Quality of Brazilian mango fruit in relation to optical properties non-destructively measured by time-resolved reflectance spectroscopy.

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Introduction

Mango is one of the most important fruit crops in Brazil and about 25% of the production is exported, mainly to USA and Europe (Pinto et al., 2004). A major challenge is to minimise quantitative and qualitative losses during the supply chain of fresh mangoes while ensuring an acceptable ripening. Quality of mangoes is strictly dependent on proper maturity at harvest 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 (Sivakumar et al., 2011). Differences among cultivars and growing conditions have precluded universal maturity indices. Recently, Padda et al. (2011) showed that firmness, flesh colour and total soluble solids content are the best parameters to assess ripening of mangoes but these factors have the disadvantage of being destructive.

Time-resolved reflectance spectroscopy (TRS) is a non-destructive technique which simultaneously quantifies the internal optical properties of fruit through the absorption (i.e. pigments) and the scattering (i.e. physical structure) coefficients by probing the pulp at a depth of 1-2 cm with no or limited influence from the skin (Torricelli et al., 2008). The absorption coefficient (μa) measured at harvest at 630-690 nm, near the chlorophyll peak, allowed selection of fruit of different maturity showing different quality attributes as regards chemical composition and sensory characteristics during shelf-life and after storage (Torricelli et a., 2008). In nectarines, the absorption coefficient measured at 670 nm (μa670) can be considered an effective maturity index as it is linked to the biological age of the fruit; in fact, it has been successfully used to predict the softening rate of nectarines during shelf-life allowing selection of fruit for different market destinations (Eccher Zerbini et al., 2009). On the other hand, the scattering coefficient (μ's) gave an insight into the textural properties of the fruit pulp. In apples, Vanoli et al. (2009) and Rizzolo et al. (2010) found that μ's measured in the range between 750 and 790 nm were related to the mechanical properties of fruit (firmness, stiffness, intercellular spaces) as well as to pectin composition and sensory attributes related to structure (firm, crispy, mealy and juicy).

The possibility of using the TRS technique to assess maturity in mangoes has been previously explored but the results were not always completely satisfactory. Pereira et al. (2010) measured Brazilian mangoes at 630 nm and, by converting μa630 into the biological shift factor, it was only possible to explain about 70% of the variation in softening behaviour. When μa630 was employed to sort mangoes into different maturity classes, it was possible to obtain fruit with different pulp colour characteristics, showing different colour rate changes during postharvest ripening; however, in the last ripening period, the increase in pulp yellowness interfered with chlorophyll measurements (Vanoli et al., 2011a).

Two problems have to be considered when measuring mangoes: the presence of a green layer (2-3 mm) under the peel which attenuates the TRS signal intensity mainly in the chlorophyll range (630-690 nm), and the carotenoid accumulation in the pulp during mango ripening, especially in more mature fruit, which affect the chlorophyll estimate (Spinelli et al., 2012; Vanoli et al., 2011a).

In order to overcome these problems, in this work we tested the TRS technique in the 540-900 nm range with the aim of assessing the quality of Brazilian mangoes during shelf-life.

Materials and Methods

One hundred and twenty mangoes (cv Tommy Atkins, harvested in Pernambuco, Brazil and immediately transported by plane to Milan, Italy) were individually measured by TRS at 540 nm on two opposite sides in the fruit equatorial region and ranked by decreasing μa540, averaged over the two sides, that is from more (high μa540) to less (low μa540) mature fruit. Then, mangoes were randomised into 4 batches of 30 fruits and analysed after 0, 1, 2 and 5 days of shelf-life at 20°C (d0, d1, d2 and d5). At each time of analysis, fruit were measured by TRS in

the 540-900 nm range on two opposite sides and analysed at the same points for flesh firmness (Instron UTM model 4301, crosshead speed 200 mm/min, 8 mm diameter plunger) and pulp colour (Spectrophotometer CM-2600d, Minolta; L*,a*,b*, C*, H°). Fruit of d5 (batch 4) were measured by TRS in the 540-900 nm range after 0, 1, 2 and 5 days at 20°C in order to follow the changes of the optical properties during the shelf-life period on the same fruit.

A TRS setup developed at Politecnico di Milano was used. The light source is a fibre laser (SC450-6W, Fianium) 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 540-900 nm. Light is delivered to the sample by a multimode graded-index fibre. Diffuse remitted light is detected by 1 mm fibre. The light is detected with a photomultiplier and a time-correlated single-photon counting board (HPM-100-50, SPC-130; Becker & Hickl). A model for photon diffusion in turbid media was used to analyse TRS data to assess the bulk optical properties (absorption coefficient, μa, and reduced scattering coefficient, μs') of samples. Convolution of the photon diffusion model with the instrument response function (measured facing the injection and the collection fibres) is performed before fitting the experimental data. An approximation to the Mie theory is used to relate the reduced scattering coefficient to the structural properties of the diffusive sample: $\mu s' = A\,(\lambda/\lambda_0)^{-B}$, where λ is the wavelength, A is the scattering coefficient at wavelength λ_0 = 600 nm, and B is a parameter related to the size of scatterers.

TRS absorption and scattering spectra were elaborated by The Unscrambler X (v. 10.0.1; Camo, Norway) in order to build partial least square (PLS) regression models for firmness and pulp colour. TRS spectra were also analysed by linear discriminant analysis to classify the samples on the basis of the day of shelf-life (Statgraphics, v. 7; Manugistic Inc., Rockville MD, USA). Firmness and pulp colour data were processed by analysis of variance and means were compared by Tukey's test.

Results and Discussion

Fruit characteristics

Flesh firmness (Table 1) significantly decreased during shelf-life. At d0, 70% of mangoes had firmness greater than 20N, at d1 56% of fruit softened below 20N, while at d5 all fruit had firmness lower than 20N with 89% of mangoes softening below 10N (Fig. 1). This means that at d0 only 30% of fruit are ready to eat and that at d5 all mangoes are ready for consumption.

Pulp colour turned from greenish-pale yellow to yellow-orange during the shelf-life period: a*, b* and C* parameters significantly increased while L* and H° significantly decreased from d0 to d5 at 20°C (Table 1).

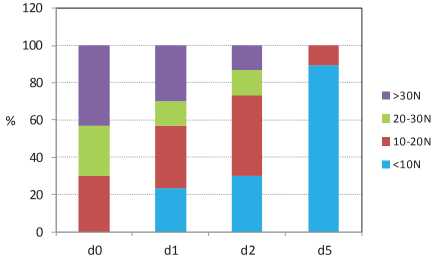

Figure 1. Frequency of 'Tommy Atkins' mangoes according to firmness classes at d0, d1, d2 and d5 of shelf-life

TRS spectra characteristics

Figure 2 shows the absorption and scattering coefficients in the 540-900 nm spectral range measured at d0 and d5, and the absorption coefficient measured at 540 nm and at 650 nm during the 5 days of shelf-life of mangoes belonging to batch 4.

Absorption spectra showed two maxima: the first at 540 nm and the second at 670 nm. On average, the μa540 values increased while those at 670 nm decreased with shelf-life: fruit at d0 were characterised by the highest values of μa670 and the lowest for μa540; the opposite was true for mangoes at d5. The increase of μa540 values during shelf-life could be due to carotenoid biosynthesis as reported by Vasquez-Caicedo et al. (2006) which found in 'Tommy Atkins' mangoes an 18.9 % increase in the amount of total β-carotenes during postharvest fruit ripening together with a dynamic interconversion of the plastid structures. The 'Tommy Atkins' cultivar is also particularly rich in all-trans-violaxanthin and 9-cis-violaxanthin (Ornelas-Paz et al., 2007). In various mango cultivars, it was found that the concentrations of these carotenoids increased in an exponential manner during fruit ripening and were highly correlated with the mesocarp a* (positive) and H° (negative) values (Ornelas-Paz et al., 2008). In our work, TRS absorption spectra reflected the changes in pulp colour. High and negative correlations were found between H° and μa540 while lower but positive correlations were observed between H° and μa670 (Table 2), confirming our previous results on 'Tommy Atkins' and 'Palmer' mangoes (Vanoli et al., 2011a; Spinelli et al., 2012).

Optical properties measured by TRS also include the estimate of scattering phenomena. The scattering spectra of 'Tommy Atkins' mangoes were flat and slightly decreased with increasing wavelength (Fig. 2). On average, scattering spectra decreased with shelf-life. The density of the scatterers (parameter A) significantly decreased during the shelf-life period while the size of the scatterers (parameter B) was constant up to d1, significantly increased at d2, and remained constant until d5 (Fig. 3). Changes in scattering parameters reflected changes

Table 1. Firmness and pulp colour data of 'Tommy Atkins' mangoes during shelf-life at 20°C (mean ± standard error).

days at 20°C	firmness (N)	*L**	*a**	*b**	*C**	*H°*
0	46.1 ± 7.2	82.7 ± 0.3	-0.32 ± 0.56	52.3 ± 0.9	52.4 ± 0.9	90.7 ± 0.6
1	27.8 ± 4.6	81.5 ± 0.6	1.53 ± 0.54	54.0 ± 0.8	54.1 ± 0.8	88.6 ± 0.5
2	16.9 ± 1.8	81.0 ± 0.3	2.58 ± 0.49	56.5 ± 0.8	56.6 ± 0.8	87.6 ± 0.5
5	8.0 ± 0.4	78.8 ± 0.3	5.41 ± 0.36	60.5 ± 0.7	60.7 ± 0.7	84.9 ± 0.3

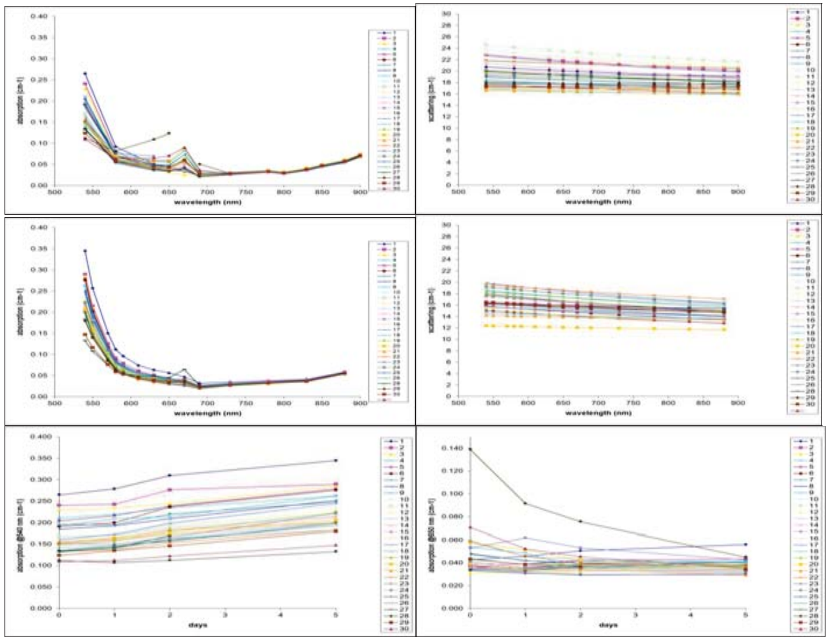

Figure 2. *Top row*: absorption coefficient (*left*) and reduced scattering coefficient (*right*) in the 540-900 nm spectral range at d0 for 30 fruits (batch 4). *Middle row*: absorption coefficient (*left*) and reduced scattering coefficient (*right*) in the 540-880 nm spectral range at d5 for 30 fruits (batch 4). *Bottom row*: absorption coefficient at 540 nm (*left*) and 650 nm (*right*) during shelf-life for 30 fruits (batch 4). Fruits are ranked from 1 to 30 on the basis of decreasing absorption coefficient at 540 nm corresponding to decreasing maturity.

which occurred in the pulp structure due to mangoe softening during shelf-life. Softening is accompanied by the enzymatic cell wall breakdown and so the density of the scattering particles in the fruit flesh decreased, as reported for apples and tomatoes (Qin and Lu, 2008; Vanoli et al., 2011b). As a consequence of this phenomenon, the light scattering at the cell interfaces is reduced leading to fewer scattering events in the tissue (Bobelyn et al., 2008). Actually, the situation in fruit pulp is much more complex than just described, as the scattering centres of a fruit are not expected to be homogeneous spheres and the parameters A and B do not assess the real size of scattering centres in the tissue; they are average equivalent parameters that could be related to physical characteristic of fruit. In our work, considering the days of shelf- life together, a significant positive correlation between firmness and µs ‘880 was found, but the highest r was observed at d1 when firmness classes were evenly represented within the sample (Table 2; Fig. 3). Significant correlations were also found between firmness and μ_a measured at 650 and 670 nm (Table 2).

Table 2. Correlation coefficients (*r*) between μ_a540, μ_a650or μ_a 670, μ'_s 88 and firmness and pulp *H°* value of ‘Tommy Atkins’ mangoes at d0, d1, d2 and d5 of shelf-life.

		μ_a540	μ_a650 or μ_a670	μ'_s880
H° pulp	d0	-0.799***	0.798***	0.138
	d1	-0.890***	0.396*	0.595***
	d2	-0.894***	0.529**	0.243
	d5	-0.902***	-0.588***	0.337
	d0-d5	-0.858***	0.537***	0.551***
firmness	d0	-0.470**	0.759***	0.412*
	d1	-0.413*	0.604***	0.701***
	d2	-0.470**	0.435*	0.391*
	d5	-0.595***	-0.329	0.457*
	d0-d5	-0.487***	0.677***	0.609***

Different PLS models were built to predict pulp colour and firmness of ‘Tommy Atkins’ mangoes considering either scattering spectra alone, absorption spectra alone, or absorption spectra coupled with scattering spectra or with A and B parameters of the scatterers (Table 3). Poor results were obtained for all the quality characteristics using scattering coefficients alone: the highest R2cv was 0.40 for a*pulp. On the contrary, good correlations (R2cv = 0.9) were obtained between TRS absorption spectra and pulp colour (a*, H°). However, the performance of the models was improved adding scattering spectra or A and B parameters of the scatterers to absorption coefficients, reaching R2cv =0.90 and 0.93 for a* and H°, respectively. PLS regression models for pulp b* and C* presented lower coefficients of determination (0.70 - 0.74) in contrast to Subedi et al. (2007) who predicted flesh b* values with R2=0.88 - 94; however, these workers found that the optimal wavelengths to model pulp b* did not include the visible wavelengths, upon which b* is based, but the longer wavelengths. This result was attributed to low penetration through the fruit tissue. In contrast, our results confirmed that the TRS technique actually measures the optical properties of the fruit pulp even in the visible range, as shown by the relationships between pulp colour and the absorption coefficients measured at 540, 650 and 670 nm (Table 2). As for firmness, the best prediction model (R2cv=0.73) was achieved coupling absorption coefficients to the A and B parameters of the scattering centers, even if RMSECV values remained high (Table 3). Many authors explored the possibility of using VIS/NIR spectroscopy to predict firmness of different mango cultivars with opposite results. Valente et al. (2009) found that good prediction results for firmness depend on maturity degree and range of firmness of mangoes as the best results (R2=0.62) were obtained for ripe fruit with firmness lower than 20N. Better results (R2=0.77) were found by Jha et al. (2006) by using a handheld colorimeter.

Table 3. Performance of different regression models on original TRS spectral data for prediction of pulp colour and firmness of ‘Tommy Atkins’ mangoes.

Dependent variable	TRS parameters	N° of factors	Calibration		Cross-validation	
			R^2_{cal}	RMSEC	R^2_{cv}	RMSECV
*a** polpa	μ_a540-880	3	0.91	1.02	0.89	1.13
	μ'_s540-880	5	0.47	2.46	0.40	2.63
	μ_a540-880+ μ'_s540-880	5	0.92	0.95	0.90	1.09
	μ_a540-880+MIE *A B*	5	0.92	0.94	0.90	1.07
*b** polpa	μ_a540-880	2	0.70	2.87	0.63	3.17
	μ'_s540-880	3	0.35	4.21	0.32	4.32
	μ_a540-880+ μ'_s540-880	4	0.73	2.70	0.65	3.18
	μ_a540-880+MIE *A B*	3	0.73	2.70	0.68	3.01
*C** polpa	μ_a540-880	2	0.71	2.85	0.64	3.18
	μ'_s540-880	3	0.35	4.27	0.31	4.45
	μ_a540-880+ μ'_s540-880	4	0.75	2.68	0.67	3.07
	μ_a540-880+MIE *A B*	3	0.74	2.69	0.68	3.09
H° polpa	μ_a540-880	3	0.91	1.01	0.90	1.07
	μ'_s540-880	5	0.47	2.47	0.39	2.66
	μ_a540-880+ μ'_s540-880	7	0.94	0.80	0.93	0.94
	μ_a540-880+MIE *A B*	7	0.94	0.79	0.93	0.91
firmness	μ_a540-880	7	0.71	14.9	0.65	16.5
	μ'_s540-880	2	0.41	21.1	0.39	21.6
	μ_a540-880+ μ'_s540-880	3	0.73	14.2	0.70	15.3
	μ_a540-880+MIE *A B*	4	0.76	13.6	0.73	14.5

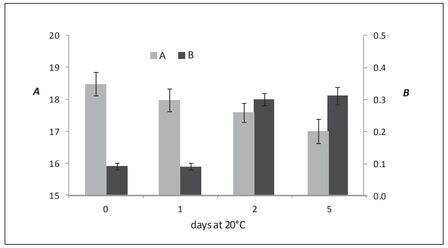

Figure 3. Density (A) and size (B) of the scatterers of ‘Tommy Atkins’ mangoes during shelf-life at 20°C (bars refer to standard error)

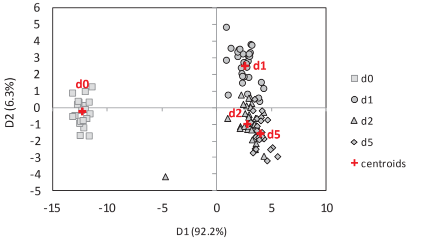

Figure 4. Classification of ‘Tommy Atkins’ mangoes as a function of day of shelf-life using discriminant analysis (see Table 3)

TRS absorption and scattering coefficients and A and B parameters of the scatterers were also used as explanatory variables in the discriminant analysis in order to classify mangoes according to the day of shelf-life. Better classification performance was obtained by including both absorption and scattering spectra (Table 4). In Fig. 4 the values of the two discriminant functions (Table 4) for every fruit and the group (day of shelf-life) centroids are plotted. Function D1 discriminated mangoes at d0 from the others while function D2 discriminated d1 fruits from the others (Fig. 4). Both functions allowed segregation of immature from mature fruit as most of the mangoes at d0 and at d1 were not ready for consumption. Mangoes at d0 shelf-life were all correctly classified while fruit belonging to d1, d2 and d5 were correctly classified in about 90% of the cases (Table 4).

Conclusion

The results of this research show that the TRS technique in the range of 540-900 nm can be used to non-destructively estimate the quality of mango fruit imported from Brazil. The absorption coefficient measured in the visible range is sensitive to carotenoid and chlorophyll contents of the pulp, pigments which have been shown to be essential in determining the maturity of mango fruit. TRS is also able to assess the properties related to scattering, allowing evaluation of this type of variability and hence the generation of more robust calibrations. By using both absorption and scattering optical properties, TRS is able to predict with good accuracy the pulp colour of mangoes and to sort fruit with different maturity degrees.

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