Adv. Hort. Sci., 2023 37(1): 75-82 DOI: 10.36253/ahsc-13943

AHS
Advances in Horticultural Science

Non-destructive determination of ripening in melon fruit using time-resolved spectroscopy

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Key words: Carotenoids, *Cucumis melo* L., ethylene, juiciness, quality, TRS.

Abstract: **The aim of this work was to explore the feasibility of time-resolved reflectance spectroscopy (TRS) in determining the ripening degree and the quality of orange-fleshed melons. Sixty 'Honey Moon' melons were measured by TRS in the 540-1064 nm range and classified as less (LeM), medium (MeM), and more (MoM) mature according to increasing values of μ_a540. MoM fruit showed yellower peel color, slightly more orange pulp, higher juiciness and higher carotenoid contents than LeM ones. MoM fruit also showed higher internal ethylene concentration and lower firmness than LeM ones, even if the differences were not significant. The μ_a540 was positively related to internal ethylene, carotenoid accumulation, and juiciness, indicating that μ_a540 was linked to different ripening processes in melons. However, the relationship between μ_a540 and total carotenoid content was not as high as expected due to the low variability of pulp color and of carotenoid content. Changes in flesh color toward a more orange shade were accompanied by increased juiciness and ethylene production and by carotenoid accumulation, while changes in peel color were associated with changes in flesh firmness and juiciness. In conclusion, the absorption coefficient measured at 540 nm (μ_a540) by TRS could be used to sort melons in different ripening degrees; however, its applicability will need to be evaluated on a larger number of fruits and on other varieties.**

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Citation:
VANOLI M., CORTELLINO G., PICCHI V., BUCCHERI M., GRASSI M., LOVATI F., MARINONI L., LEVONI P., TORRICELLI A., SPINELLI L., 2023 - *Non-destructive determination of ripening in melon fruit using time-resolved spectroscopy.* - Adv. Hort. Sci., 37(1): 75-82.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 10 November 2022
Accepted for publication 20 January 2023

1. Introduction

Melon (*Cucumis melo* L.) fruit are particularly appreciated by consumers for their sweetness, flavour, texture, and attractive flesh color, as well as for their nutritional and phytochemical properties. Melons are a good source of carotenoids, ascorbic acid, and phenolic compounds (Gómez-García *et al*., 2020; Manchali *et al*., 2021; Singh *et al*., 2022). Melon quality depends on the balance between sugars, organic acids, volatiles, and texture characteristics (Kyriacou *et al*., 2018). These traits are strongly affected by genotype, agro-climate conditions, harvest matu-

rity, post-harvest handling, and storage conditions (Kyriacou *et al*., 2018). Melons picked at the optimal harvest maturity have premium quality as maturity at harvest has a large impact on sugars, volatiles, and texture. During ripening, rind color changes from green to yellow, orange, or creamy yellow, depending on melon genotype; mesocarp color turns from pale green to orange in orange-fleshed melons, along with carotenoid accumulation; firmness and acidity decrease, while sucrose as well as soluble solids, vitamin C and pH markedly increase (Beaulieu and Lea, 2007; Tristan *et al*., 2022). Melons harvested mature develop the strongest flavour, whereas fruit harvested early develop a less aromatic flavour: esters are predominant in ripe melons, aldehydes are present at higher concentration in immature fruit while a sharp increase of alcohols is typical of overripe melons (Senesi *et al*., 2005; Beaulieu and Lea, 2007; Vallone *et al*., 2013; Lignou *et al*., 2014). During ripening, the sensory scores for color intensity, fruity aroma, juiciness, and sweetness increased while the sensory scores for firmness and sourness generally decreased (Vallone *et al*., 2013). In climacteric melons, ethylene was produced at higher levels in fully ripe fruits and was negatively related to firmness and positively related to sensory juiciness and fruity flavour and aroma (Senesi *et al*., 2005; Vallone *et al*., 2013).

For more than two decades, several efforts have been made to measure quality characteristics of melons in a nondestructive way (Zeb *et al*., 2021). In industrial fruit sorting, accuracy, cost, and detection speed are important factors. Thus, spectroscopic techniques being fast, simple, and cost-effective, have been widely studied. In melons, Vis-NIR spectroscopy (Sanchez *et al*., 2014; Lu *et al*., 2015; Khurnpoon and Sirisomboon, 2018; Zeb *et al*., 2021), computer vision (Calixto *et al*., 2022) and hyperspectral imaging (Sun *et al*., 2017; Cho *et al*., 2022) were studied with less or more successful results for predicting soluble solids, moisture, pulp color, and firmness and for classifying fruit according to different sweetness degree or as suitable or not for harvesting. Among spectroscopic techniques, time-resolved reflectance spectroscopy (TRS) is gaining increasing interest in assessing fruit quality. Due to its accuracy in measuring optical properties in deep tissues it allows the evaluation of maturity and internal defects of horticultural products with a relatively thick surface layer (Lu *et al*., 2020). TRS allows the complete optical characterization of a diffusive medium through the measurements of the absorption (μ_a) and of the scattering (μ_s) coefficients by probing flesh at a depth of 1-2 cm with no or limited influence from the skin (Cubeddu *et al*., 2001; Rizzolo and Vanoli, 2016). While scattering is related to the structural properties of fruits and vegetables, absorption depends on the chemical composition of the tissue, mainly on the presence of pigments such as chlorophylls, anthocyanins, and carotenoids (Lu *et al*., 2020). TRS has been mainly applied in post-harvest studies on fruit and vegetables for estimating fruit ripening, for the detection of internal defects, and for discriminating fruit with different texture and sensory characteristics (Rizzolo and Vanoli, 2016). The absorption coefficient measured at 670 nm (μ_a670) at harvest was shown to be a maturity index for various fruit, such as peaches, nectarines, plums, pears, and apples, as it declines with fruit ripening (Vanoli and Buccheri, 2012). The μ_a540 (carotenoid tail) has been used as a non-destructive maturity index for mangoes, as it was able to classify intact mangoes of different cultivars according to pulp color and to the contents of total and individual carotenoids (Rizzolo and Vanoli, 2016; Vanoli *et al*., 2018).

TRS has not yet been studied on melons, so the aim of this work was to explore the feasibility of TRS in determining the ripening degree and the quality of orange-fleshed melons.

2. Materials and Methods

Fruit

The experiment was carried out on 'Honey Moon' (*Cucumis melo* L. cantalupensis) melons. 'Honey Moon' fruits have round shape, medium size, a smooth grey-green skin that turns yellow when fully ripe. Fruits has firm, deep orange flesh and show high sugar content and a pleasant and almost exotic aroma. In order to have high variability in fruit maturity, 60 melons were picked and selected in a packinghouse in Sermide (Mantova-Italy) on a peel color basis (15 fruit for four color stages: blue, gray-green, yellow-green and yellow). Then fruit were immediately transported to the CREA-IT lab in Milan, measured by TRS in the 540-1064 nm range and classified as less (LeM), medium (MeM) and more (MoM) mature according to increasing μ_a540 values (low μ_a540=less mature fruit; high μ_a540=more mature fruit). All the 60 melons were also individually evaluated for color (peel and flesh) and for standard matu-

rity indices (flesh firmness, soluble solids content, acidity). Among the 60 melons, 20 fruits covering the whole range of μ_a540 (i.e., the highest, the lowest and 18 intermediate values of μ_a540), were chosen for internal ethylene concentration, total carotenoid content and juiciness analyses.

Time-resolved reflectance spectroscopy (TRS)

Whole melons were measured by TRS on two opposite sides in the fruit equatorial region. A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Zhao *et al*., 2022) was employed. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light pulses, with duration of a few picoseconds. Two custom-made filter wheels loaded with overall 14 band-pass interference filters (MaxLine series, Semrock, NY, USA, and TECHSPEC series, Edmund Optics, New Jersey, USA) are used for spectral selection in the range 540-1064 nm. Light is delivered to the sample by a 200 µm core step-index fiber and collected by a 1 mm core graded-index fiber; interfiber distance was set to 1.5 cm. A pair of filter wheels identical to the previous one is used for cutting off the fluorescence signal originated from the sample when it is illuminated in the visible spectral region. The light then is detected by a customized Silicon PhotoMultiplier module (Martinenghi *et al.*, 2016) and the photon time-of-flight distribution is 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 100 ps and the typical acquisition time is 1 s per wavelength. A semi-infinite model for photon diffusion in a turbid medium was used to analyze TRS data to assess the bulk optical properties of the samples (Martelli *et al*., 2009) to obtain the estimates of μ_a and μ_s at each wavelength.

Peel and flesh color

Color was measured on the equatorial region on two opposite side of whole fruit and, after cutting longitudinally the melons, on two opposite portions of the flesh, 1.5 cm from the fruit edge. A CM2600D spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) with the primary illuminant D65 and 2° observer, was used to perform the color measurements in the L^* (lightness) a^* (green-red), b^* (yellow-blue) color space. From a^* and b^* values, hue ($h°$) and chroma (C^*) were computed according to $h° =$ arctangent $(b^*/a^*) \times 360/(2\times3.14)$ and $C^* = (a^{*2} + b^{*2})^{-2}$. In the flesh also the color spectra (350-740 nm) were considered. Color readings were averaged for each fruit.

Standard maturity indices and juiciness

Flesh firmness

After having cut the melons into two parts along the longitudinal axis, flesh firmness was measured on two opposite sides in the equatorial part of the fruit (around 1,5 cm from the fruit edge) using an 8 mm diameter plunger mounted on a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at a crosshead speed of 200 mm min^{-1}. The two measurements were averaged for each fruit.

Soluble solids content (SSC) and titratable acidity

Two longitudinal slices around 4 cm thick, taken from two opposite sides of each fruit (in the same places of the firmness measurements) were frozen for the evaluation of soluble solids content (SSC) and of titratable acidity. After thawing, SSC was determined on few juice drops from each slice by using an automatic refractometer (RFM81, Bellingham-Stanley Ltd., England). Titratable acidity (TA) was measured by titrating 10 g of juice plus 50 mL of distilled water with 0.1 N NaOH to pH 8.

Juiciness

Juiciness was measured on pulp cylinders (diameter = 15 mm; height = 10 mm) taken from two opposite sides of the equatorial part of the fruit. Each cylinder was compressed between two plates with a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at a deformation rate of 100 mm min^{-1} by a compression of 50% of the original height of the cylinder (Eccher Zerbini *et al*., 1999). This method correlated with sensory analysis, and only the juice that could be easily and quickly released by the pulp cylinder was measured. Juiciness was calculated as the ratio between the juice weight spilled out after compression and the cylinder's original weight.

Internal ethylene concentration

1 mL sample of internal gas was taken from the seed cavity of each melon using a syringe equipped with a 15 cm long, 15-gauge needle. The sample was injected in a DANI GS 86.10 gas chromatograph and analyzed for the ethylene content following the conditions reported by Rizzolo *et al*. (2005), using a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in × 200 cm Alltech Italia, Sedriano, Italy), column temperature 100°C, injection temperature 100°C, and a flame ionization detector temperature

of 225°C. Ethylene was identified and quantified by relating the peak area of the sample to that of a 10 μL L^{-1} external standard and was expressed as ppm.

Total carotenoid content

Two slices per fruit were frozen for total carotenoid content analysis. 1 g of frozen sample was rapidly homogenized (Ultra-Turrax, IKA-Werk, Germany) in 5 ml of an ice-cold solution of hexane: acetone:ethylacetate (2:1:1) containing 1% of 1% butylated hydroxitoluene (BHT) in methanol. The homogenate was sonicated for 10 min, centrifuged at 10000 *g* for 15 min at 4°C and the organic phase was collected. The absorbance of the organic phase, after proper dilution, was measured spectrophotometrically at 450 nm. Total carotenoid content was estimated using the molar extinction coefficient for b-carotene in hexane (139200 l mol^{-1} cm^{-1}), as reported in Craft and Soares (1992).

Statistical analysis

Data were submitted to analysis of variance (Statgraphics ver. 7, Manugistic Inc., Rockville, MD, USA) considering TRS maturity class as a source of variation, and means were compared by Tukey's test at P≤0.05%. The relationships between μ_a540 and quality characteristics and the relationships among quality parameters were studied using regression analysis. For each parameter, the model with the higher performance was considered. Only the models with correlation coefficient r>0.50 were considered.

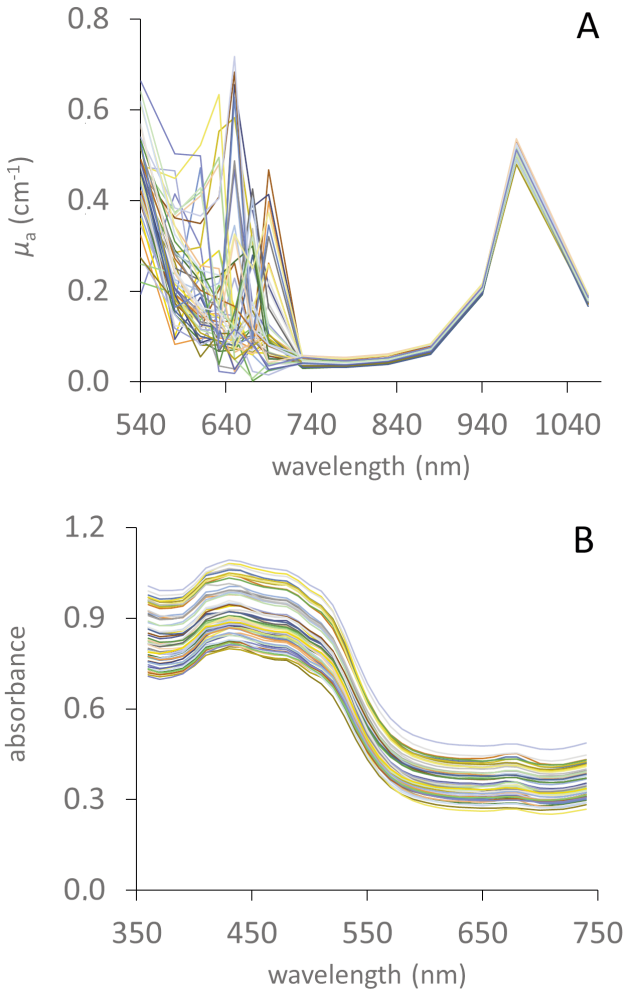

Fig. 1 - TRS absorption spectra (A) and pulp color spectra (B) of 'Honey Moon' melons.

3. Results

TRS absorption spectra

TRS absorption spectra of 'Honey Moon' melons (Fig. 1A) showed a maximum at 980 nm (corresponding to water), high absorption values at 540 nm (corresponding to the tail of carotenoids) and very high variability in the chlorophyll absorption range (610-690 nm).

The variability in the chlorophyll absorption was not linked to the presence of chlorophyll in the pulp but depended on the presence of a green layer (about 3.5-7 mm) between the rind and the pulp, which interfere with TRS measurements that pass through the pulp, up to 2 cm. The color spectra of the pulp confirmed the TRS data, showing a very low absorption in the chlorophyll range and a very high absorption in the carotenoid range (400-500 nm) (Fig. 1B). Then, the absorption coefficient measured at 540 nm (μ_a540) was chosen as a possible ripening index for melons due to its correlation to the carotenoid content in the pulp, as previously found by Rizzolo and Vanoli (2016) and Vanoli *et al*. (2018) in mangoes. The μ_a540 ranged from 0.194 to 0.663 cm^{-1}. Melons were ranked according to increasing μ_a540 (increasing maturity) and sorted into three maturity classes: low (μ_a540 = 0.383±0.008 cm^{-1}), medium (μ_a540 = 0.451±0.004 cm^{-1}) and more mature (μ_a540 = 0.,537±0.014 cm^{-1}).

Peel and flesh color

Peel and pulp color significantly changed with TRS maturity classes (Table 1). Peel color turned from green to yellow-green with advancing TRS maturity class, as b^* and C^* values increased and $h°$ values decreased from LeM to MoM melons. Pulp color became slightly more orange, as b^* and $h°$ were lower in MoM fruit than in LeM ones. There was no

Table 1 - Peel and pulp color parameters of 'Honey Moon' melons in relation to TRS maturity classes

Maturity stage	*L**	*a**	*b**	*C**	*h°*
Peel					
Less mature	72.4 a	-6.1 a	23.3 b	24.2 b	106.5 a
Medium mature	73.4 a	-5.4 a	26.0 ab	26.7 ab	103.4 ab
More mature	76.0 a	-5.4 a	28.8 a	29.4 a	101.2 b
Pulp					
Less mature	62.1 a	14.0 a	33.9 ab	36.7 a	67.6 a
Medium mature	63.2 a	14.4 a	34.7 a	37.6 a	67.5 a
More mature	60.0 a	14.4 a	33.3 b	36.3 a	66.6 b

Means in the same column followed by different letters are statistically different at P≤0.05 (Tukey's test).

main change in the pulp color of melons, as *b** ranged from 29.3 to 38.1 and *h°* from 64.5 to 70.2.

Standard maturity indices, juiciness, internal ethylene and total carotenoids content

Flesh firmness ranged from 7.09 to 27.55 N, SSC from 7.4 to 12.2% and acidity from 1.70 to 5.57 g l^{-1} citric acid. Flesh firmness, SSC and acidity were not significantly affected by TRS maturity classes (Fig. 2 A, B, C). However, firmness and acidity were lower and SSC was higher in MoM fruit than in LeM ones. Juiciness ranged from 6.86 to 22.27% and was significantly higher in MoM fruit than in the LeM ones (Fig. 2 D). Internal ethylene concentration ranged from 51.9 to 153.3 ppm. It did not significantly change with TRS maturity class, even if the LeM fruit showed the lowest concentration and MoM ones the highest (Fig. 2E). Total carotenoids content ranged from 16.95 to 28.25 mg Kg^{-1} fw; it was significantly higher in MoM fruit than in LeM ones (Fig. 2F).

Regression analysis

The results of regression analysis between μ_a540 and quality parameters are summarized in Table 2. The μ_a540 was significantly related to juiciness (r=0.53), internal ethylene concentration (r=-0.86) and total carotenoid content (r=0.66). The μ_a540 was also positively related to skin color (except for *a**)

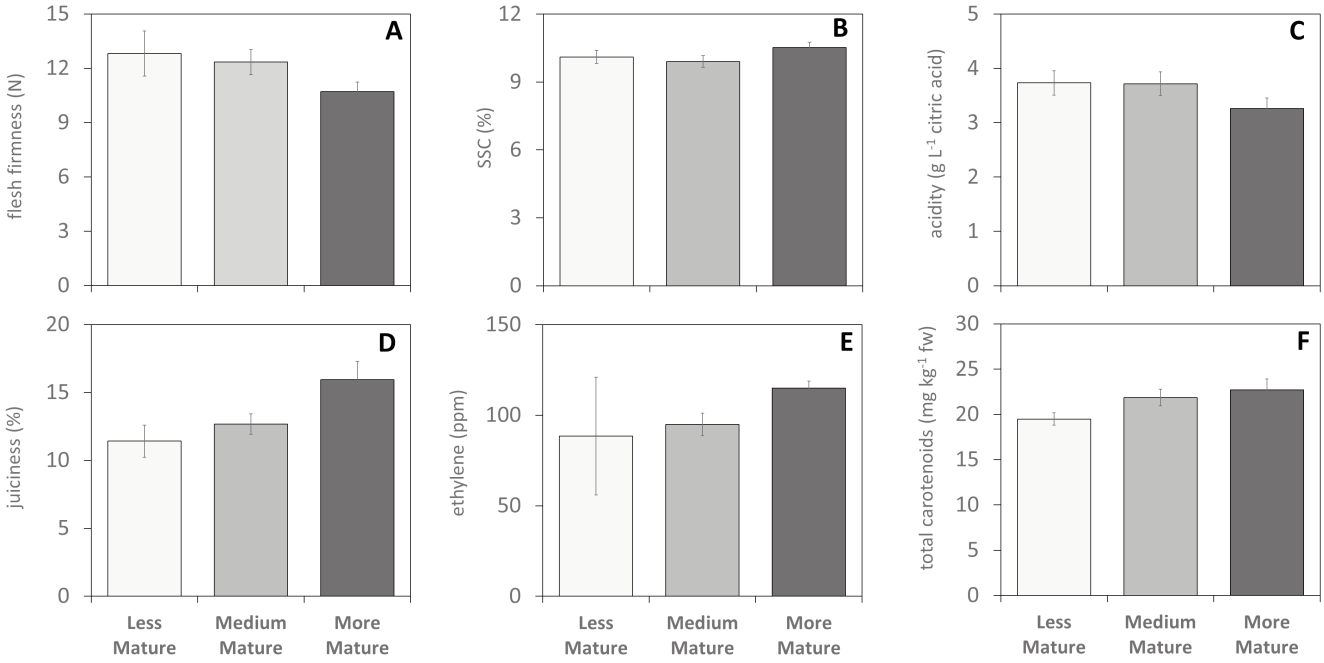

Fig. 2 - Flesh firmness (A), soluble solids content (B), acidity (C), juiciness (D), internal ethylene concentration (E) and total carotenoid content (F) in 'Honey Moon' melons in relation to TRS maturity classes. Bars refer to SE.

Table 2 - Regression models between μ_a540, juiciness, internal ethylene concentration and total carotenoid contents

	r	P	Model type
Juiciness	0.531	*	DR
Internal ethylene	-0.864	***	RX
Total carotenoids	0.664	**	DR

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), and model type.
DR= double reciprocal; RX=reciprocal X.

Table 3 - Regression models between firmness, juiciness, internal ethylene concentration, total carotenoid content and pulp color

	r	P	MT
Firmness			
*b** peel	0.616	***	RY
*C** peel	0.604	***	RY
h° peel	0.576	***	LIN
Juiciness			
firmness	0.733	***	RX
*b** peel	0.565	*	DR
*C** peel	0.548	*	DR
h° peel	0.538	*	RX
h° pulp	0.577	*	RX
Ethylene			
*a** pulp	-0.617	*	RY
Carotenoids			
juiciness	0.640	*	DR
*a** pulp	0.679	**	DR
h° pulp	-0.881	***	LIN

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) and MT= model type.
RY=reciprocal Y; LIN=linear; RX=reciprocal-X; DR=double reciprocal.

with r<0.5. No significant correlation was found between μ_a540 and pulp color, flesh firmness, SSC and acidity.

Significant relationships were also found among peel color, firmness and juiciness (Table 3). Firmness was related to *b** peel (r=0.62), *C** peel (r=0.60) and to *h*° peel (r=0.58); juiciness was related to *b** peel (r=0.57), *C** peel (r= 0.55) and to *h*° peel (r= 0.54). As for pulp color, total carotenoid content was positively related to *a** pulp (r=0.68) and negatively to *h*° pulp (r= −0.88); internal ethylene was related to *a** pulp (r= −0.61) and juiciness to *h*° pulp (r= 0.58). Significant relationships were also found between juiciness and total carotenoids (r= 0.64) and between juiciness and firmness (r= 0.73).

4. Discussion and Conclusions

'Honey Moon' melons used in this experiment showed pulp color similar to that of fruit of the same cultivar picked at commercial maturity, even if firmness, acidity and SSC had lower values (Cavicchi and Pasotti, 2004). Total carotenoid content showed values typical of orange-fleshed fruit (Saladie *et al*., 2015; Singh *et al*., 2022). Internal ethylene concentration confirms that 'Honey Moon' is a climacteric genotype, as there was an increase in ethylene production with advancing fruit maturity (Senesi *et al*., 2005; Vallone *et al*., 2013; Saladie *et al*., 2015).

TRS measurements of melons showed some problems. In fact, the absorption in the chlorophyll range cannot be used as a maturity index as already done for other fruits, such as apples, pears, peaches and nectarines (Rizzolo and Vanoli, 2016), since chlorophyll was almost absent in the melon pulp while it was present in a layer just under the peel. On the other hand, μ_a540 was able to distinguish MoM melons from LeM ones, as MoM fruit showed yellower peel color, slightly more orange pulp, higher juiciness and higher carotenoid contents, in agreement with Kyriacou *et al*. (2018), Senesi *et al*. (2005) Beaulieu and Lea (2007), Vallone *et al*. (2013), Saladie *et al*. (2015) and Tristan *et al*. (2022). MoM fruit also showed higher internal ethylene concentration and lower firmness than LeM ones, even if the values showed high variability and the differences were not significant. The μ_a540 was also positively related to internal ethylene, carotenoid accumulation and juiciness, indicating that μ_a540 is linked to different ripening processes in melons. However, the relationship between μ_a540 and total carotenoid content was not as high as expected (r=0.66) and as previously found in mangoes when r ranged from 0.78 to 0.91 depending on the cultivar (Vanoli *et al*., 2018). It seems that in 'Honey Moon' melons, the range of μ_a540 (0.194-0.663 cm^{-1}) is quite similar to that of mangoes (0.117-0.835 cm^{-1}), while the variability in total carotenoids

(16.95-28.25 mg Kg^{-1} fw) and in pulp color ($h°$ pulp= 64.5-70.2) was narrower in melons than in mangoes (total carotenoids= 5-56 mg Kg^{-1} fw; $h°$ pulp=71-104). Moreover no correlation was found in melons between μ_a540 and pulp color while in mangoes μ_a540 was negatively related to $h°$ pulp with r= 0.83-0.98 (Vanoli *et al*., 2018). In 'Honey Moon' fruit, with advancing TRS ripening degree, the changes in flesh color toward a more orange shade were accompanied by increased juiciness values and ethylene production and by carotenoid accumulation, while changes in peel color toward a yellow shade were associated with fruit softening.

In conclusion, the absorption coefficient measured at 540 nm (μ_a540) by the TRS technique could be used to sort melons in different ripening degrees; however, its applicability will need to be evaluated on a larger number of fruits and on other varieties.

Acknowledgements

This study was funded from the Italian Ministry of Agriculture, Agridigit project, subproject Agrofiliere (D.M. 36503/7305/2018, approved on 20 December 2018).

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