

# Quality and nutraceutical properties of mango fruit: influence of cultivar and biological age assessed by Time-resolved Reflectance Spectroscopy

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

**Key words:** absorption coefficient, ascorbic acid, carotenoid composition, *Mangifera indica* L., total antioxidant capacity, total phenols.

**Abstract:** The content and composition of the main antioxidants in the pulp of mangoes depend also on cultivar and maturity degree, the latter being non-destructively evaluated by the absorption coefficient measured by Time-resolved Reflectance Spectroscopy (TRS) at 540 nm ( $\mu_a540$ ). Aiming at evaluating the levels of antioxidants [carotenoids (CAR), phenols (TPC), ascorbic acid (AA)] and antioxidant capacity (TAC) in relation to  $\mu_a540$  maturity class, selected 'Haden' and 'Palmer' mangoes were measured for  $\mu_a540$  by TRS, classified based on  $\mu_a540$  value as less (LeM), medium (MeM) and more (MoM) mature and analyzed for pulp firmness, pulp color ( $a^*$ ,  $h^\circ$ , Yellowness Index), CAR (total and composition by HPLC-DAD), TPC, AA and TAC. 'Palmer' fruit had higher TPC, AA and TAC than 'Haden' mangoes. On average MoM fruit showed higher TPC, total CAR, total *all-trans*-violaxanthin esters and *all-trans*- $\beta$ -carotene than MeM and LeM fruit. LeM fruit did not have compounds belonging to the 9-*cis*-violaxanthin group, while *cis*- $\beta$ -cryptoxanthin was approx. 19% of total carotenoids. In MoM mangoes the main carotenoid was *all-trans*- $\beta$ -carotene (53%), followed by total *all-trans*-violaxanthin esters (30%), 9-*cis*-violaxanthin group (8%) and *cis*- $\beta$ -cryptoxanthin (6%). The  $\mu_a540$  significantly correlated ( $r=0.78-0.94$ ) with total CAR, *all-trans*- $\beta$ -carotene, *all-trans*-violaxanthin no.3 (both cultivars), TPC, *all-trans*-violaxanthin no.1, no.2, no.6 ('Haden'), and 9-*cis*-violaxanthin no.2, no.3 ('Palmer'). Our results indicate that TRS is suitable to non-destructively measure the pulp color of mangoes and to sort fruit with different ripening degree and nutraceutical properties.

## 1. Introduction

Mango (*Mangifera indica* L.) is a climacteric fruit belonging to the family of *Anacardiaceae* grown particularly in tropical and subtropical coun-

tries, with an estimated world production of 42 million tons per year (FAO, 2015). Brazil is one of the first ten largest mango producers, and more than 25% of its production is exported to Europe (Mitra, 2016). Appreciated for its excellent eating quality due to attractive flesh color, juicy texture and sweet flavor, mango provides high contents of bioactive compounds including carotenoids, phenolic compounds, ascorbic acid and reducing sugars (Rocha Ribeiro *et al.*, 2007; Manthey and Perkins-Veazie, 2009).

Carotenoids are responsible for the yellow-orange color of mango pulp and their content and composition depend mainly on cultivar and maturity degree, along with edaphic and climatic factors and postharvest handling, processing and storage conditions (Ornelas-Paz *et al.*, 2007, 2008; Manthey and Perkins-Veazie, 2009; Hewavitharana *et al.*, 2013; Liu *et al.*, 2013; Vásquez-Caicedo *et al.*, 2006; Vanoli *et al.*, 2016). During mango fruit ripening, biosynthesis of carotenoids occurs, due to chloroplasts differentiation into chromoplasts by disintegration of the thylacoid membranes and by the development of new pigment-bearing structures (Vásquez-Caicedo *et al.*, 2006). This process leads to carotenoids accumulation, which is usually accompanied by color changes of the pulp turning from white to yellow-orange (Ornelas-Paz *et al.*, 2008; Vásquez-Caicedo *et al.*, 2006). The accumulation of carotenoids in the mesocarp shows an exponential behavior during fruit ripening and cultivar-specific relationships between total or individual carotenoid (*all-trans*- $\beta$ -carotene, *all-trans*-violaxanthin and 9-*cis*-violaxanthin) contents and mesocarp color ( $a^*$ ,  $h^\circ$ ) were established in different mango cultivars (Vásquez-Caicedo *et al.*, 2006; Ornelas-Paz *et al.*, 2008). Similarly to carotenes, ascorbic acid content and total phenolic content vary according to the cultivars, maturity stage and cultural practices (Rocha Ribeiro *et al.*, 2007; Valente *et al.*, 2011; Liu *et al.*, 2013; Oliveira *et al.*, 2016; Septembre-Malaterre *et al.*, 2016).

In order to withstand shipping to distant markets and at the same time to have the optimum eating quality when ripe, mango fruit are harvested at the hard green stage, after having reached the physiological maturity, but before the onset of the climacteric rise. If mango fruit are immature at harvest, they do not reach an eating quality when ripe and, hence, the discrimination between mature and immature fruit at harvest is very important from the marketing point of view in order to minimize qualitative losses during the supply chain. Fruit shape ("shoulders" should be full), pulp color and firmness are the most used

maturity indices for mangoes, but differences among cultivars and growing conditions have precluded universal maturity indices. On the other hand, the current industry measurements of firmness and pulp color have the disadvantage of being destructive of fruit; hence, the development of a non-destructive technique could help the growers to pick fruit at the proper maturity degree for the different market destinations.

Among the non-destructive techniques able to assess the maturity degree of fruit, Time-resolved Reflectance Spectroscopy (TRS) is gaining increasing interest (Nicolai *et al.*, 2014). TRS is a non-destructive optical technique which can separate the effect of light absorption, due to chemical compounds such as pigments and water, and light scattering, due to microscopic changes in refractive index caused by membranes, vacuoles, starch granules, organelles, and air. By measuring photon time-of-flight distribution with picoseconds temporal resolution, the absorption ( $\mu_a$ ) and reduced scattering ( $\mu_s$ ) coefficients in the VIS-NIR wavelength range are quantified, 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). TRS has been used to study the internal fruit attributes related to maturity in apples, peaches, nectarines and pears (Rizzolo and Vanoli, 2016). In nectarines, the  $\mu_a$  measured at harvest at 670 nm ( $\mu_{a670}$ ), near the chlorophyll-*a* peak, can be considered an effective maturity index as it is linked to the biological age of the fruit (Tijskens *et al.*, 2007) and has been successfully used to predict the softening rate during shelf life in nectarines and to select fruit for different market destinations (Eccher Zerbini *et al.*, 2009).

Previous studies carried out on mango fruit have demonstrated the potential of TRS for the non-destructive determination of pulp color (Vanoli *et al.*, 2013; Rizzolo *et al.*, 2016; Vanoli *et al.*, 2016), as well as the possibility of using TRS absorption in both the carotenoids (540 nm) and chlorophyll-*a* (670 nm) spectral regions to classify mango fruit according to maturity and to predict the ripening of individual fruit (Eccher Zerbini *et al.*, 2015).

The present work aimed at evaluating the levels of antioxidants and antioxidant capacity in the pulp of two cultivars of Brazilian mangoes in relation to fruit maturity class assigned according to the  $\mu_{a540}$  value, along with selected quality parameters. The relationships between  $\mu_{a540}$  maturity index and total carotenoid content, total phenolic compounds, ascorbic acid and the fourteen carotenoid com-

pounds identified were also studied.

## 2. Materials and Methods

### Mango fruit

On November 2011, 'Haden' and 'Palmer' mangoes were picked in experimental orchards of EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais) in Minas Gerais state (Brazil) and transported to Milan (Italy) by plane soon after harvest. On arrival at CREA-IT lab (about 5-7 days from harvest), 60 fruits of 'Haden' and 90 fruits of 'Palmer' without defects were selected and measured by TRS at 650 nm ('Haden') or at 690 nm ('Palmer') on two opposite sides in the fruit equatorial region and ranked by decreasing  $\mu_a$  averaged over the two sides, that is from less (high  $\mu_a$ ) to more mature fruit (low  $\mu_a$ ). 'Haden' fruit were put at 20°C for 2 days, while 'Palmer' mangoes were randomized into 3 batches of 30 fruits, corresponding to 0, 4 and 11 days of shelf life at 20°C, in order to have the whole range of  $\mu_a$  690 in each batch. Sub-samples of 10 fruits, covering the whole range of  $\mu_a$ , were selected for 'Haden' and for 'Palmer' batch held for 4 days at 20°C. Each selected fruit was measured by TRS at 540 nm on two opposite sides in the equatorial region; in the same positions of TRS measurements, skin was removed by a slicer, and, after measuring pulp color and firmness, the whole fruit were immediately deep frozen and kept at -30°C until carotenoid, ascorbic acid and total phenolic extractions.

### Time-resolved Reflectance Spectroscopy (TRS)

A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Spinelli *et al.*, 2012) was used. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light picoseconds pulses, with duration of few tens of picoseconds. A custom-made filter wheel loaded with 14 band-pass interference filters (NT-65 series; Edmund Optics) 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 and diffuse remitted light is collected by 1 mm fiber placed at 1.5 cm distance from the illumination point. A second filter wheel identical to the first one is used for cutting off the fluorescence signal originated when illuminating the fruit in the visible spectral region. The light then is detected with a photomultiplier (HPM-100-50, Becker & Hickl, Germany) and the photon time-of-flight distribution was measured by a time-correlated single-photon counting

board (SPC-130, Becker & Hickl, Germany). The instrumental response function has a full width at half maximum of about 260 ps and the typical acquisition time is 1 s per wavelength. A model for photon diffusion in turbid media was used to analyze TRS data to assess the bulk optical properties of the samples (Martelli *et al.*, 2009) to obtain the estimates of  $\mu_a$  and  $\mu_s$  at each wavelength.

### Firmness and pulp color

Flesh firmness was measured using a Instron UTM model 4301 penetrometer (crosshead speed 200 mm min<sup>-1</sup>, 8 mm diameter plunger). Data were averaged per fruit.

Pulp color was measured with a spectrophotometer (CM-2600d, Minolta Co., Japan), using the primary illuminant D65 and 2° observer in the  $L^*$ ,  $a^*$ ,  $b^*$  color space. Hue ( $h^\circ$ ) was computed from  $a^*$  and  $b^*$  values according to:

$$h^\circ = \arctangent(b^*/a^*) \times 360 / (23 \times 14)$$

Yellowness index ( $I_Y$ ) was computed as:

$$I_Y = [81.2746X - 1.0574Z] / Y \times 100$$

after converting  $L^*a^*b^*$  parameters into the XYZ color space (Jha *et al.*, 2006). Data were averaged per fruit.

### Carotenoids, ascorbic acid, total phenols and total antioxidant capacity analysis

Carotenoids, ascorbic acid, total phenols and total antioxidant capacity analysis were carried out on frozen fruit after 30 min thawing at ambient temperature, by slicing pulp portions without peel near the positions of TRS measurements and pooling the slices coming from the two fruit sides.

Carotenoids were extracted following the procedure described by Picchi *et al.* (2012) with slight modifications. Briefly, on individual fruit, 2 g of pulp (two replicates) was extracted with 10 mL of a solution of hexane:acetone:ethyl acetate (2:1:1 v/v/v) containing 100  $\mu$ L of 1% butylhydroxytoluol (BHT) in methanol, to prevent carotenoid oxidative degradation, and centrifuged at 4°C at 15,000 rpm for 20 min. The extracts were stored at -80°C until spectrophotometric and high-performance liquid chromatographic (HPLC) analyses.

Total carotenoid content (CAR) was determined measuring absorbance at 450 nm using a spectrophotometer (UV-UVIDEC 320, Jasco, Japan). The hexane:acetone:ethyl acetate solution was used as the blank. Total carotenoid content was estimated from a standard curve of *all-trans*- $\beta$ -carotene and data were expressed as milligrams of  $\beta$ -carotene

equivalent ( $\beta$ -car) per kilogram of fresh weight (mg  $\beta$ -car kg FW<sup>-1</sup>).

Carotenoid composition was determined on extracts according to Azevedo-Meleiro and Rodriguez-Amaya (2004) with some modifications. A Jasco (Tokio, Japan) HPLC system consisting of a PU-1580 liquid chromatographic pump coupled to LG 1580-04 quaternary gradient unit, a model AS 2055-plus autosampler and an MD 2010-plus multi-wavelength detector was used. Separations were performed on an Inertsil ODS-3 column (4.6 mm i.d.  $\times$  250 mm length, particle diameter 5  $\mu$ m, GL Science) at the temperature of 40°C which was maintained using a Jasco Co-1560 Intelligent Column thermostat. The sample injection volume was 80  $\mu$ L. The column was eluted with 20% methanol and 80% of a gradient mixture of acetonitrile (A) and ethyl acetate (B) at the flow rate of 0.6 mL min<sup>-1</sup>, with 10% B at 0-25 min, 10-20% B at 25-35 min, 20-50% B at 35-40 min, 50% B at 40-45 min, 50-10% B at 45-50 min. Spectra of all peaks were recorded in the 200-600 nm wavelength range, and peak areas were monitored at 450 nm. Carotenoids (Table 1) were identified by comparing their retention times and spectral characteristics with those of standards (*all-trans*- $\beta$ -carotene and violaxanthin, obtained by pansy petals) and with those reported in the literature (Ornelas-Paz *et al.*, 2007, 2008), considering the three maximum absorbance wavelengths ( $\lambda_{\max}$ ) and the spectral fine structure (% III/II), which is the percentage of the peak height of the longest wavelength absorption band ( $\lambda_{\max}$  III) to that of the middle absorption band ( $\lambda_{\max}$  II), taking

the maximum of the valley between peak II and peak III as the baseline (Sajilata *et al.*, 2008). Carotenoids were quantified referring to the total carotenoid content estimated spectrophotometrically on the same extract in conjunction with the chromatogram percent composition and data were expressed as milligrams of  $\beta$ -carotene equivalent ( $\beta$ -car) per kilogram of fresh weight (mg  $\beta$ -car kg FW<sup>-1</sup>). All the measurements were carried out in triplicate. The vitamin A value, expressed as retinol equivalent (RE) was estimated from *all-trans*- $\beta$ -carotene and *cis*- $\beta$ -cryptoxanthin amounts using as conversion figures 6  $\mu$ g for Car and 12  $\mu$ g for Crypt (Capra, 2006).

Ascorbic acid was extracted following the procedure described by Robles-Sánchez *et al.* (2009 a) with slight modifications. Briefly, on individual fruit, 2 g of pulp (two replicates) was homogenized with 10 mL of 6% (w/v) aqueous solution of metaphosphoric acid (MPA), vortexed for 30 s, and centrifuged at 4°C at 15,000 rpm for 20 min and the extracts were kept at -20°C till HPLC analysis. Ascorbic acid was determined on just thawed extracts according to the conditions reported by Rizzolo *et al.* (2002), using a Jasco (Tokio, Japan) HPLC system consisting of a PU-980 liquid chromatographic pump, a model AS 1055-10 autosampler and an UV-Vis 15770 detector set at 254 nm, coupled to an Inertsil ODS-3 column (4.6 mm i.d.  $\times$  250 mm length, particle diameter 5  $\mu$ m, GL Science) at the temperature of 30°C, which was eluted with 0.02 M orthophosphoric acid at a flow rate of 0.7 mL min<sup>-1</sup>. Ascorbic acid was estimated from a standard curve of L-ascorbic acid in 6% MPA and data were

Table 1 - Retention time (R<sub>t</sub>, min), spectra characteristics [ $\lambda_{\max}$  (nm) in the mobile phase, obtained by DAD, spectral fine structure (% III/II)] and name abbreviation of tentatively identified compounds according to Ornelas-Paz *et al.* (2007, 2008)

Peak no.	R <sub>t</sub>	$\lambda_{\max}$ I	$\lambda_{\max}$ II	$\lambda_{\max}$ III	% III/II	Tentative identification	Abbreviation
1	5.24-5.89	419	439	471	82	unknown	UNK
2	5.92-5.97	415	439	471	82	<i>all-trans</i> -violaxanthin various esters	Viol no. 1
3	6.03-6.09	415	439	471	100	<i>all-trans</i> -violaxanthin various esters	Viol no. 2
4	6.11-6.19	415	443	471	90	<i>cis</i> - $\beta$ -cryptoxanthin	Crypt
5	6.23-7.29	415	439	471	93	<i>all-trans</i> -violaxanthin various esters	Viol no. 3
6	7.40-7.83	411	435	463	75	9- <i>cis</i> -violaxanthin	9-viol no. 1
7	8.03-8.77	415	435	467	83	9- <i>cis</i> -violaxanthin	9-viol no. 2
8	9.40-10.31	415	439	467	84	<i>all-trans</i> -violaxanthin various esters	Viol no. 4
9	10.32-11.15	411	435	463	80	9- <i>cis</i> -violaxanthin	9-viol no. 3
10	33.48-36.19	419	439	471	n.c.	<i>all-trans</i> -violaxanthin various esters	Viol no. 5
11	37.97-40.61		451	479	23	<i>all-trans</i> - $\beta$ -carotene	Car
12	40.90-43.60	419	439	471	100	<i>all-trans</i> -violaxanthin various esters	Viol no. 6
13	43.71-43.99	415	435	467	100	9- <i>cis</i> -violaxanthin	9-viol no. 4
14	44.00-44.60	415	439	467	100	<i>all-trans</i> -violaxanthin various esters	Viol no. 7

expressed as milligram per kilogram of fresh weight (mg kg FW<sup>-1</sup>). All the measurements were carried out in triplicate.

Total phenol content (TPC) and total antioxidant capacity (TAC) were determined on the same extract (two replicates/fruit) obtained by homogenizing 2 g of pulp with 10 mL of acidic ethanol (ethanol:0.04 M HCl, 1:1 v/v), vortexed for 30 s and centrifuged at 4°C at 15,000 rpm for 20 min. Extracts were kept at -20°C till total phenol content and antioxidant capacity determinations. TPC was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999) based on the reduction of a phosphowolframate phosphomolibdate complex by phenolics to blue reaction products, and measuring absorbance at 730 nm using a spectrophotometer (UV-UVIDEC 320, Jasco, Japan). The TPC was estimated from a standard curve of gallic acid and data were expressed as milligrams of gallic acid equivalents (GAE) per kilogram of fresh weight (mg GAE kg FW<sup>-1</sup>). All the measurements were performed in triplicate. TAC was evaluated using the free radical 1,1-dyphenyl-2-picrylhydrazil (DPPH•) according to Brand-Williams *et al.* (1995) with modifications. Fifty microlitres of sample extract or Trolox standard solution (0.02-0.8 mM) were added to 2 mL of ethanol and 550 µL of DPPH• solution (0.05 mM in ethanol) and, during 5 min of incubation, the absorbance at 517 nm was measured with a Jasco 7800 UV/VIS spectrophotometer (Jasco Europe S.r.l., Cremella, LC, Italy). The DPPH scavenging capacity of the samples was calculated using a standard curve of Trolox, and expressed as micromoles of Trolox equivalents (TE) per kilogram of fresh weight (µmol TE kg FW<sup>-1</sup>). All the measurements were performed in triplicate.

#### Statistical analysis

The Statgraphics v. 5.2 (Manugistic Inc., Rockville, MD, USA) software package was used. Data were submitted to multifactor analysis of variance (ANOVA) considering cultivar, TRS maturation class and their interaction as source of variation. In addition, one-way ANOVA was used to study the main factors (cultivar, TRS maturity class), and the TRS maturity class within each cultivar. Percentage data of carotenoids were analyzed after arcsine transformation. Means were compared by 95 percent Bonferroni's test. Relationships between  $\mu_a540$  and pulp color parameters and between  $\mu_a540$ ,  $a^*$ ,  $h^\circ$ ,  $l_y$  and ascorbic acid, TPC, TAC and carotenoids were studied using regression analysis. For each parameter, the model with the higher performance was considered.

### 3. Results

#### TRS optical properties

In 'Palmer' fruit,  $\mu_a690$  at harvest ranged from 0.074 cm<sup>-1</sup> for the less mature fruit to 0.021 cm<sup>-1</sup> for the more mature ones and decreased to 0.061 cm<sup>-1</sup> and 0.019 cm<sup>-1</sup>, respectively, after 4 days of shelf life at 20°C; concomitantly, after shelf life,  $\mu_a540$  ranged from 0.117 cm<sup>-1</sup> for the least mature fruit to about 0.33 cm<sup>-1</sup> for the most mature ones. In 'Haden' mangoes,  $\mu_a650$  ranged at harvest from 0.231 cm<sup>-1</sup> to 0.030 cm<sup>-1</sup>, with the majority of the fruit in the 0.030-0.065 cm<sup>-1</sup> range; after 2 days of shelf life at 20°C,  $\mu_a650$  decreased only in less mature fruit, whereas in all the other mango fruit it increased to values ranging from 0.036 cm<sup>-1</sup> to 0.053 cm<sup>-1</sup>, while the  $\mu_a540$  values after shelf life ranged from 0.157 cm<sup>-1</sup> for the least mature fruit to 0.835 cm<sup>-1</sup> for the most mature ones.

The  $\mu_a540$  maturity index, related to carotenoids content, was then used to classify the selected fruit within each cultivar in three TRS maturity classes: less mature (LeM) with low  $\mu_a540$ , more mature (MoM) with high  $\mu_a540$  and medium mature (MeM) with intermediate values of  $\mu_a540$ . Cultivar and TRS maturity class influenced the value of  $\mu_a540$  maturity index (Table 2); on average  $\mu_a540$  was higher in cv. Haden ('Haden': 0.400±0.025 cm<sup>-1</sup>; 'Palmer': 0.248±0.025 cm<sup>-1</sup>) and in the MoM class in both cultivars, with MoM 'Haden' fruit being characterized by the highest  $\mu_a540$  value.

#### Quality parameters

TRS maturity class and cultivar greatly affected  $a^*$  and  $h^\circ$  pulp color parameters and had only a slight influence on firmness, probably due to the high standard error values, whereas  $l_y$  depended only on maturity class (Table 2). On average, 'Palmer' fruit had lower firmness and  $a^*$  value, and higher  $h^\circ$  than 'Haden' fruit. In 'Palmer' mangoes firmness did not vary with maturity class, while in 'Haden' firmness showed the highest values in LeM fruit and the lowest in MeM and in MoM ones. MoM 'Palmer' fruit had higher  $a^*$  and  $l_y$  and lower  $h^\circ$  than LeM and MeM maturity classes, whereas LeM 'Haden' fruit had lower  $l_y$  than MoM fruit, and  $a^*$  increased and  $h^\circ$  decreased from LeM to MeM and MoM maturity classes.

#### Ascorbic acid, total phenolic content and total antioxidant capacity

AA content and TAC were significantly influenced only by cultivar, while TPC depended by both cultivar

and maturity class (Table 3). On average, ‘Palmer’ mangoes had higher AA, TPC and TAC than ‘Haden’ fruit, and LeM fruit had lower TPC than MoM mangoes (Fig. 1).

The average AA values were approx. 190 mg kg FW<sup>-1</sup> for ‘Haden’ and 390 mg kg FW<sup>-1</sup> for ‘Palmer’ and AA content did not change with maturity class in ‘Palmer’ mangoes while in ‘Haden’ showed the highest values in MeM fruit (Fig. 1). TPC was higher in ‘Palmer’ than in ‘Haden’ cv., being on average approx. 316 and 264 mg kg FW<sup>-1</sup> in ‘Palmer’ and

cultivars showed 9 common carotenoids (Fig. 2) out of 14 peaks tentatively identified by comparing spectral characteristics with those previously reported using a similar mobile phase (Table 1). The carotenoid pattern includes seven *all-trans*-violaxanthin (Viol) and four 9-*cis*-violaxanthin (9-Viol) containing compounds, *cis*- $\beta$ -cryptoxanthin and *all-trans*- $\beta$ -carotene.

The most abundant carotenoid in both cultivars was *all-trans*- $\beta$ -carotene (Tables 4 and 5), representing 49-56% of the total carotenoid content, followed

Table 2 - Absorption coefficient at 540 nm ( $\mu_a540$ , cm<sup>-1</sup>), flesh firmness (N) and pulp colour parameters ( $a^*$ ; hue,  $h^\circ$ ; yellowness index,  $I_Y$ ) of ‘Palmer’ and ‘Haden’ mangoes of less mature (LeM), medium mature (MeM) and more mature (MoM) TRS maturity classes and results of multifactor ANOVA (F-ratio value and P-value)

Cultivar	Maturity class	$\mu_a540$	Firmness	$a^*$	$h^\circ$	$I_Y$
Palmer	LeM	0.196±0.027 b	8.33 ±0.55 a	0.86±0.79 b	89.13±0.75 a	130.7±1.7 b
	MeM	0.241±0.006 ab	7.70±0.45 a	3.64±1.03 b	86.85±0.89 a	153.3±1.6 ab
	MoM	0.310±0.017 a	6.12±0.63 a	9.84±1.75 a	81.72±1.30 b	165.7±9.5 a
Haden	LeM	0.191±0.011 b	37.29±9.89 a	2.98±1.62 c	87.18±1.55 a	130.0±6.0 b
	MeM	0.336±0.033 b	10.47±3.62 a	10.60±1.44 b	80.32±1.15 b	159.7±6.6 a
	MoM	0.677±0.158 a	5.85±0.65 b	20.46±1.12 a	72.37±0.84 c	191.2±6.1 a
ANOVA						
A: cultivar		18.88 ***	4.81*	28.85 ***	32.20 ***	3.94 NS
B: maturity class		23.52 ***	4.85 *	36.79 ***	32.25 ***	27.18 ***
A × B		9.21 **	3.98 *	3.94 *	4.16 *	2.01 NS

Mean±SE. Within each cultivar, means followed by different letters are statistically different (Bonferroni's test, P≤0.05). P-value of F-ratio: NS=not significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

Table 3 - Multifactor analysis of variance (F-ratio and P-value) for ascorbic acid (AA), total carotenoids (CAR), total phenolic content (TPC) and total antioxidant capacity (TAC)

Factors	AA	CAR	TPC	TAC
<i>main factors</i>				
Cultivar (A)	38.79 ***	0.48 NS	5.12 *	32.75 ***
Maturity class (B)	0.75 NS	7.28 **	4.20 *	1.85 NS
<i>interaction</i>				
A × B	0.68 NS	0.98 NS	0.81 NS	0.53 NS

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001; NS=not significant.

‘Haden’ fruit, respectively, as well as it was higher in MoM fruit from both cultivars. ‘Palmer’ fruit were characterized by higher TAC showing on average 2.59 times greater than that from ‘Haden’. TAC had significant positive correlations with TPC ( $r=0.66$ ,  $p=0.002$ ) and AA ( $r=0.89$ ,  $p<0.0001$ ).

### Carotenoids

Total carotenoids (CAR) depended only by maturity class (Table 3), with LeM fruit having less CAR than MoM ones (Fig. 1). The chromatographic carotenoid patterns of LeM and MoM maturity classes in both

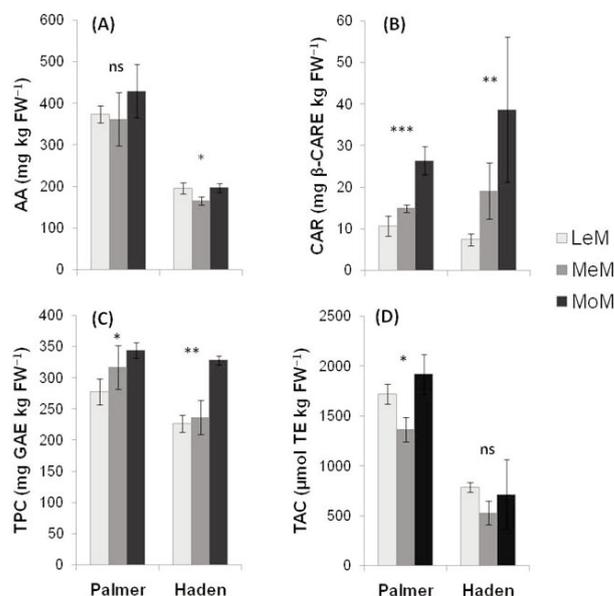


Fig. 1 - (A) Ascorbic acid (AA), (B) total carotenoids (CAR), total phenolic content (TPC) and total antioxidant capacity (TAC) in ‘Palmer’ and ‘Haden’ mangoes in function of  $\mu_a540$  maturity class (LeM, less mature; MeM, medium mature; MoM, more mature). Bars refer to SE. Within each cultivar ANOVA results are indicated as follows: \*, \*\*, \*\*\*: significant at P≤0.05, 0.01, 0.001, respectively; ns, not significant.

by *cis*- $\beta$ -cryptoxanthin (6-18%) and Viol no.3 (11-16%). The content of *all-trans*-violaxanthins was higher than that of 9-*cis*-violaxanthins in both cultivars.

The content of Viol no.3, no.4 and no.6, 9-Viol no.4 and *all-trans*- $\beta$ -carotene, as well as the sums of *all-trans*-violaxanthins ( $\Sigma$ Viol) and of 9-*cis*-violaxanthins ( $\Sigma$ 9-Viol) depended only on maturity

class, that of 9-Viol no.1 only on cultivar, whereas those of 9-Viol no.2 and no.3 on both cultivar and maturity class (Table 4). In fact 'Haden' mangoes had higher amounts of 9-Viol no.3 (Table 4) and had lower proportion of 9-Viol no.4 than 'Palmer' fruit (Table 5). Viol no. 1 and 9-Viol no.1 were present only in 'Haden' and 9-Viol no. 2 only in 'Palmer' fruit.

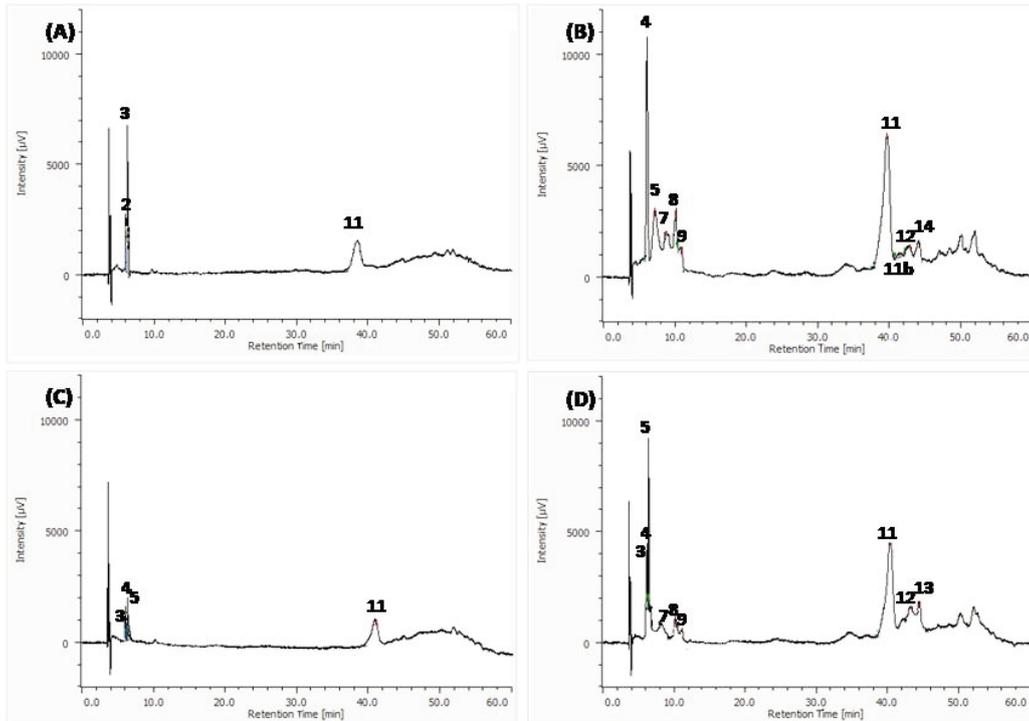


Fig. 2 - Typical chromatographic patterns at 450 nm of carotenoid extracts of (A) LeM 'Palmer', (B) MoM 'Palmer', (C) LeM 'Haden' and (D) MoM 'Haden' mangoes. For peak assignment see Table 1.

Table 4 - Carotenoid compounds of 'Palmer' and 'Haden' mangoes (mg  $\beta$ -CARE kg FW<sup>-1</sup>) and vitamin A value (RE 100 g FW<sup>-1</sup>) influence of cultivar and of TRS maturity class and results of multifactor ANOVA (F-ratio value and P-value). For identification data of each carotenoid see Table 1

	Cultivar		Maturity class			ANOVA		
	Palmer	Haden	Less mature	Medium mature	More mature	Cultivar (A)	Maturity class (B)	A × B
Viol no. 1	0±0 a	0.49±0.49 a	0 ±0 a	0±0 a	0.99±0.99 a	3.25 (*)	2.89 (*)	2.89 (*)
Viol no. 2	1.14±0.46 a	0.87±0.68 a	0.68 ±0.40 a	0.49±0.38 a	2.15±1.33 a	0.08 NS	2.11 NS	2.48 NS
Crypt	2.58±0.55 a	1.82±0.38 a	1.66 ±0.37 a	2.69±0.47 a	2.18 ±0.97 a	1.53 NS	0.89 NS	0.39 NS
Viol no. 3	2.43±0.31 a	2.49±0.55 a	1.53±0.28 b	2.82±0.67 ab	3.26±0.30 a	0.07 NS	3.08 (*)	2.37 NS
9-viol no. 1	0±0 b	0.81±0.42 a	0±0 a	0.67±0.42 a	0.68±0.68 a	4.91 *	1.36 NS	1.36 NS
9-viol no. 2	0.33±0.16 a	0±0 b	0±0 b	0.06±0.06 ab	0.52±0.26 a	10.91 **	6.23 *	6.23 *
Viol no. 4	0.84±0.16 a	0.91±0.40 a	0.27±0.13 b	0.88±0.40 ab	1.72±0.37 a	0.65 NS	4.90 *	0.89 NS
9-viol no. 3	0.09±0.06 b	0.35±0.15 a	0±0 b	0.21±0.11 ab	0.56±0.25 a	7.62 *	7.57 **	2.35 NS
Car	9.36±1.14 a	9.63±2.72 a	5.05±0.67 b	8.85±1.81 ab	16.65±3.47 a	0.81 NS	8.37 **	1.03 NS
Viol no. 6	0.64±0.14 a	0.56±0.23 a	0.08±0.08 b	0.74±0.16 ab	1.12±0.25 a	0.12 NS	10.19 **	1.66 NS
9-viol no. 4	0.55±0.19 a	0.22±0.15 a	0±0 b	0.55±0.21 a	0.67±0.28 a	1.58 NS	4.15 *	0.99 NS
Viol no. 7	0.15±0.10 a	0.19±0.10 a	0.07±0.07 a	0.17±0.11 a	0.30±0.18 a	0.19 NS	0.72 NS	0.97 NS
$\Sigma$ Viol	5.20±0.76 a	5.51±1.95 a	2.64±0.74 b	5.11±1.22 ab	9.54±2.82 a	0.88 NS	5.69 *	2.26 NS
$\Sigma$ 9-viol	0.98±0.35 a	1.38±0.67 a	0±0 b	1.49±0.48 ab	2.43±1.06 a	1.06 NS	4.39 **	0.32 NS
Vitamin A value	258.2±22.3 a	162.0±45.3 a	85.6±11.4 b	149.7±30.5 ab	279.3±57.2 a	0.40 NS	8.38 **	1.01 NS

Mean±SE; 0=not detected. Within cultivar and within TRS maturity class means followed by different letters are statistically different (Bonferroni's test, (\*) P<0.10; \* P<0.05; \*\*P<0.01; ns =not significant).  $\Sigma$ Viol= total all-trans-violaxanthin esters;  $\Sigma$ 9-Viol= total 9-cis-violaxanthin.

Table 5 - Carotenoid composition (percent to total carotenoids) of 'Palmer' and 'Haden' mangoes: influence of cultivar and of TRS maturity class and results of ANOVA (F-ratio value and P-value). For identification data of each carotenoid see Table 1

	Cultivar		Maturity class			ANOVA		
	Palmer	Haden	Less mature	Medium mature	More mature	Cultivar (A)	Maturity class (B)	A × B
Viol no. 1	0±0 a	0.87±0.87 a	0±0 a	0±0 a	0.36±0.36 a	3.25 (*)	2.89 (*)	2.89 (*)
Viol no. 2	6.74±2.84 a	3.13±1.49 a	2.51±0.64 a	1.04±0.43 a	2.89±0.58 a	0.21 NS	0.36 NS	1.65 NS
Crypt	14.87±3.01 a	15.40±2.77 a	18.18±0.53 a	15.31±0.07 a	5.88±1.04 a	0.01 NS	1.80 NS	1.40 NS
Viol no. 3	14.32±2.05 a	16.30±2.07 a	16.33±0.18 a	15.96±0.09 a	11.31±0.07 a	0.04 NS	1.51 NS	2.63 NS
9-viol no. 1	0±0 b	2.44±1.30 a	0±0 a	1.122±0.26 a	0.25±0.25 a	7.84 *	2.48 NS	2.48 NS
9-viol no. 2	1.36±0.60 a	0±0 b	0±0 b	0.06±0.06 ab	1.08±0.20 a	14.55 **	5.95 *	5.95 *
Viol no. 4	4.23±0.58 a	3.17±0.95 a	1.27±0.17 a	3.07±0.12 a	5.51±0.01 a	0.54 NS	2.49 NS	0.40 NS
9-viol no. 3	0.33±0.22 b	1.24±0.46 a	0±0 b	0.46±0.11 ab	1.21±0.08 a	7.51 *	8.04 **	2.49 NS
Car	50.62±1.03 a	54.25±2.31 a	55.65±0.08 a	48.86±0.02 b	53.26±0.02 ab	3.22 (*)	3.28 (*)	3.38 (*)
Viol no. 6	3.20±0.71 a	1.98±0.68 a	0.07±0.07 b	3.27±0.10 a	3.57±0.005 a	1.66 NS	11.66 **	0.62 NS
9-viol no. 4	2.63±0.91 a	0.71±0.52 b	0±0 b	1.73±0.23 a	1.14±0.20 ab	4.52 *	6.20 **	2.26 NS
Viol no. 7	0.77±0.51 a	0.95±0.51 a	0.06±0.06 a	0.25±0.11 a	0.56±0.21 a	0.19 NS	0.46 NS	1.24 NS
∑Viol	29.25±3.30 a	26.41±3.04 a	25.030±0.42 a	28.13±0.09 a	28.76±0.07 a	0.22 NS	0.17 NS	4.27 *
∑9-viol	4.31±1.33 a	4.39±1.71 a	0±0 b	6.07±0.19 a	5.79±0.21 a	0.01 NS	13.50 **	0.00 NS

Mean±SE; 0=not detected. Within cultivar and within TRS maturity class means followed by different letters are statistically different (Bonferroni's test, (\*) P<0.10; \* P<0.05; \*\*P<0.01; ns =not significant). ∑Viol= total all-trans-violaxanthin esters; ∑9-Viol= total 9-cis-violaxanthin.

LeM mangoes had no 9-cis-violaxanthins and were characterized by lower contents of Viol no.3, Viol no.4, Viol no.6 and all-trans-β-carotene than MoM fruit, but higher proportion of all-trans-β-carotene than MeM ones (Tables 4 and 5). These carotenoids increased with advancing maturity degree, showing the highest contents in MoM mangoes. On average all-trans-β-carotene corresponded to 53% of total carotenoids; the all-trans-β-carotene proportion was not significantly affected by cultivar, whereas on average was lower in MeM fruit than in LeM ones, while MoM mangoes were not statistically different from fruit of the other two maturity classes (Table 5).

The vitamin A value did not differ between cultivar, but significantly increased with maturity class from 86 of LeM fruit to 279 RE 100 g<sup>-1</sup> of MoM mangoes (Table 4).

**Regression analysis**

The results of regression analysis between μ<sub>a</sub>540 and pulp color parameters and between μ<sub>a</sub>540, a\*, h°, l<sub>v</sub> and ascorbic acid, TPC, TAC and carotenoids differed for the two cultivars and data are summarized in Tables 6 and 7, reporting the type of the model having the best performance.

The μ<sub>a</sub>540 was positively related to a\* and l<sub>v</sub> and

negatively to h° (Table 6) with r ranging from 0.83 to 0.87 for 'Palmer' fruit and approx. 0.98 for 'Haden' cultivar. In 'Palmer' mangoes (Table 7) μ<sub>a</sub>540, a\*, h° and l<sub>v</sub> were related to total carotenoids, Viol no.3 and no.4, 9-Viol no.2 and no.3, all-trans-β-carotene, ∑9-Viol and vitamin A value with lower r values (0.62-0.84) for μ<sub>a</sub>540 respect to those found for pulp color parameters (0.74-0.96). Only l<sub>v</sub> was related to Viol no.6, 9-Viol no.4 and ∑Viol with r≥0.7, and only a\* was related to TPC, but with r<0.6. In contrast, in 'Haden' fruit μ<sub>a</sub>540, a\*, h° and l<sub>v</sub> were related to total

Table 6 - Results of regression analysis between absorption coefficient at 540 nm (μ<sub>a</sub>540) and pulp color parameters

μ <sub>a</sub> 540	'Palmer'			'Haden'		
	r	P	MT	r	P	MT
a*	0.831	**	L	0.975	***	E
h°	0.826	**	RX	0.976	***	Sc
l <sub>v</sub>	0.872	**	Ln	0.977	***	DR

For each regression, the following data are given: r = correlation coefficient, P = significance of the model (\*\*\*, P<0.001; \*\*, P<0.01) and MT= model type (DR= doble reciprocal, E= exponential, L= linear, Ln= logarithmic-X, M= multiplicative, RX= reciprocal-X, Sc = S-curve).

carotenoids, Viol no.2, no.3, no.4 and no.6, 9-Viol no.1 and no.3, *all-trans*- $\beta$ -carotene,  $\Sigma$ Viol,  $\Sigma$ 9-Viol, vitamin A value and TPC, with higher *r* values (0.72-0.95) for  $\mu_a540$  than for pulp color parameters. In addition, only  $\mu_a540$  was related to 9-Viol no. 4.

No significant relationships were found between  $\mu_a540$ , *a*<sup>\*</sup>, *h*<sup>o</sup> and *l<sub>v</sub>* and ascorbic acid and TAC, whatever the cultivar, suggesting that  $\mu_a540$  was able to reveal the carotenoids content in the pulp, as this wavelength corresponds to the tail of carotenoid absorption.

independently from cultivar. Rizzolo *et al.* (2016) also showed that  $\mu_a540$  maturity index, related to carotenoids content, successfully classified ‘Tommy Atkins’ mangoes at harvest.

As for quality parameters, ‘Palmer’ mangoes had firmness values typical of fully ripe fruit (Beaulieu and Lea, 2003), independently from maturity class, whereas in ‘Haden’ fruit LeM class showed firmness values typical of firm-ripe stage and MeM and MoM classes values typical of ready-to-eat or ripe fruit (Eccher Zerbini *et al.*, 2015). Pulp color parameters

Table 7 - Results of regression analysis between absorption coefficient at 540 nm ( $\mu_a540$ ), pulp color parameters and total carotenoid (CAR), total phenolic compounds (TPC), carotenoid compounds (for identification data see Table 1) and vitamin A value

	$\mu_a540$			<i>a</i> <sup>*</sup>			<i>h</i> <sup>o</sup>			<i>l<sub>v</sub></i>		
	<i>r</i>	P	MT	<i>r</i>	P	MT	<i>r</i>	P	MT	<i>r</i>	P	MT
<b>‘Palmer’</b>												
CAR	0.78	*	L	0.909	***	L	0.902	***	RX	0.897	***	L
Viol no.3	0.814	**	L	0.789	*	L	0.783	*	RX	0.735	*	L
9-Viol no.2	0.839	**	L	0.959	***	L	0.958	***	RX	0.823	**	L
Viol no.4	0.625	(*)	L	0.908	***	L	-0.912	***	L	0.784	*	L
9-Viol no.3	0.765	*	L	0.871	**	L	0.871	**	RX	0.778	*	L
Car	0.793	**	L	0.913	***	Sy	-0.909	***	Sy	0.888	**	Sy
Viol no.6	–			–			–			-0.745	*	RX
9-Viol no.4	–			–			–			-0.847	**	RX
$\Sigma$ Viol	0.576	(*)	Log	–			–			0.693	*	Log
$\Sigma$ 9-Viol	0.812	**	L	0.873	**	L	0.86	**	RX	0.954	***	L
Vitamin A value	0.792	**	L	0.915	***	Sy	-0.912	***	Sy	0.887	**	L
TPC	–			0.575	(*)	Sy	–			–		
<b>‘Haden’</b>												
CAR	0.912	***	L	0.855	**	E	0.854	**	Sc	0.876	***	E
Viol no.2	0.853	**	L	0.618	(*)	L	0.634	*	RX	0.601	(*)	L
Viol no.3	-0.824	**	Sc	0.723	*	E	-0.742	*	E	-0.805	**	Sc
9-Viol no.1	0.756	*	L	0.666	*	L	0.661	*	RX	0.718	*	L
Viol no.4	0.815	**	Sx	0.789	**	L	0.79	**	RX	0.823	**	L
9-Viol no.3	0.95	***	L	0.83	**	L	0.834	**	RX	0.848	**	L
Car	0.924	***	L	0.823	**	Sy	0.817	**	Sc	0.837	**	Sy
Viol no.6	0.944	***	L	0.862	**	L	0.862	**	RX	0.873	***	L
9-Viol no.4	0.700	*	L	–			–			–		
$\Sigma$ Viol	0.937	***	L	0.885	***	Sy	-0.886	***	E	0.905	***	Sy
$\Sigma$ 9-Viol	0.848	**	L	0.723	*	L	0.721	*	RX	0.756	*	L
Vitamin A value	0.923	***	L	0.823	**	Sy	0.817	**	Sc	0.838	**	Sy
TPC	0.867	**	L	0.758	*	L	0.761	**	RX	0.766	**	L

For each significant regression, the following data are given: *r*= correlation coefficient, P= significance of the model (\*\*\*, P< 0.001; \*\*, P<0.01; \*, P< 0.05; (\*), P<0.10) and MT= model type (DR= doble reciprocal, E= exponential, L= linear, Ln= logarithmic-X, Log= logistic, RX= reciprocal-X, Sc= S-curve, Sx= square-root-X, Sy= square-root-Y).

#### 4. Discussion and Conclusions

The absorption coefficient measured at 540 nm ( $\mu_a540$ ) showed different value ranges between ‘Haden’ and ‘Palmer’ mangoes and it was able to distinguish more mature fruit from less mature ones

differed among maturity classes, confirming previous results obtained for ‘Tommy Atkins’ cultivar. In fact Rizzolo *et al.* (2011) and Vanoli *et al.* (2011) found that LeM ‘Tommy Atkins’ mangoes were characterized by higher *h*<sup>o</sup> and lower *a*<sup>\*</sup> and *l<sub>v</sub>* than MoM fruit. Moreover, Vanoli *et al.* (2011) found that with fruit

ripening at 20°C  $h^\circ$  decreased and  $I_V$  increased, confirming that the trend of pulp color observed in our work with the TRS maturity classes was actually due to a different ripening degree.

The average AA values found for 'Palmer' and 'Haden' fruit are comparable with the data by Rocha-Ribeiro *et al.* (2007) for the same cultivar, and with AA content reported for other cultivars by Liu *et al.* (2013) and Elsheshetawy *et al.* (2016). However, within the same variety, AA content may vary according to climatic conditions, cultural practices, maturity stage and postharvest factors. For 'Keitt' cultivar Ibarra-Garza *et al.* (2015) found that AA content varied from about 1300 mg kg FW<sup>-1</sup> in fruit soon after harvest, to about 2500 mg kg FW<sup>-1</sup> till 8 days of ripening at room temperature, followed by a 54% decrease in fully-ripe fruit. Similarly, Robles-Sánchez *et al.* (2009 b) reported for 'Ataulfo' fruit stored for 15 days at 12°C a 50% decrease of AA content at the end of shelf-life.

The TPC contents found in this work are in agreement with Rocha-Ribeiro *et al.* (2007), even if these Authors reported lower AA contents than those found in our work for the same cultivars. No data on TPC content in mangoes in relation to TRS maturity classes, having same harvest time and same post-harvest management, are available in the literature. However, the TPC increasing trend from less to more mature fruit class found in this work is in agreement with the results for the final period of shelf life/storage reported by Ibarra-Garza *et al.* (2015) and Robles-Sánchez *et al.* (2009 b) when fruit are becoming softer and with a yellower pulp color. Ibarra-Garza *et al.* (2015) found higher TPC in 'Keitt' fruit at the beginning of a 10 d shelf life period at room temperature, with a sharp decrease from 2 to 4 days of shelf life, followed by a slight but significant TPC increase till the end of shelf life; a similar trend was also found by Robles-Sánchez *et al.* (2009 b) for whole and fresh-cut 'Ataulfo' mangoes stored at 5°C for 15 days.

In agreement with data obtained for TPC and AA, 'Palmer' fruit showed higher TAC than 'Haden' ones. The positive correlations of TAC with TPC and AA found in this work are in agreement with literature data. In fact Silva and Sirasa (2018) reported significant correlations between ascorbic acid and TPC and FRAP and DPPH scavenging activity measured for several fruit species, and Palafox-Carlos *et al.* (2012) between DPPH scavenging activity and TPC in 'Ataulfo' mangoes; on the other hand, Liu *et al.* (2013) and Ibarra-Garza *et al.* (2015) found in man-

goes correlation between phenolic concentration and antioxidant activity measured with other methods (FRAP, ORAC), but not between antioxidant activity and ascorbic acid content; in contrast Rocha-Ribeiro *et al.* (2007) reported that DPPH radical scavenging activity of mango extracts was strongly correlated with ascorbic acid content, but not with phenolic content. Liu *et al.* (2013) suggested that the difference in relationships between antioxidant activity and ascorbic acid and phenolic compound contents found among authors could be due to a masking effect of phenolics present in far higher concentration than ascorbic acid. Our results suggest that in this work the antioxidant activity can be attributed more to ascorbic acid than to total phenols.

No data on total carotenoids in relation to TRS maturity classes are available in the literature. Vanoli *et al.* (2016) for 'Palmer' and 'Haden' mangoes reported a wide fruit-to-fruit variability in CAR content for both cultivars and that CAR content had an increasing trend with  $\mu_a540$  value measured on fruit belonging to the same harvest date, i.e. that CAR content increases with advancing maturity degree. Similarly, an exponential increase in the carotenoid content with fruit ripening has been reported for 'Ataulfo' mangoes (Ornelas-Paz *et al.*, 2008) and nine Thai cultivars (Vásquez-Caicedo *et al.*, 2005). As for the chromatographic carotenoid patterns, the tentative identification of peaks was carried out by comparing spectral characteristics with those previously reported using a similar mobile phase. Seven peaks were tentatively identified as *all-trans*-violaxanthin (439 nm maximum absorption wavelength) and four as 9-*cis*-violaxanthin (435 nm maximum absorption wavelength) containing compounds. The spectral maximum for peak 4 was similar to that reported for *cis*- $\beta$ -cryptoxanthin. A standard mixture of *all-trans*- $\beta$ -carotene was used for the identification of peak 11; the retention time and the spectroscopic characteristics of reference material were identical to those observed for peak 11 in all the samples. In general, the spectral fine structures (% III/II values in Table 1) found in this work are in agreement with the values reported in the literature (Ornelas-Paz *et al.*, 2007, 2008). In both cultivars the proportions of *all-trans*- $\beta$ -carotene and *cis*- $\beta$ -cryptoxanthin to total carotenoid content, as well as the higher content of *all-trans*-violaxanthins than that of 9-*cis*-violaxanthins are very similar to findings reported in 'Ataulfo', 'Keitt', 'Tommy Atkins' and 'Kent' mangoes (Mercadante and Rodriguez-Amaya, 1998; Pott *et al.*, 2003; Ornelas-Paz *et al.*, 2008). The differences in

carotenoid composition among the maturity classes found in this research are consistent with literature data. Previous researches on carotenoid composition of various mango cultivars carried out by Godoy and Rodriguez-Amaya (1989), Mercadante and Rodriguez-Amaya (1998), Yahia *et al.* (2006), and Ornelas-Paz *et al.* (2007) have shown that generally the most important carotenoid in mango is *all-trans*- $\beta$ -carotene and its proportion to total carotenoids depends on cultivar and fruit maturity stage. Ibarra-Garza *et al.* (2015) reported for 'Keitt' fruit that *all-trans*- $\beta$ -carotene corresponded to 33% of total carotenoids in unripe fruit at harvest, ranged from 37 to 44% during the first 6 day-period of ripening at room temperature and reached 61% in fully ripe fruit. The contents of *all-trans*- $\beta$ -carotene in 'Haden' and 'Palmer' mangoes were similar to those reported by Rocha-Ribeiro *et al.* (2007) for the same cultivars and by Mercadante and Rodriguez-Amaya (1998) for 'Keitt' and 'Tommy Atkins' fruit, but lower than those found for 'Haden' and other cultivars by Ornelas-Paz *et al.* (2007). Also the amounts of *all-trans*- and 9-*cis*-violaxanthins in both cultivars were lower than those found by Ornelas-Paz *et al.* (2007) for 'Haden' and other cultivars, but similar to those reported by Low *et al.* (2015) for 'Kensington Pride' mangoes. The amounts of *cis*- $\beta$ -cryptoxanthin in both cultivars were far higher than the maximum of 0.1  $\mu\text{g g}^{-1}$  reported by Mercadante and Rodriguez-Amaya (1998) for 'Keitt' and 'Tommy Atkins' mangoes from mature-green to ripe stages, indicating that in the fruit of this experiment, not only  $\beta$ -carotene, but also *cis*- $\beta$ -cryptoxanthin was a contributor to the vitamin A value for these fruit.

The differences in the single carotenoid concentrations respect to literature data for the same cultivar could be due to the different maturity degree of mango fruit. In fact, referring to pulp color parameters, the fruit used in our experiment had pulp  $a^*$  values similar to those reported by Rocha-Ribeiro *et al.* (2007) for the same cultivars and in this case the carotenoid amounts were similar, whereas pulp  $h^\circ$  values for 'Haden' fruit were higher than those reported by Ornelas-Paz *et al.* (2007), indicating a less advanced ripening degree consistent with the lower carotenoid content of our results.

The results of regression analysis showed that  $\mu_a540$  was positively related to  $a^*$  and  $l_V$  and negatively to  $h^\circ$  pulp color parameters, confirming the results obtained by Spinelli *et al.* (2012, 2013) and

Vanoli *et al.* (2016) for the same cultivars and by Vanoli *et al.* (2013) for 'Tommy Atkins' mangoes. Color changes in the pulp of mango fruit are usually accompanied by carotenoid accumulation. In this work significant correlations between  $\mu_a540$ , pulp color parameters  $a^*$ ,  $h^\circ$ ,  $l_V$  and carotenoids were found for both cultivars. Vanoli *et al.* (2016) found an increasing trend of total carotenoids content with  $\mu_a540$  in 'Palmer' and 'Haden' mangoes; they also found high positive correlations between total carotenoids and  $a^*$  and  $l_V$  and a higher negative correlation with  $h^\circ$  following a logarithmic-law function with higher correlation in 'Palmer' than in 'Haden' cv., confirming the better relationships of pulp color in 'Palmer' than in 'Haden' fruits. High correlations between pulp color and *all-trans*- $\beta$ -carotene, *all-trans*-violaxanthin and 9-*cis*-violaxanthin were also observed in 'Ataulfo' and in 'Manila' mangoes (Ornelas-Paz *et al.*, 2008) with the highest correlation coefficients for  $a^*$  and  $h^\circ$  parameters; in 'Manila' mangoes the best results were associated with the concentrations of *all-trans*-violaxanthin and 9-*cis*-violaxanthin, while in 'Ataulfo' with *all-trans*- $\beta$ -carotene, confirming that there is a cultivar specific relationship between pulp color and carotenoids content. Similar correlations were also found by Vasquez-Caceido *et al.* (2005) in 9 Thai mango cultivars (power law functions) and by Bicanic *et al.* (2010) in 21 mango homogenates (second order polynomial dependence).

Differently from carotenoids, ascorbic acid content and total antioxidant capacity for both cultivars and TPC for 'Palmer' were not related to pulp color, measured both by  $a^*$ ,  $l_V$  and  $h^\circ$  color parameters and  $\mu_a540$ ; this is not surprising as literature reported that AA and TPC contents with shelf life does not follow a well-defined increasing or decreasing trend (Robles-Sánchez *et al.*, 2009 b; Ibarra-Garza *et al.*, 2015).

In conclusion our results confirmed that the absorption coefficient at 540 nm ( $\mu_a540$ ) can be used as a non-destructive maturity index for mangoes. In fact it was able to classify intact fruit of two mango cultivars according to pulp color, the destructive maturity index commonly used for mangoes, as well as according to the contents of total carotenoids and of individual carotenoid compounds and vitamin A value. The good correlations between  $\mu_a540$ , pulp color parameters and carotenoids indicate that TRS is a suitable tool to sort fruit with different ripening degree, having specific carotenoid pattern.

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