flesh, dry matter content of the flesh, total soluble solids, titratable acidity, and contents of biochemical compounds).

Parameters of the different relationships were estimated by nonlinear regression. All statistical analyses were computed using R software (*22*).

RESULTS AND DISCUSSION

Effect of Fruit Position in the Canopy on Quality, Maturity, and Chlorophyll Fluorescence. Table 1 presents various physical and biochemical parameters related to fruit quality and maturity measured at harvest and after ripening on mangoes from two positions in the tree canopy and harvested at a maturity stage based on the age after full bloom. At harvest, the hue angle (*23*) measured both on the peel and on the flesh of mango within the canopy was significantly higher than the one of mango from the top of the canopy. The high values indicate a greener color of the peel and the flesh for fruits from within the canopy. The low values for fruits from the top of the canopy indicate a red-purple color of the peel due to the effect of light exposure during fruit growth and the subsequent stimulation of the synthesis of anthocyanin pigments (*24*). The color of peel and flesh was less affected by the fruit position in ripe fruits because all fruits developed during the ripening period a yellow to red and yellow to orange coloration at both the peel and flesh levels, respectively. Similarly, Murray et al. (*25*) observed on plums that the shade effect on fruit color became nonsignificant after storage and ripening as well. Fruit density, which comes closer to 1 as the fruits ripen, was higher in well-exposed fruits. Dry matter content was higher in the flesh of fruits from the top of the canopy. All of these indicators show that fruit maturity at harvest was affected by the fruit position in the tree canopy. Only fruit firmness, which is used as a maturity indicator, too, was not affected by position. Therefore, the harvesting stage based on the age of mango from full bloom was not able to represent the observed difference in fruit maturity according to fruit position in the tree.

The differences according to fruit position in the canopy observed at harvest for quality traits linked to gustatory quality were still significant after ripening at 20 °C only for the total sugar content. In ripe fruit from the well-exposed treatment, the highest content of soluble sugars, which represent the main carbohydrates in mango flesh, shows that the fruits located at the top of the canopy had a higher energy value at consumption. This difference was consistent with these observed for indicators of

Table 1. Effects of the Fruit Position in the Canopy (FP Effect) on Quality and Maturity Indices[a]

		100 shaded leaf/fruit ratio	100 well-exposed leaf/fruit ratio	FP effect
H_{peel} (°C)	at harvest	109.2 ± 7.6	39.3 ± 9.8	***
	ripe	58 ± 14.4	24.8 ± 2.7	§
H_{flesh} (°C)	at harvest	105.9 ± 1.3	97.4 ± 1.4	**
	ripe	89.1 ± 2.0	86.2 ± 0.4	ns
density	at harvest	0.977 ± 0.003	0.992 ± 0.007	*
	ripe	0.991 ± 0.006	0.999 ± 0.003	ns
firmness (newton)	at harvest	72.4 ± 6.3	75.7 ± 10.2	ns
	ripe	6.8 ± 0.4	9.4 ± 0.4	*
DM (%)	at harvest	12.0 ± 0.7	17.7 ± 1.0	***
	ripe	13.9 ± 1.3	17.7 ± 1.1	§
soluble sugars (g/100 g)	at harvest	2.7 ± 0.2	4.0 ± 0.3	**
	ripe	8.0 ± 0.4	10.1 ± 0.2	*
starch (g/100 g)	at harvest	6.2 ± 0.5	8.6 ± 0.9	*
	ripe	0.6 ± 0.1	1.4 ± 0.2	*
TSS (°Brix)	at harvest	6.7 ± 0.3	9.3 ± 0.7	**
	ripe	14.4 ± 1.4	16.7 ± 0.4	ns
organic acids (g/100 g)	at harvest	1.48 ± 0.1	1.47 ± 0.1	ns
	ripe	0.47 ± 0.2	0.37 ± 0.1	ns
TA (mequiv/100 g)	at harvest	31 ± 1.1	25.2 ± 2.9	*
	ripe	5.5 ± 3.7	3.4 ± 1.6	ns
TSS to acid ratio	at harvest	1.9 ± 0.2	2.7 ± 0.3	*
	ripe	21.0 ± 5.5	31.1 ± 6.3	ns

[a] Values are the mean ± SE of six fruits. ns, §, *, **, and *** correspond to the effect of fruit position in the canopy: nonsignificant and significant at $P < 0.10$, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

fruit maturity at harvest, suggesting a delay in the maturity stage of fruits grown under limiting light conditions. This delay may be attributable to lower temperatures and reduced carbohydrate availability. In fact, as shaded fruit-bearing branches received less light, net photosynthesis of leaves from this treatment is lower. Moreover, in the case of mango, leaf nitrogen on a leaf area basis (calculated as the product between leaf nitrogen on a mass basis and the mass-to-area ratio), which reflects photosynthetic capacity, was lower in the shaded leaves (*26*) and involved a reduced carbon supply for shaded fruits.

Assessment of maturity stage based on fruit age calculated in days after full bloom or with the degree-day method is useful in the case of fruits with a grouped flowering period and with little variability in growing conditions, such as banana (*27*) or pineapple (*28*). These indices of fruit harvest provide only an estimate of physiological age for an average fruit in a tree because exposure of fruit-bearing branches or fruits themselves, as well as carbohydrate availability, varied according to their position in the canopy.

To evaluate differences of physical and chemical characteristics according to fruit position, fluorescence parameters were measured on fruit still attached to the tree prior to harvest. At 120 DAFB, values of F_o, F_m, F_v, and F_v/F_m were significantly lower for fruits of the 100 leaves per fruit treatment from the top of the canopy than for those from within the canopy (**Table 2**). As reported on mango (*14*) and other fruits, a decrease in chlorophyll fluorescence parameters occurs during ripening. Therefore, the lower values of chlorophyll fluorescence parameters measured at 120 DAFB on fruits from the top of the canopy suggest that they had reached a further stage of maturity when compared to fruits within the canopy. Chlorophyll fluorescence measurements support results from physical and biochemical measurements, which indicated that fruit at the top of the canopy was more mature. These results show that the usual methods based on degree-days,

Table 2. Effects of Fruit Position in the Canopy on Chlorophyll Fluorescence Parameters at Harvest[a]

	100 shaded leaf/fruit ratio	100 well-exposed leaf/fruit ratio
F_o	328.2 ± 8.8	273.0 ± 7.6
F_m	1926.6 ± 41.0	1489.7 ±36.0
F_v	1598.4 ± 33.6	1216.6 ± 30.3
F_v/F_m	0.830 ± 0.002	0.816 ± 0.003

[a] Values are the mean ± SE of 30 fruits. The effect of fruit position in the canopy was significant at $P < 0.001$ for the four chlorophyll fluorescence parameters.

for instance, are not accurate for estimating maturity of fruits from a complex canopy. Chlorophyll fluorescence measurements can be applied as a nondestructive technique reflecting mango maturity, as other alternative approaches such as NIR spectroscopy developed on mango (*11*).

Changes in Chlorophyll Fluorescence Parameters and Respiratory Rates of Fruits Attached to the Tree. The patterns of changes in fluorescence parameters, F_o (**Figure 1A**), F_m (**Figure 1B**), and F_v (**Figure 1C**), as a function of the number of days before fruit fall, were similar and could be represented by monomolecular functions. Moreover, a unique relationship was obtained for each considered parameter, regardless of the fruit position in the canopy, as the 95% confidence intervals of parameters estimated from data of the two treatments taken separately and together overlap (data not shown). This relationship can be split into three parts. First is a constant phase up to 20 days before fruit fall (DFF), during which all parameters remain constant, at maximal mean values of 341 ± 9, 1936 ± 41, and 1600 ± 34 for F_o, F_m, and F_v, respectively. The two last phases corresponded to the decrease in chlorophyll fluorescence parameters and can be split according to the rate of decrease. Thus, fluorescence parameters slowly decreased from 12 to 8 DFF for F_o and from 10 to 6 DFF for F_m and F_v, and then sharply declined until the fruit fell from the tree.

Changes in the F_v/F_m ratio according to the number of DFF (**Figure 1D**) followed a different pattern: no decrease of F_v/F_m was observed on fruits attached to the tree, regardless of the growth conditions. This parameter varied between 0.81 and 0.84 and tended to be lower 1−3 days before fruit fall. A relatively stable F_v/F_m ratio was observed in papaya during the early stages of the ripening process (*13*−*29*). The relatively invariant F_v/F_m ratio that represents the maximal photochemical quantum yield of photosystem II was weakly correlated to all maturity-associated parameters, as noted for white grapevine (*28*). It was especially affected during fruit senescence when damage at the thylakoid membrane level occurred and when color changes became visible (*30*). For cv. 'Cogshall', visible changes in skin color corresponding to the disappearance of the color green and the appearance of yellow first occurred near the apex zone (referred to as the "yellow point") between 1 and 3 DFF (data not shown). A ca. 40% decrease in F_o, F_m, and F_v occurred before any visible changes became apparent.

These changes in chlorophyll fluorescence parameters were assessed for the first time on fruits attached to trees as early indicators of fruit maturity. Thus, they are potentially exploitable for determining harvesting stages. Decreases in fluorescence parameters have already been observed, especially on fruits detached from the tree, illustrating the effects of ripening and senescence on chlorophyll content and the loss of integrity of chloroplast membranes, limiting photosynthetic capacity (*14*,*17*). In a recent study, chlorophyll fluorescence parameters were assessed at different harvest dates and correlated with sugar concentrations because sugar content is the standard parameter

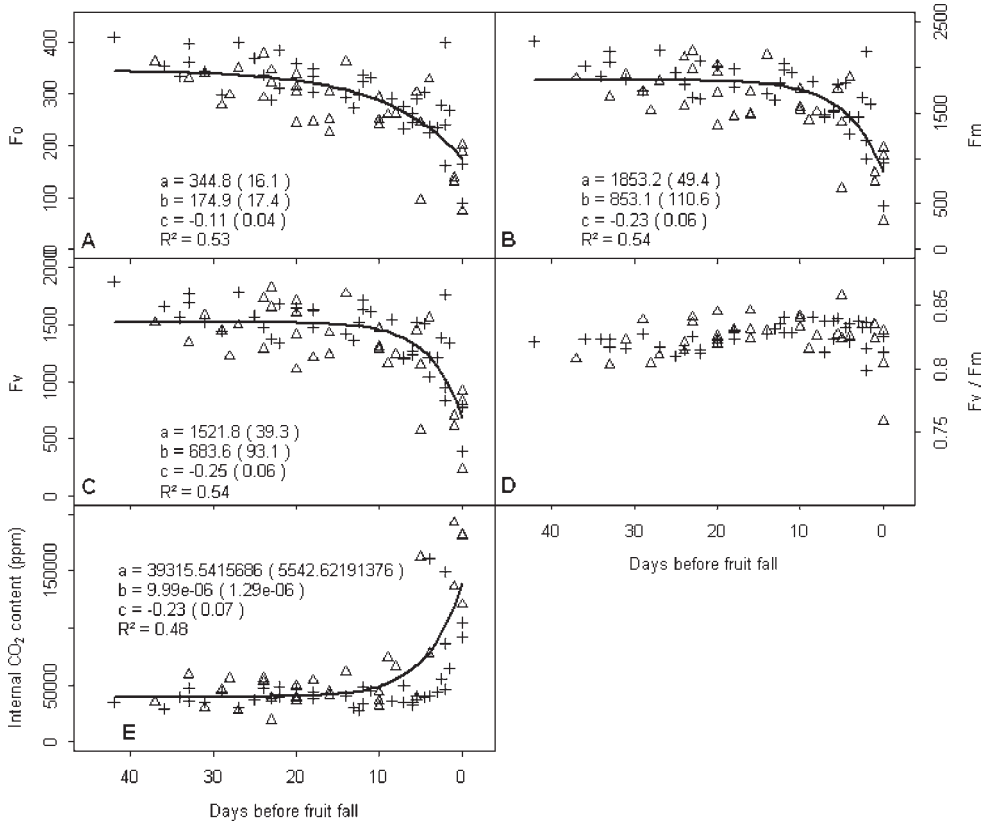

Figure 1. Changes in chlorophyll fluorescence parameters: F_o (**A**), F_m (**B**), F_v (**C**), and F_v/F_m (**D**), and internal concentration of carbon dioxide (**E**) as a function of days before the fruit falls from the tree (DFF), measured on fruits from well-exposed fruits from branches with a 100 leaf-to-fruit ratio (△) and shaded fruits from branches with a 100 leaf-to-fruit ratio (+). Lines represent the best empirical relationship between chlorophyll fluorescence parameters or internal concentration of carbon dioxide and the number of days before fruit fall (equation of the type $a - (a - b) \times e^{(c\text{-}DFF)}$ and $a + 1/(b \times e^{(c\text{-}DFF)})$ for changes in chlorophyll fluorescence parameters and internal concentrations of carbon dioxide, respectively). For each equation, the means and standard errors (SE in parentheses) of fitted parameters and R^2 are provided.

for evaluation of maturity stage for white grapevine in the field (*20*).

The variability in chlorophyll fluorescence parameters observed on fruits attached to the tree may be explained by the different exposure histories of each fruit. This resulted in various degrees of sustained photoinhibition, which affects F_m more than F_o (*31*). However, in our study, the effect of light exposure on chlorophyll fluorescence was limited because measurements were made early in the morning and the fruit peel was dark-adapted for 2 h. Variability in chlorophyll fluorescence can also be caused by the photoprotective quenching, which is mainly attributable to the xanthophyll cycle. Sudden exposure to sun rapidly initiates this process, and these changes influence chlorophyll fluorescence, as has already been observed in apple peel (*32*). F_o and F_m are decreased in direct proportion by photoprotective quenching, whereas photodamage normally increases F_o and decreases F_m (*33*).

The pattern of changes in internal concentration of carbon dioxide as a function of the number of days before fruit fall was represented by a unique inverse monomolecular function, regardless of fruit growth conditions (**Figure 1E**). The function presents three phases: (1) a steady state, at a constant internal concentration of 35000−40000 ppm, up to 20 DFF; (2) a slow increase in internal carbon dioxide of the fruit (instead of a slow decrease as for chlorophyll fluorescence) between 12 and 8 DFF; and (3) a strong increase in internal concentration (instead of a strong decrease as for chlorophyll fluorescence) until the fruit falls from the tree. Thus, it can be stated that the respiratory rise of mango cv. 'Cogshall' occurred on the tree and was initiated before any visible change in fruit color appeared.

By comparing these changes in chlorophyll fluorescence parameters and in respiratory activity we found that at the same time that F_o, F_m, and F_v decrease, the internal concentration of carbon dioxide increases, namely, during the last 10 days before fruit fall. **Figure 2** shows the relationship between the respiratory activity and the chlorophyll fluorescence parameter F_v, obtained from data monitored during two supplementary years on fruits from various growth conditions, namely, the fruit position in the canopy and carbohydrate supply. The same relationships versus F_o and F_m were obtained with similar parameters of fitting goodness (R^2 = 0.70 and 0.71 for the relationship between internal CO_2 and F_o and internal CO_2 and F_m, respectively). The relationship versus F_v was represented to take the decrease in both F_o and F_m parameters into account. This relationship suggests that the decrease in chlorophyll fluorescence parameters is correlated with mango maturity.

The concomitant decrease in F_o and F_m and the increase in internal carbon dioxide content in mangoes attached to the tree can be interpreted as a decline in chlorophyll content associated with the climacteric rise of the fruit. In apple, ethylene was observed to increase before the decrease in chlorophyll fluorescence during fruit maturation and ripening (*17*). The parameter F_m has not decreased during ripening when apples were treated with an ethylene inhibitor such as 1-methylcyclopropene with a high application frequency during fruit storage, suggesting that chlorophyll content was only slightly reduced and that ethylene played a major role in the degradation of chlorophyll (*34*). This hypothesis is supported by studies on the regulation of the activity of the enzyme implicated in chlorophyll catabolism,

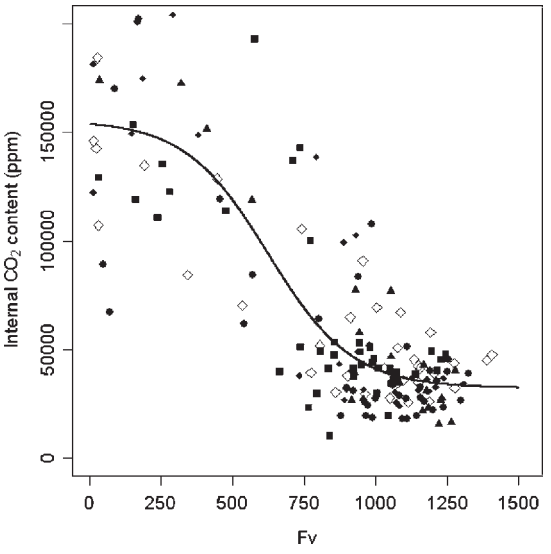

Figure 2. Nonlinear relationship (equation of the type $a + b/(1 + e^{[(F_v - c)/d]})$, with $a = 32231.42 \pm 4999.54$, $b = 123086.66 \pm 10863.39$, $c = 626.26 \pm 43.16$, $d = 145.96 \pm 35.23$, and $R^2 = 0.71$) between variable chlorophyll fluorescence F_v and internal concentration of carbon dioxide measured on mangoes attached to the tree: from 10 (▲), 25 (●), and 100 (■) leaf-to-fruit ratio treatments in the 2005 growing season and from well-exposed (◇) and shaded (◆) fruit from branches with a 100 leaf-to-fruit ratio in the 2006 growing season.

chlorophyllase, during fruit ripening. Ethylene appears to enhance the degreening of fruit peel through the de novo synthesis of chlorophyllase protein (*35*). The rather constant F_v/F_m ratio accompanied by clearly dropping absolute fluorescence (F_o and F_m) as a function of the physiological age suggests coordinated chlorophyll degradation leaving the remaining photosystem II active. In fact, the alteration in photosynthetic activity due to the release of chlorophyll from protein−pigment photosynthetic complex may affect both F_m and F_v/F_m parameters. Further experimentations are required to achieve a direct correlation between chlorophyll content and fluorescence parameters and to study the role of chlorophyllase during fruit maturation throughout changes in chlorophyll fluorescence parameters and in contents of internal ethylene and sugar on fruit attached to the tree.

In conclusion, our observations demonstrate that changes in chlorophyll fluorescence parameters on fruits attached to trees are correlated with changes in fruit maturity, regardless of the fruit growth conditions, such as the position of the fruit and the fruit-bearing branch in the canopy or the carbon supply for fruit growth. The chlorophyll fluorescence method evaluates maturity of individual fruits, whereas the degree-day method gives only an average of fruit maturity at the tree or orchard level. By being independent of growing conditions, this nondestructive method provides fruit maturity for harvesting mango with a better accuracy than the degree-day method. Moreover, in the study we suggested that the chlorophyll fluorescence method can be used on fruits before harvest, which is quite novel, previous studies having focused exclusively on the potential of this method as a postharvest tool for sorting.

The chlorophyll fluorescence method has the potential to become a nondestructive indicator of the harvesting stage of mango and possibly other fruit species. Further investigations will be conducted on mango cv. 'Cogshall' to determine processes involved in the observed changes of chlorophyll fluorescence parameters, in particular, the role of ethylene in the degradation of chlorophyll in the peel. Another step will consist in finding ways to free one's self from the need to dark-adapt prior to measurement of chlorophyll fluorescence parameters. The chlorophyll fluorescence technique should be compared to alternative approaches, such as NIR spectroscopy, by studying spectral response at the wavelength of chlorophyll absorption, to assess mango maturity. Eventually, the potential of this method will be assessed on mango cultivars other than 'Cogshall'.

ACKNOWLEDGMENT

We gratefully acknowledge C. Soria (CIRAD, UR HortSys, Reunion Island) for technical assistance during the field experiments and J. Minier (CIRAD, UMR Qualisud, Reunion Island) for assistance with the biochemical analyses. We thank G. Wagman for revising the manuscript and improving the English.

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Received for review March 31, 2010. Revised manuscript received June 4, 2010. Accepted June 5, 2010.