Nondestructive assessment of fruit biological age in Brazilian mangoes by time-resolved reflectance spectroscopy in the 540–900 nm spectral range

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ABSTRACT

Time-resolved Reflectance Spectroscopy (TRS) in the 540–900 nm spectral range has been tested in order to assess nondestructively the biological age of Brazilian mangoes. To this purpose a TRS set-up has been used to measure absorption and scattering coefficients of 60 intact mango fruits (cultivar 'Haden'), harvested in Minas Gerais, Brazil, and transported by plane to Milan, Italy. Flesh firmness, pulp color, and absorbance spectra by reflectance were determined on the same fruit, while ethylene and CO_2 production rates, and respiratory quotient were determined on a subsample of the same fruit. Absorption spectra of intact fruit showed high variability at 540–600 nm, near carotenoids absorption and in the 630–690 nm range, near chlorophyll-*a* absorption, in agreement with the reflectance measurements on the pulp. Firmness ranged from 5 to 108 N, but the majority of the fruit was less firm than 20 N. The pulp color showed a greenish yellow hue in less mature fruit, and an orange hue in more mature fruit. Good correlations (R^2>0.8) were found between the ethylene production rate and the ratio of the absorption coefficient at 540 nm to any of the absorption coefficients at 630, 650, 670, 690 nm, as well as between color parameters and μ_a540. Our results underline that TRS in the 540–900 nm range can be a useful tool to assess nondestructively the biological age of Brazilian mangoes.

1 Introduction

The discrimination between mature and immature mangoes at harvest is important from the marketing point of view, as fruit picked too early are more sensitive to chilling injury and may fail to ripen, while fruit harvested at a late maturity stage have a reduced shelf life and greater susceptibility to disease. Fruit shape, skin and pulp color, and firmness are the most used maturity indices for mangoes, but there is a lack of reliable quality and harvest criteria (Padda *et al.*, 2011; Subedi *et al.*, 2007; Sivakumar *et al.*, 2011). Furthermore, the measurements of firmness and pulp color have the disadvantage to be destructive analyses. Pulp color turns from light green to yellow-orange during fruit ripening, due to chlorophyll breakdown and to biosynthesis of carotenoids.

Time-resolved Reflectance Spectroscopy (TRS) is a nondestructive optical technique which quantifies the absorption (*i.e.* pigments) and scattering (*i.e.* structure) coefficients in the VIS–NIR wavelength range, by probing pulp at a depth of 1–2 cm with no or limited influence from the skin (Cubeddu *et al.*, 2001, Torricelli *et al.*, 2008).

In nectarines, the absorption coefficient measured at 670 nm, near the chlorophyll-*a* absorption peak, can be considered an index of the fruit biological age (Tijskens *et al.*, 2007) and has been successfully used to predict fruit softening rate during shelf life, and, hence, to select fruit for different market destinations (Eccher Zerbini *et al.*, 2009).

In a previous work on 'Tommy Atkins' mango fruit, the absorption coefficient at 630 nm (related to chlorophyll-*b* content), converted into the biological shift factor, was used for softening rate prediction, but the

model explained only 70% of the variation in softening development (Pereira *et al.*, 2010). However, when the absorption coefficient at 630 nm was used to sort mangoes into different maturity classes, fruit with different pulp color characteristics having different color rate changes were selected, until the increase of pulp yellowness did not interfere with chlorophyll measurements (Vanoli *et al.*, 2011). Furthermore, it was ascertained that the 2–3 mm green layer under the mango peel attenuates the TRS intensity signal, mainly in the chlorophyll range (Spinelli *et al.*, 2012).

This work aimed at testing whether TRS can be used to assess nondestructively the biological age of Brazilian mangoes. In particular, diffuse reflectance measurements were performed in the spectral range 540–900 nm, which encompasses the absorption characteristics of the two main chromophores that are linked to the ripening of mango fruit: carotenoids and chlorophyll. The TRS measurements were compared to more standard properties of fruit, such as pulp color and firmness, and to metabolic measurements, such as ethylene and CO_2 production rates and respiratory quotient.

2 Material and Methods

2.1 Instrumentations and methods

The schematic of the TRS setup developed at Politecnico di Milano and used for measurements is shown in figure 1 (Spinelli *et al.*, 2012). The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light picosecond pulses, adjustable in power by a variable neutral-density attenuator. A filter wheel loaded with 14 band-pass interference filters is used for spectral selection in the range 540–940 nm. Light is delivered to the sample by means of a multimode graded-index fiber. Diffuse remitted light is collected by 1 mm fiber. The light then is detected with a photomultiplier (HPM-100-50, Becker&Hickl, Germany) and the photon distribution of time-of-flight is measured by a time-correlated single-photon counting board (SPC-130, Becker&Hickl, Germany). A model for photon diffusion in turbid media was used to analyze TRS data to assess the bulk optical properties of samples (Martelli *et al.*, 2009).

Pulp color was measured in the L* a* b* color space with a spectrophotometer (CM-2600d, Minolta Co., Japan), using the primary illuminant D65 and 2° Standard Observer, acquiring the reflectance spectrum from 360 to 740 nm at 10 nm intervals. Chroma (C*) and hue (H°) were computed from a* and b* values according to $C^* = [(a^*)^2+(b^*)^2]^{-2}$ and $H^\circ = \arctan(b^*/a^*)$. Reflectance data (R) were converted into absorbance (A) using $A = \log(100/R)$. Furthermore, the yellowness index (I_Y) was also computed, according to the expression: $I_Y = [(1.2746X - 1.0574Z)/Y]\times100$, after converting the L* a* b* parameters into the *XYZ* color space (Jha *et al.*, 2006).

Flesh firmness was measured using a penetrometer (Instron UTM model 4301, crosshead speed 200 mm/min, 8 mm diameter plunger) after skin removal, in position corresponding to the TRS readings.

Ethylene and CO_2 production rates were measured by putting fruit in 1.7 L gastight glass jars (one fruit per jar) for 2 h at 20°C; then, for the determination of the ethylene content, 1 ml of the headspace gas was sampled and analyzed using a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in × 200 cm) at a column temperature of 100°C and FID detection. Quantitative data were obtained by relating the ethylene peak area to that of a 10 µL/L standard and were expressed as picomoles per kilogram per second. For the analysis of respiratory gases (CO_2, O_2), the jar was directly connected to the MicroGC MTI (model P-200, Hewlett-Packard), and the CO_2 production and the O_2 uptake rates were expressed as nanomoles per kilogram per second in standard conditions. GC data were corrected for fruit mass, void volume, temperature and pressure of the jar and the time of production. Respiratory quotient (RQ) was computed as the ratio between CO_2 production and O_2 uptake rates.

2.2 Mango fruit

Mango fruit (cultivar 'Haden') were harvested in a commercial orchard in Minas Gerais, Brazil, and immediately transported by plane to Milan, Italy. At arrival, 60 fruits without defects were selected and individually measured by means of the TRS set-up for the absorption coefficient at 650 nm (μ_a650) as the signal-to-noise ratio observed at 670 nm (*i.e.* on the chlorophyll-*a* peak) was too low to guarantee reliable TRS measurements. Then, mangoes were ordered on the basis of decreasing μ_a650, *i.e.* increasing maturity, and hence classified based on the ranking order as (class ranking, μ_a650, mean ± std err) less mature (LM, rank 1-20, 0.0863 ± 0.0112 cm^{-1}), medium mature (MM, rank 21-40, 0.0389 ± 0.0006 cm^{-1}) and very mature (VM, rank 41-60, 0.0321 ± 0.0048 cm^{-1}).

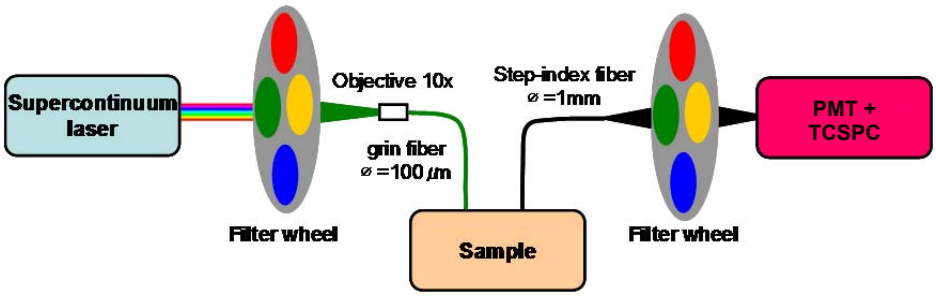


Fig. 1 Scheme of the TRS instrumental setup: PMT is for photomultiplier tube, TCSPC is for time-correlated single-photon counting electronics read-out.

Then, for each intact fruit, the optical properties in the 540–900 nm spectral range were measured by means of the TRS set-up on two opposite sides in the fruit equatorial region. At the same positions, also flesh firmness and pulp color were assessed. Pulp color was measured after removing with a blade a segment of skin with underlying pulp. For the measurement of ethylene and CO_2 production rates and respiratory quotient, a subsample of 20 fruits, covering the whole range of $\mu_a 650$, was selected.

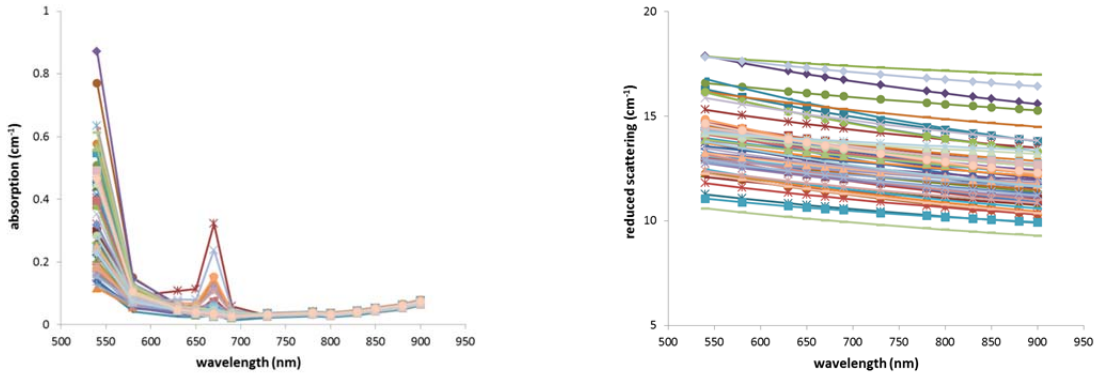
3 Results and discussion

3.1 TRS measurements

Figure 2 shows the absorption coefficient and the reduced scattering coefficient in the 540–900 nm spectral range for the 60 mango fruits. As for the absorption properties (Fig. 2a), the contribution to the absorption spectra of the 3 main tissue chromophores present in these fruit, that is carotenoids, chlorophyll-*a* and water is clear. In particular, absorption spectra shows a high variability in the 540–600 nm spectral range, near the carotenoid absorption, while in the 630–690 nm region, in correspondence of the chlorophyll absorption peak, the variability is more limited. This feature of absorption spectra is related to a high variability of carotenoid content in these fruit (Azzollini, 2013), and it is compatible with the fact that they are in a quite advanced state of ripeness. In contrast, no differences among fruit were visible above 700 nm. As for the reduced scattering coefficient, the spectra decrease slowly with the wavelength increase, while the reduced scattering values span over a quite wide range: from about 10 to 18 cm^{-1} (compare Fig. 2b). Again, this feature is related to the advanced maturity state of these fruit, as scattering decreases with fruit ripening.

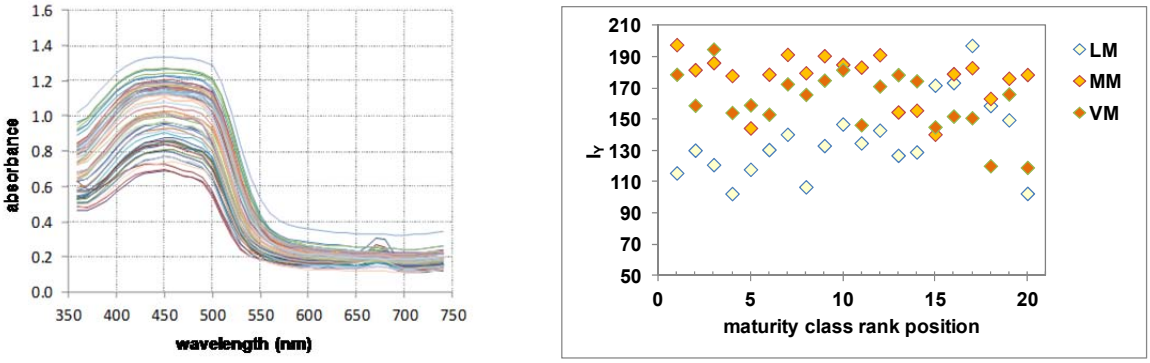
3.2 Color and firmness measurements

The absorbance spectra assessed by means of the spectrophotometer on the pulp of the 60 mango fruits are reported in Fig. 3a. The pulp color shows a maximum of absorption in the spectral range 400–500 nm, and another one, even if smaller, at 670 nm. These features of the absorbance spectra are in agreement with what observed by TRS.



(a) (b)

Fig. 2 Absorption (a) and reduced scattering (b) spectra assessed by means of the TRS set-up in the wavelength range 540–900 nm for the 60 mango fruits.



(a) (b)

Fig. 3 Absorbance spectra (a) and I_Y (b) of the pulp of the 60 mango fruits.

As for the color parameters linked to the pulp color, their ranges of variability are reported in Table 1. Differences in color parameters were found between LM class and the other two, with less mature fruit being characterized by lower a*, b*, C* and I_Y and higher H°. Fig. 3b shows the value of I_Y of the pulp of the 60 mango fruits as a function of the rank position within each maturity class: as can be seen, five out of twenty fruits of LM class (ranks 15–19) had I_Y values in the same range of the other two maturity classes, and the I_Y of the MM and VM maturity classes were in the same range, indicating that these fruit were in an advanced maturity stage. This pattern of the fruit batch was confirmed also by the firmness measurements (Fig. 4). Even if the firmness varied from 5 to 108 N, the majority of the mango fruit (45 out of 60) had firmness values lower than 20 N, which are characteristic of ready to eat or overripe fruit.

Table 1 Pulp color parameters (mean ± std err) of 'Haden' mango fruit.

Parameter	LM class (n=20)	MM class (n=20)	VM class (n=20)	batch mean (n=60)
a*	5.69±1.24	14.83±1.01	11.04±1.06	10.52±0.79
b*	56.28±1.23	63.27±0.52	61.04±0.75	60.20±0.63
C*	56.78±1.36	65.12±0.66	62.2±0.83	61.37±0.72
H°	84.71±1.09	76.93±0.83	79.89±0.93	80.51±0.68
I_Y	136.6±5.5	176.0±3.6	160.9±4.3	157.8±3.3

3.3 Ethylene production, CO_2 production and O_2 uptake rates

In Fig. 5 the average values and standard errors of ethylene and CO_2 production rates, together with the respiratory quotient are reported for the three different maturity classes. Results for ethylene production rate are similar to those reported in literature (Zheng *et al.*, 2007; Zaharah & Singh, 2011): LM mangoes produce less ethylene than MM and VM mangoes (Fig. 5a), and less CO_2 than VM mangoes (Fig. 5b). As for the respiratory quotient, only the MM fruit present an average value larger than 1.2 together with a high variability (Fig. 5c), suggesting a change from aerobic to anaerobic respiration in some fruit.

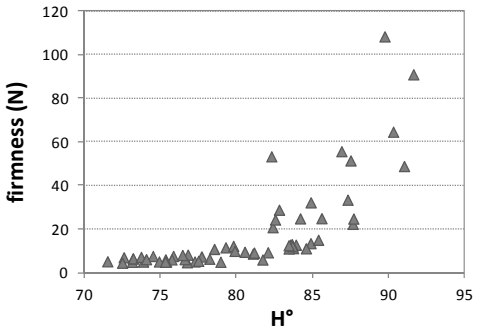
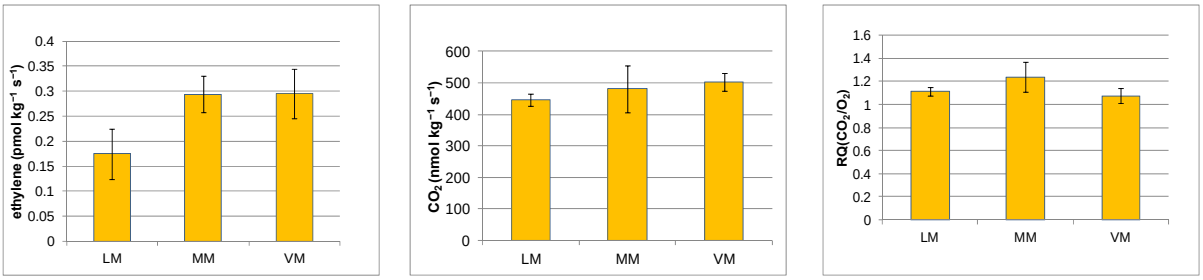


Fig. 4 Pulp firmness as a function of the H° pulp color parameter for the 60 mango fruits.



(a) (b) (c)

Fig. 5 Production rate of ethylene (a) and CO_2 (b), and respiratory quotient (c) as a function of the mango maturity class: less mature (LM), medium mature (MM) and very mature (VM). Bars represent standard deviations.

3.4 Correlation analysis

The relationships between the ethylene production rate and the optical properties have been considered. Due to the possible influence of the carotenoids on the estimation of the absorption coefficient at 630, 650, 670 and 690 nm, where the absorption peak of chlorophyll-*a* is located, we studied the correlations between the ethylene production and the ratio of the absorption coefficient at 540 to μ_a630, μ_a650, μ_a670, μ_a690. From Fig. 6a, we observe that as the ratios of absorption coefficients increased, which means more carotenoids and less chlorophyll, hence more ripe fruit, the ethylene production rate increased. This is in accordance with the climacteric behavior of mango fruit, where the production of ethylene drives the ripening syndrome, characterized by many symptoms, among which the most evident are decrease of chlorophyll, increase of carotenoids, and softening. The correlation coefficients result larger than those between ethylene production rate and individual absorption coefficients (data not shown). As for color parameters and firmness, the correlations with μ_a540 were considered (Fig. 6b). High correlation coefficients were obtained for color parameters, linked to carotenoids concentrations, whereas for firmness an $R^2<0.75$ was observed.

4 Conclusions

Even though the 60 mango fruits exhibited an advanced maturity state as confirmed by firmness measurements, it was already possible to find important information about the TRS technique: firstly, the absorption spectra determined by TRS on the intact fruit are in agreement with the absorbance spectra of the mango pulp assessed by means of the spectrophotometer. This fact confirms that the TRS is able to determine the optical properties of the pulp, with no influence due to the mango skin (Spinelli *et al.*, 2012). Secondly, in the absorption spectra the two spectral regions, 540–600 nm and 630–690 nm, which are sensitive to carotenoids and chlorophyll content respectively, show a useful variability that can be exploited for the assessment of the biological age. This was confirmed by the good positive correlation between the ethylene production rate of mango fruit and the ratio of the absorption coefficient at 540 nm to an absorption coefficient in the 630–690 nm spectral region, as well as between color parameters and μ_a540.

Therefore it can be concluded that TRS in the 540–900 nm range can be a useful tool to assess in a nondestructive way the biological age of Brazilian mangoes.

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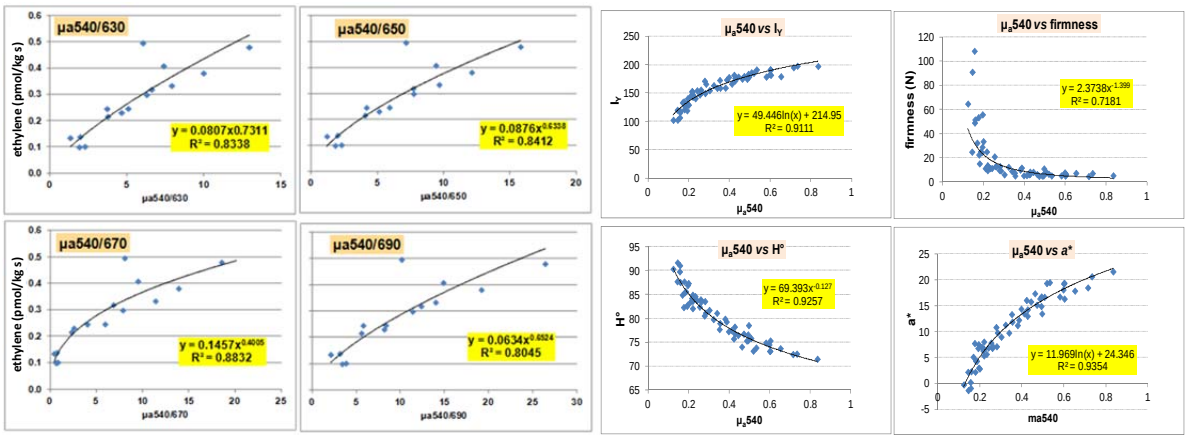

(a) (b)

Fig. 6 (a) correlations between the ethylene production rate of mangoes and the ratios μ_a540/μ_a630, μ_a540/μ_a650, μ_a540/μ_a670, μ_a540/μ_a690. (b) correlations between I_Y, H°, a*, firmness and μ_a540.

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