Adv. Hort. Sci., 2020 34(1S): 71-80 DOI: 10.13128/ahsc-7666

AHS
Advances in Horticultural Science

Anthocyanin and carotenoid contents assessed by time-resolved reflectance spectroscopy in potato tubers (*Solanum tuberosum* L.) with different flesh colors

M. Vanoli [1] (*), L. Spinelli [2], A. Torricelli [2, 3], A. Ibrahim [4], B. Parisi [5], R. Lo Scalzo [1], A. Rizzolo [1]

[1] *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Ingegneria e Trasformazioni agroalimentari (CREA-IT), Via Venezian, 26, 20133 Milano, Italy.*

[2] *Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche (IFN-CNR), Piazza Leonardo da Vinci, 32, 20133 Milano, Italy.*

[3] *Politecnico di Milano, Dipartimento di Fisica, Piazza Leonardo da Vinci, 32, 20133 Milano, Italy.*

[4] *Agricultural Engineering Research Institute (AEnRI), Agricultural Research Center (ARC), Nadi El-Seid St., 12311 Dokki-Giza, Egypt.*

[5] *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Cerealicoltura (CREA-CI), Via di Corticella, 133, 40128 Bologna, Italy.*

(*) **Corresponding author:**
maristella.vanoli@crea.gov.it

Citation:
VANOLI M., SPINELLI L., TORRICELLI A., IBRAHIM A., PARISI B., LO SCALZO R., RIZZOLO A., 2020 - *Anthocyanin and carotenoid contents assessed by time-resolved reflectance spectroscopy in potato tubers (Solanum tuberosum L.) with different flesh colors.* - Adv. Hort. Sci., 34(1S): 71-80

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 18 December 2019
Accepted for publication 13 May 2020

Key words: absorption spectra, flesh color, partial least square regression, *Solanum tuberosum L.*, TRS.

Abstract: **This work aimed at studying the relationships between the absorption spectra acquired by time-resolved reflectance spectroscopy (TRS) and the carotenoid (CAR) and/or the anthocyanin (ANT) contents in 9 potato genotypes with different flesh color (white, yellow, red, purple). Fifty whole and intact tubers/genotype were non-destructively measured by TRS in the 540-980 nm range; white- and yellow-fleshed were ranked according to increasing μ_a540, the red ones according to μ_a670 and the purple ones according to μ_a780. Then, 5 tubers/genotype, corresponding to the highest, the lowest and 3 intermediate values of each μ_a range, were analyzed for flesh color and CAR and ANT contents. In white- and yellow-fleshed genotypes, μ_a540 ranged from 0.078 to 0.207 cm^{-1}, showing the highest value in 'Melrose' and in 'ISCI 133/12-1' and the lowest ones in 'Romantica' and in 'CN 07.16.3'. In red-fleshed tubers, μ_a670 ranged from 0.049 to 0.146 with no significant differences between genotypes; in purple-fleshed genotypes, μ_a780 ranged from 0.147 to 0.473, showing the highest values in 'Bleuet'. CAR content ranged between 0.071 to 5.937 mg kg^{-1} FW, displaying the highest amounts in the deep yellow genotypes 'Melrose' and 'ISCI 133/12-1' and the lowest ones in the white 'CN 07.16.3' and in the dark purple 'Bleuet' tubers. ANT content ranged from 31.63 to 798.44 mg kg^{-1} FW in red-purple genotypes, having the highest values in 'Bleuet'. By using TRS spectra and PLS analysis, it was possible to predict CAR (R^2_{CV}=0.79, RMSECV=0.89) and ANT (R^2_{CV} =0.81, RMSECV=95.53) contents and flesh color ($h°$) in yellow-fleshed genotypes (R^2_{CV} =0.93, RMSECV=0.67) and purple genotypes (R^2_{CV} =0.82, RMSECV=1.63).**

1. Introduction

Potatoes are grown throughout the world and are consumed in large quantities. Potatoes present wide biodiversity, with approximately 5000 known varieties, most of them developed through man selection (Fernandez-Orozco *et al*., 2013). Potatoes account for only about 2% of the food energy supply; however, they are the predominant staple for many countries.

Potato is mainly composed of water (80%) and carbohydrate, with starch being the most abundant; contributes up to 3.3% of dietary fiber, shows low amount of proteins and aminoacids with excellent nutritional value and is also rich in vitamins (ascorbic acid, folic acid, niacin, riboflavin, thiamine, pyridoxine) and in minerals such as potassium, phosphorous and calcium (Burlingame *et al.*, 2009; Fernandez-Orozco *et al.*, 2013; Zaheer and Akhtar, 2016). In addition to ascorbic acid, potatoes contain several phytochemicals such as polyphenols, anthocyanins, flavonoids, carotenoids, tocopherols, and alpha-linoleic acid, which have beneficial effects on human health due to their antioxidant activity (Ezekiel *et al.*, 2013; Zaheer and Akhtar, 2016). Although other fruits and vegetables have antioxidant content higher than that of potatoes, considering the large quantities in which potatoes are consumed throughout the world, their contribution to the human diet is very significant. Phytochemical content in potatoes is affected by various factors such as genotype, cultivation conditions and methods (organic *vs* conventional), developmental stage, postharvest storage, cooking and processing conditions (Lachman *et al.*, 2012; Ezekiel *et al*., 2013; Murniece *et al.*, 2013). Generally, the skin and/or the fleshes of potatoes varieties are white, yellow, or deep yellow. However, the introduction and availability of pigmented potatoes in which skin and/or fleshes are red, purple, blue, or orange have attracted consumers over the last two decades due to their high antioxidant content in terms of anthocyanins, carotenoids and total phenolics (Tierno *et al.*, 2016). The coloration pattern of the skin and fleshes of colored potatoes is variable, i.e., the skin alone may be pigmented, or the flesh may be partially or entirely pigmented.

Potato cultivars with white flesh contained fewer carotenoids as compared to cultivars with yellow or orange flesh (Ezekiel *et al.*, 2013; Fernandez-Orozco *et al.,* 2013; Kaspar *et al.*, 2013; Murniece *et al*., 2013). Carotenoid concentrations in white- and purple-fleshed potatoes were similar, while yellow potatoes having a 45-fold greater carotenoids concentration compared to white and purple potatoes (Kaspar *et al*., 2013; Hejtmankova *et al*., 2013). Lutein, zeaxanthin, violaxanthin and neoxanthin are the major carotenoids present in potatoes and β-carotene is present in trace amounts (Lu *et al.*, 2001; Ezekiel *et al*., 2013; Hejtmankova *et al*., 2013; Kaspar *et al*., 2013). Both total and individual carotenoid contents were positively correlated with tuber yellow intensity (Lu *et al*., 2001; Murniece *et al*., 2013).

Anthocyanins are present in considerable amounts in purple-red pigmented potatoes and their concentrations are considerably higher in the skin than in the flesh (Ezekiel *et al*., 2013). Purple-fleshed potatoes had higher anthocyanins compared to red-fleshed potatoes, while low or non-detectable amounts were found in yellow and white-fleshed cultivars (Nayak *et al.*, 2011, Lachman *et al*., 2012; Ezekiel *et al*., 2013; Kaspar *et al*., 2013; Kita *et al*., 2013; Lachman *et al*., 2013; Tierno *et al*., 2015, 2016; Akyol *et al*., 2016). The most common anthocyanins present in potatoes are pelargonidin, malvidin, petunidin, cyanidin, peonidin and delphinidin (Lachman *et al.,* 2012; Hejtmankova *et al*., 2013; Akjol *et al.,* 2016). Red-fleshed genotypes contain predominantly acylated glycosides of pelargonidin, while purple-fleshed clones contain predominantly acylated glycosides of petunidin, malvidin and peonidin (Lachman *et al.*, 2012; Hejtmankova *et al*., 2013; Kita *et al.,* 2013; Akyol *et al*., 2016; Tierno *et al*., 2016). Especially due to anthocyanins, pigmented potatoes also exhibit higher antioxidant activity in comparison to common yellow-fleshed potatoes (Lachman *et al*., 2009; Nayak *et al*., 2011; Lachman *et al*., 2012).

Anthocyanin and carotenoid contents are generally determined by analytical methods, such as gas-liquid chromatography (GLC), HPLC and UV-VIS spectrophotometry. These techniques, however, are costly and time-consuming and are not suitable for on-line applications in the food industry. Consequently, rapid, accurate, and non-destructive techniques have been studied to monitor antioxidant amounts in potato tubers. However, most of the published papers concern the estimation of dry matter, starch, proteins and sugars in potatoes and only a few articles deal with the prediction of anthocyanin and carotenoid contents in raw and processed potatoes (López *et al*., 2013). NIR spectroscopy applied on whole tubers was able to accurately identify samples containing different levels of soluble phenolics, anthocyanins and hydrophilic antioxidant capacity

belonging to a collection of 18 purple- and red-fleshed potatoes and to predict the total phenolic content in 98 potato varieties (López *et al.,* 2014; Tierno *et al*., 2016). Total and individual carotenoids, anthocyanins as well as total phenolics and antioxidant activity have been estimated with good/high accuracy by NIR, hyperspectral imaging, infrared and Raman spectroscopy during drying process, in homogenized potato chips and in lyophilized potatoes (Shiroma-Kian *et al*., 2008; Bonierbale *et al*., 2009; Liu *et al*., 2017; Mazurek *et al*., 2017; Escuredo *et al*., 2018; Sebben *et al.,* 2018). NIR was also used to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein and β-carotene (Bonierbale *et al.,* 2009).

Among non-destructive optical techniques, Time-resolved Reflectance Spectroscopy (TRS) is gaining increasing interest (Nicolai *et al*., 2014). TRS has been mainly applied in postharvest studies for estimating fruit maturity, for discriminating fruit having different texture and sensory characteristics and for the detection of internal defects in fruits and vegetables (Rizzolo and Vanoli, 2016). TRS, in combination with proper models of photon migration, 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 *et al*., 2016). While scattering is related to the structure, absorption depends on the chemical composition of the tissue, mainly on the presence of pigments such as chlorophylls, anthocyanins and carotenoids. TRS absorption spectra measured in the 540-780 nm range were successfully used to predict total carotenoids content in mangoes in combination with partial least squares regression achieving a R^2_{cv}=0.83 and 0.93 depending on the cultivars (Vanoli *et al.,* 2016). In 'Haden' and 'Palmer' mangoes, the absorption coefficient measured by TRS at 540 nm (μ_a540), in correspondence of the tail of carotenoid absorption, significantly correlated (*r*=0.78-0.94) with total carotenoids, *all-trans*-β-carotene, *all-trans*-violaxanthin no.3, *all-trans*-violaxanthin no.1, no.2, no.6 ('Haden'), and 9-*cis*-violaxanthin no.2, no.3 ('Palmer') (Vanoli *et al.*, 2018). Furthermore, high positive correlations were also found among μ_a540 and *a** and yellowness index (*r*=0.83-0.98), as well as high but negative correlation between μ_a540 and H° (*r*=-0.83-0.98) (Rizzolo *et al*., 2016; Vanoli *et al*., 2016, 2018). The absorption coefficient measured in the 500-580 nm range was also related to the presence of anthocyanins, as found in plums and in red-fleshed peaches (Rizzolo and Vanoli, 2016).

The aim of this work was to investigate the relationships between TRS absorption spectra and carotenoids and/or anthocyanin contents in nine potato genotypes with white, yellow, red and purple flesh color.

2. Materials and Methods

Potato tubers

The experiment was carried out on 9 potato genotypes: 7 commercial varieties and 2 belonging to CREA-CI breeding programme. The 9 genotypes showed different flesh color: 2 had purple flesh ('Bleuet', 'Salad Blue'); 2 red flesh ('Magenta Love', 'ISCI 218/3'), 4 yellow flesh ('ISCI 133/12-1', 'Doribel', 'Melrose', 'Romantica') and 1 white flesh ('CN 07.16.3'), whose traits are reported in Table 1.

All the potato genotypes were grown in the experimental field in Budrio (Bologna Province, Northern Italy), 44°32'14" N - 11°32'03" E - 28 m a.s.l. in accordance to the Emilia-Romagna Region's IPM

Table 1 - Potato genotype characteristics

Genotype	Dealer	Ploidy	Skin colour	Flesh colour	Weight (g) mean ± SD	GMD (mm) mean ± SD
CN 07.16.3	Bernard SAS, France	2n=4x=48	yellow	white	203.5 ± 61.9	68.4 ± 4.7
Romantica	Danespo A/S, Denmark	2n=4x=48	dark red	cream	191.7 ± 42.7	68.4 ± 4.7
Doribel	Pizzoli spa, Italy	2n=4x=48	yellow	cream	203.8 ± 42.9	68.1 ± 4.8
Melrose	Romagnoli F.lli spa, Italy	2n=4x=48	reddish brown	deep yellow	187.0 ± 36.5	66.5 ± 4.1
ISCI 133/12-7	Not on the market yet	2n=4x=48	yellow	deep yellow	196.0 ± 60.4	67.7 ± 6.5
ISCI 218/3	Not on the market yet	2n=4x=48	red	red with yellow pigmentation	104.6 ± 24.5	55.1 ± 4.4
Magenta Love	GM Sottotetti srl, Italy	2n=4x=48	red	red	132.0 ± 36.0	58.4 ± 5.2
Salad Blue	D.T. Brown Seeds, United Kingdom	2n=4x=48	blue	parti-coloured purple	88.0 ± 18.8	51.5 ± 3.3
Bleuet	NewStyle Potatoes BV, The Netherlands	2n=4x=48	blue	deep purple	149.3 ± 37,7	62.5 ± 5.1

Guidelines. Potatoes were harvested at full maturity on August 13, 2018 by a mechanical potato digger and stored at 4°C, 90% relative humidity, up to February 14, 2019. At storage removal, 50 potatoes/genotype without external defects were selected, and the diameters (x=longest axis, y= longest axis normal to x; z= longest axis normal to y) were measured. Geometrical Mean Diameter (GMD) of each tuber was calculated according to Mohsenin (1986) as following:

$$GMD = (xyz)^{1/3}$$

Then each tuber was measured by TRS on two opposite sides in the central region in the 540-980 nm range for white- and yellow-fleshed ones, in the 670-980 nm range for red-fleshed and in the 780-980 nm range for purple-fleshed ones. Within each genotype, white- and yellow tubers were ranked according to increasing μ_a540, the red ones according to μ_a670 and the purple ones according to μ_a780. Then, 5 tubers/genotype, corresponding to the highest, the lowest and 3 intermediate values of μ_a540 (yellow), μ_a670 (red) and μ_a780 (purple) were selected for physical-chemical analyses. Each tuber was cut in half and the flesh was measured for color in correspondence of the two TRS measurement points; then samples were immediately deep frozen at -20°C until carotenoids (CAR) and anthocyanin (ANT) analysis.

Time-resolved Reflectance Spectroscopy (TRS)

A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Torricelli *et al*., 2015) was used. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light picosecond pulses, with the duration of a few tens of picoseconds. A custom-made filter wheel loaded with 14 band-pass interference filters (NT-65 series, Edmund Optics, New Jersey, USA) is used for spectral selection in the range 540-940 nm. Light is delivered to and collected from the sample 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 originating from the sample when it is illuminated 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 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 260 ps and the typical acquisition time is 1 s per wavelength. A model for photon diffusion in a spherical 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.

Flesh color

Flesh 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. From *a** and *b** values, hue (*h*°) was computed according to:

$$h° = \text{arctangent}\,(b^*/a^*) \times 360/(2\times 3.14).$$

Carotenoids and anthocyanin analysis

CAR and ANT analyses were carried out on individually frozen samples by slicing flesh portion after skin removal.

For CAR analysis, 1 g of flesh was extracted with 2 mL of NaCl 20% in water and 4 mL of a solution of hexane/acetone/ethyl acetate 2:1:1 v/v/v (Picchi *et al.,* 2012). For ANT analysis, 1 g of flesh was extracted with 4 mL of ethanol/water 50:50 acidified with HCl, final concentration 0.2 M, pH=1.2 (Giusti and Wrolstad, 2001). Then the mixtures were accurately stirred, mixed, centrifuged at 4890 *g* for 5 minutes at 4°C, and the supernatants were used for the spectrophotometric analysis. The extracts were stored at -20°C until spectrophotometric analysis (Jasco, model V-630, Deutschland GmbH, Pfungstadt, Germany).

Total carotenoid content (CAR) was determined by measuring the absorbance at 441 nm and quantified considering the Epsilon value of 2540 g 100 g^{-1} for zeaxanthin (Baurernfeind *et al.,* 1971). CAR data were expressed as mg of zeaxanthin equivalent (ZE) per kilogram of fresh weight (mg ZE kg^{-1} FW).

Total anthocyanin content (ANT) was determined by measuring the absorbance at 503 nm (Giusti and Wrolstad, 2001) and quantified, considering the Epsilon values of 18420 Moles cm^{-1} for pelargonidin (Giusti and Wrolstad, 2001). ANT data were expressed as mg of pelargonidin equivalent (PE) per kilogram fresh weight (mg PE kg^{-1} FW).

Statistical analysis

Data of μ_a540, μ_a670 and μ_a780, CAR, ANT and flesh color (*h*°) were submitted to ANOVA considering genotype as factor (means compared by Tukey's test at P≤0.05%) by using the Statgraphics v. 5.2 (Manugistic Inc., Rockville, MD, USA) software package. TRS absorption spectra were processed by Unscrambler X 10.0.1 (Camo, Norway) in order to build Partial Least Square (PLS) Regression models for

CAR, ANT and *h*° prediction, without pretreatments of spectral data.

3. Results and Discussion

TRS absorption spectra

The TRS absorption spectra of the 5 selected white, yellow, red and purple tubers are illustrated in figure 1. The absorption spectra of white and yellow potatoes showed a maximum at 980 nm, corresponding to water, and high values at 540 nm, in correspondence to the tail of carotenoid absorption, as previously found by Rizzolo *et al.* (2016) and Vanoli *et al.* (2016, 2018) in mangoes. The absorption spectra of red potatoes showed a peak at 980 nm and high absorption at 670 nm, while in purple potatoes maxima were observed at 670 nm for 'Salad Blue' tubers and at 780 nm for 'Bleuet' ones, with a lower water peak. The absorption at 670 and at 780 nm could be linked to the presence of anthocyanins, as the prominent absorbance peaks of anthocyanin were around 500-550 nm (Giusti and Wolstrad, 2001) but some absorbance was also noticed above 650 nm (Laksmiani *et al*., 2016; Noda *et al.,* 2017). Contrary to what found in fruit such as apples, pears, peaches, mangoes and plums (Rizzolo and Vanoli, 2016), μ_a670 in potatoes was not linked to chlorophyll content, as no greening development was detected in tubers studied in this experiment.

In white and yellow genotypes, μ_a540 ranged from 0.078 to 0.207 cm^{-1} and showed the highest value in 'Melrose' and 'ISCI 133/12-1' and the lowest ones in 'Romantica' and 'CN 07.16.3' (Table 2). As for red-fleshed tubers, μ_a670 ranged from 0.049 to 0.146 cm^{-1}, with no significant differences between genotypes (Table 2). In purple genotypes, μ_a780 ranged from 0.147 to 0.473 cm^{-1} assuming the highest values in 'Bleuet' (Table 2).

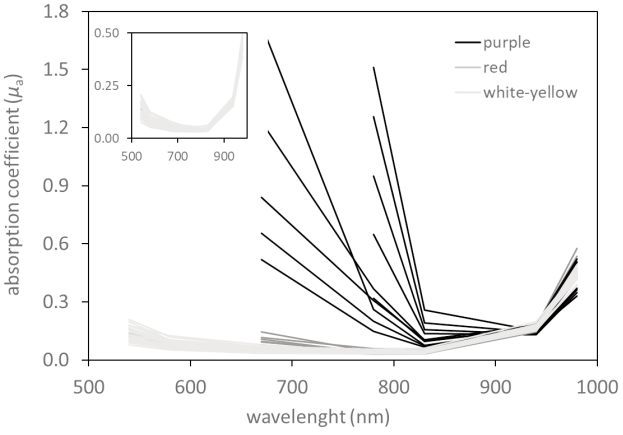

Fig. 1 - Absorption spectra of the five selected purple-red-yellow-white fleshed tubers. The inset figure shows the variability of the absorption spectra for white- and yellow-fleshed genotypes.

Table 2 - Values of the absorption coefficients measured by TRS at 540 nm (μ_a540), 670 nm (μ_a670) and 780 nm (μ_a780) used to rank white-yellow, red and purple potatoes, respectively

	Mean	Min	Max	SD
μ_a540 (cm^{-1})				
CN 07.16.3	0.104	0.078	0.136	0.023
Romantica	0.108	0.089	0.132	0.016
Doribel	0.141	0.101	0.176	0.029
Melrose	0.167	0.139	0.197	0.022
ISCI 133/12-1	0.161	0.122	0.207	0.033
μ_a670 (cm^{-1})				
ISCI 218/3	0.096	0.049	0.146	0.038
Magenta Love	0.080	0.055	0.107	0.021
μ_a780 (cm^{-1})				
Salad Blue	0.256	0.147	0.367	0.086
Bleuet	0.937	0.318	1.510	0.473

Carotenoid and anthocyanin contents

Carotenoid content (CAR) ranged from 0.071 to 5.937 mg ZE kg^{-1} FW, *i.e*. values comparable with the data found by Ezekiel *et al.* (2013), Hejtmankova *et al.* (2013) and Tierno *et al.* (2015), on other genotypes. CAR was present in white, yellow and also in red and purple (except in 'Salad Blue') genotypes, and showed the highest amounts in the deep yellow genotypes 'Melrose' and 'ISCI 133/12-1', intermediate contents in the red-fleshed 'ISCI 218/3' and in 'Magenta Love' and the lowest ones in the white genotype 'CN 07.16.3' and in the dark purple 'Bleuet' tubers (Table 3). These data confirmed that deep yellow-fleshed genotypes are usually characterized by much higher carotenoid contents than white- and red-fleshed ones (Ezekiel *et al*., 2013; Kaspar *et al.,* 2013; Tierno *et al.,* 2015; Kotíková *et al.,* 2016; Tierno *et al.,* 2016). In contrast, purple-fleshed tubers had either no carotenoids or a carotenoid content similar to that of white genotypes (Hejtmankova *et al.,* 2013; Kaspar *et al*., 2013; Kotíková *et al*., 2016).

Anthocyanins (ANT) were present in red and purple genotypes, with values ranging from 31.63 to 798.44 mg PE kg^{-1} FW (Table 3) in agreement with the findings of Ezekiel *et al.* (2013), Hejtmankova *et al.* (2013), Lachman *et al.* (2012, 2013), Kita *et al.* (2013) and Tierno *et al.* (2015) on other potato cultivars. ANT showed the highest amount in 'Bleuet' tubers,

Table 3 - Carotenoid and anthocyanin contents and pulp color (h°) of white, yellow, red and purple-fleshed potatoes

	CAR (mg ZE kg^{-1} FW)				ANT (mg PE kg^{-1} FW)				*h*° pulp			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
CN 07.16.3	0.241	0.071	0.496	0.181	nd	nd	nd	nd	97.8	97.5	98.3	0.4
Romantica	0.997	0.323	1.498	0.563	nd	nd	nd	nd	97.4	96.7	98.2	0.6
Doribel	1347	0.278	2.244	0.716	nd	nd	nd	nd	96.1	95.9	96.3	0.2
Melrose	4540	3.457	5.937	0.955	nd	nd	nd	nd	92.2	91.1	93.8	1.1
ISCI 133/12-1	4063	3.323	5.291	0.751	nd	nd	nd	nd	94.0	93.4	95.1	0.7
ISCI 218/3	2.655	2.000	3.882	0.784	88.42	47.37	130.42	29.65	55.8	44.7	65.8	7.9
Magenta Love	2.285	1.276	3.244	0.812	144.49	80.62	245.84	65.96	38.4	21.3	58.1	15.4
Salad Blue	nd	nd	nd	nd	49.05	31.63	73.05	19.74	337.0	333.6	339.7	2.3
Bleuet	0.526	0.331	0.843	0.212	529.38	328.08	798.44	176.58	331.8	328.2	335.4	2.7

nd= not detected.

while did not significantly differ among the other 3 genotypes (Table 3). The highest ANT content was usually found in dark purple genotypes (Ezekiel *et al*., 2013; Hejtmankova *et al*., 2013; Lachman *et al*., 2012, 2013; Nayak *et al.,* 2011; Tierno *et al*., 2015, 2016). 'Salad Blue', characterized by a parti-coloured purple flesh, showed lower ANT content than the deep purple 'Bleuet' genotype, but similar ANT values to the red-fleshed genotypes, as previously observed by Hejtmankova *et al.* (2013), Kita *et al.* (2013), Lachman *et al.* (2012, 2013). ANT was not detected in white and yellow genotypes (Table 3) as found by Tierno *et al.* (2015); on the other hand, Kaspar *et al.* (2013) observed that white potatoes had no ANT, whereas yellow-fleshed ones showed 20-fold lower ANT concentrations than the purple ones.

Flesh color

Considering the white and yellow genotypes, 'CN 07.16.3' and 'Romantica' exhibited the highest *h*° values, 'Melrose' and 'ISCI 133/12-1' the lowest ones and 'Doribel' intermediate values (Table 3). 'CN 07.16.3' and 'Romantica' had a pale yellow flesh, even if classified white and cream, respectively (Table 1); 'Doribel' showed a slightly yellower flesh than 'CN 07.16.3' and 'Romantica', even if classified creamy as 'Romantica' (Table 1); 'Melrose' and 'ISCI 133/12-1' tubers had the yellowest flesh color, even if the yellow intensity was higher in 'Melrose' tubers: both these genotypes were classified as deep yellow-fleshed (Table 1). The flesh color of these 5 genotypes agreed with the respective carotenoid contents: more intense was the yellow color of the flesh, the higher the CAR content. Considering all the 5 genotypes, a high negative linear (r=-0.83, p<0.001) relationship was found between *h*° and CAR content of the flesh, in agreement with Lu *et al.* (2001), reporting a strong relationship between total and individual carotenoids and tuber yellow intensity, and Murniece *et al.* (2013), finding a positive correlation between carotenoid content and the *b**coordinate of the flesh in organically and in conventionally cultivated potatoes.

As for purple genotypes, a slight but significant difference in the flesh color existed between 'Bleuet' and 'Salad Blue', as the former showed a lower *h*°, indicating a darker purple color (Table 3). In addition, 'Bleuet' also had an 11-fold greater ANT content compared to 'Blue Salad'; this higher ANT content was responsible for the deeper purple color as confirmed by the negative and significant correlation between ANT content and *h*° (r=-0.89, p<0.001). Considering red-fleshed potatoes, 'Magenta Love' showed lower *h*° than 'ISCI 218/3', confirming that the former had a red color and the latter a deep orange color due to the presence of a slightly higher CAR and a slightly lower ANT contents in the flesh (Table 3). A negative and significant correlation was found between ANT content and *h*° (r=−0.87, p<0.001) also for red genotypes. Dependence of the flesh coloration of the tubers measured by the CIELab scale with phenol flavonoid contents was also observed by Escuredo *et al.* (2018) in 35 potato varieties with different flesh color.

Partial Least Square (PLS) Regression models

TRS absorption coefficients measured at the different wavelengths were used to develop Partial least squares (PLS) regression models for predicting CAR and ANT contents and *h*° color of the potato flesh. For each parameter, the best model was selected considering the lowest root-mean-square error of cross-validation (RMSECV), combined with the lowest number of latent variables (LV) and the highest coefficient of determination in cross-validation (R^2_{CV}). The

results of PLS regressions are reported in Table 4 and in figures 2 and 3.

A good result was obtained for the prediction of CAR contents, as the PLS model had a R^2_{CV} of 0.79 and an RMSECV of 0.89, being μ_a540 and μ_a580 the important variables (Fig. 2, top). A slightly better result was achieved for ANT prediction, as the performance of the PLS model showed R^2_{CV} of 0.81 and RMSECV of 95.53 (Fig. 2, bottom). The μ_a780 and μ_a830 were the important variables. However, figure 2 (bottom) showed that samples are not equally distributed according to ANT content. There are two groups: the larger one with ANT content up to 250 mg PE kg^{-1} FW, including the red genotypes and the purple genotype 'Salad Blue', and a second group with ANT content ranging from ~300 to 800 mg PE kg^{-1} FW corresponding to the deep purple-fleshed 'Bleuet' genotype. Probably, the highest ANT content, together with highest variability of 'Bleuet' tubers, strongly affected the performance of the PLS model for ANT content prediction.

To the best of our knowledge, there are a few papers in literature dealing with the non-destructive determination of antioxidant compounds in whole tubers. Tierno *et al.* (2016) found that NIR measurements on unpeeled intact potatoes combined with PLS-DA allowed to accurately identify samples containing different levels of total phenols, total

Table 4 - Performance of PLS regression models on original TRS absorption spectral data for prediction of total carotenoids (CAR) and total anthocyanin (ANT) contents and of flesh color (*h*°)

Dependent variables	TRS parameters	Variable number	Calibration		Validation	
			R^2_C	RMSEC	R^2_{CV}	RMSECV
CAR	μ_a540-980	5	0.84	0.73	0.79	0.89
ANT	μ_a780-980	1	0.81	90.61	0.81	95.53
h° white-yellow genotypes	μ_a540-980	4	0.94	0.54	0.93	0.67
h° purple genotypes	μ_a780-980	2	0.87	1.24	0.82	1.62

R^2C= coefficient of determination between predicted and measured values in calibration;
R^2CV= coefficient of determination between predicted and measured values in cross-validation;
RMSEC= root mean square error of calibration;
RMSECV= root mean square error of cross-validation.

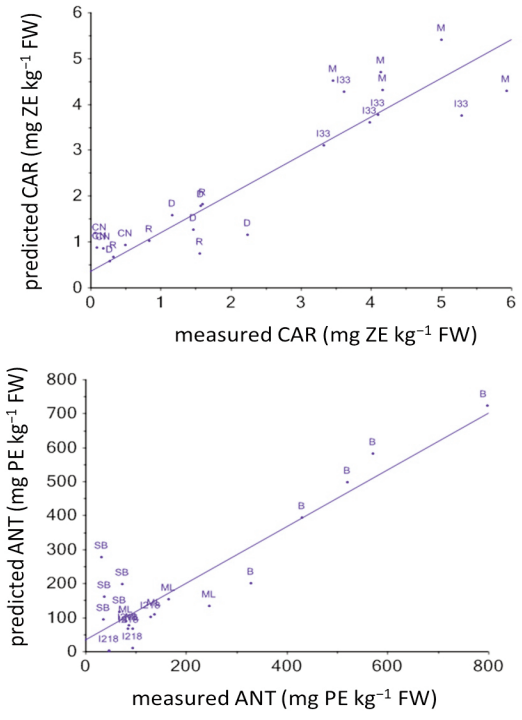

Fig. 2 - Measured and predicted CAR (top) and ANT (bottom) contents by PLS regression analysis.

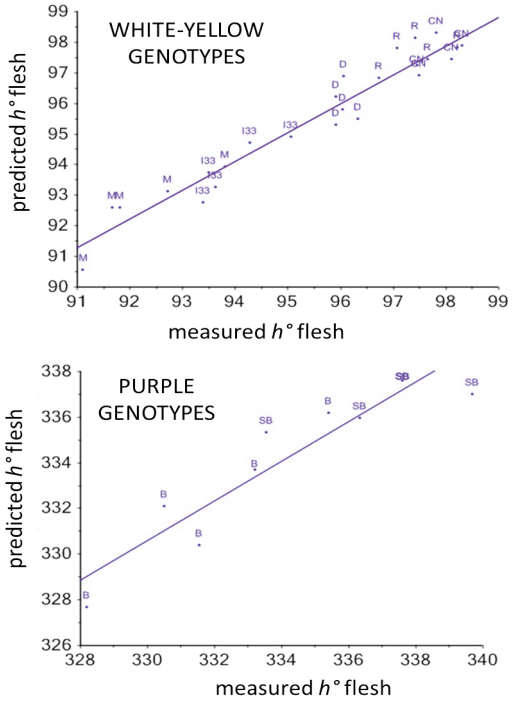

Fig. 3 - Measured and predicted flesh color of white-yellow (top) and purple (bottom) potato genotypes by PLS regression analysis.

monomeric anthocyanins and hydrophilic antioxidant capacity belonging to a collection of 18 purple- and red-fleshed potatoes. Regarding total carotenoids content, Tierno *et al.* (2016) found that NIRS was only capable of identifying samples with a high content of these compounds. Good models for predicting total phenol content have also been built by López *et al.* (2014) measuring 1157 whole potato tubers with NIR, and obtaining coefficients of determination of 0.88, 0.77 and 0.74 for calibration, cross-validation and external validation, respectively. However, when NIR technology was applied on freeze-dried and milled material (Bonierbale *et al.,* 2009; Escuredo *et al*., 2018; Liu *et al*., 2017) it was possible to successfully estimate CAR and/or ANT contents. Bonierbale *et al.* (2009), measuring 152 *Solanum phureja* germplasm accessions by NIR, estimated total carotenoids and zeaxanthin concentrations with R^2 values ranging from 0.63 to 0.92, and they were able to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein or β-carotene. Total flavonoid content was predicted by NIR with R^2=0.82 (Escuredo *et al*., 2018) and total anthocyanin amount by hyperspectral imaging in purple-fleshed sweet potato during drying process achieving a coefficient of determination for calibration of 0.868 and a coefficient of determination for prediction of 0.866 by using ten key wavelengths (637, 660, 666, 700, 729, 761, 801, 837, 892, and 957 nm) (Liu *et al*., 2017).

The flesh color prediction model for yellow genotypes (Fig. 3, top) showed the best performance, as R^2_{CV} was 0.93 and RMSECV was 0.67 and, as found for CAR content, the important variables were μ_a540 and μ_a580. PLS models were separately developed for flesh color prediction of red and purple genotypes considering the very high differences in the *h*° values, being on average, 47 for red-fleshed tubers and 335 for purple-fleshed potatoes (Table 3). A good model was obtained for the prediction of flesh color of purple genotype with R^2_{CV} = 0.82 and RMSECV = 1.62 (Fig. 3, bottom), while no significant model could be developed for red genotypes. By using NIR spectra, Escuredo *et al.* (2018) were able to estimate the *b** coordinate of the flesh with R^2=0.75 by using NIR spectra, while poor results were obtained for the *a** and *L** coordinates in lyophilized creamy, yellow and purple-fleshed potatoes; on the other hand, Mazurek *et al.* (2017), successfully modeled *L** parameter (R^2=0.992) in potato chips.

4. Conclusions

TRS was able to quantify with a reasonable accuracy carotenoid and anthocyanin contents in yellow and in red/purple fleshed-genotypes, respectively. TRS also allowed the estimation of flesh color in yellow-fleshed-genotypes, without being influenced by the different color of the skin, and in purple-fleshed ones. However, TRS was not able to predict flesh color in red-fleshed genotypes. The highly significant correlations between *h*° color coordinate and CAR and ANT contents can be used for discriminating potato tubers with different concentrations of pigments. The encouraging results of this study indicated the potential application of TRS for the non-destructive determination of carotenoid and anthocyanin contents and for the flesh color estimation in whole and intact potato tubers. However, further studies with a larger set of samples will be advisable in order to obtain better and more reliable models.

Acknowledgements

We are grateful to Science and the Technology Development Fund (STDF), Ministry of State for Scientific Research, Egypt for financial support to Ayman Ibrahim (Project ID: 25329. This research was carried out within the activity of the AGROFILIERE project funded by the Italian Ministry of Agriculture (D.M. 36503/7305/2018).

References

AKYOL H., RICIPUTI Y., CAPANOGLU E., CABONI M.F., VERARDO V., 2016 - *Phenolic compounds in the potato and its byproducts: an overview*. - Int. J. Mol. Sci., 17: 835.

BAUERNFEIND J.C., BRUBACHER G.B., KLÄUI H.M., MARUSICH W.L., 1971. - *Use of Carotenoids*. - In: ISLER O., H. GUTMANN, and U. SOLMS (eds.) *Carotenoids*. Chemische Reihe. Lehrbücher und Monographien aus dem Gebiete der Exakten Wissenschaften, vol 23, Birkhäuser, Basel, Switzerland.

BONIERBALE M., GRUNEBERG W., AMOROS W., BURGOS G., SALAS E., PORRAS E., ZUM FELDE T., 2009 - *Total and individual carotenoid profiles in* Solanum phureja *cultivated potatoes: II. Development and application of near-infrared reflectance spectroscopy (NIRS) calibrations for germplasm characterization*. - J. Food Comp. Anal., 22: 509-516.

BURLINGAME B., MOUILLÉ B., CHARRONDIÈRE E., 2009 -

Nutrients, bioactive non-nutrients and anti-nutrients in potatoes. - J. Food Comp. Anal., 22: 494-502.

CUBEDDU R., D'ANDREA C., PIFFERI A., TARONI P., TORRICELLI A., VALENTINI G., DOVER C., JOHNSON D., RUIZ-ALTISENT M., VALERO C., 2001 - *Nondestructive quantification of chemical and physical properties of fruits by time-resolved reflectance spectroscopy in the wavelength range 650-1000 nm*. - Appl. Opt., 40: 538-543.

ESCUREDO O., SEIJO-RODRÍGUEZA A., GONZÁLEZ-MARTÍN M.I., RODRÍGUEZ-FLORESA M.S., SEIJOA M.C., 2018 - *Potential of near infrared spectroscopy for predicting the physicochemical properties on potato flesh*. - Microchemical Journal, 141: 451-457.

EZEKIEL R., SINGH N., SHARMA S., KAUR A., 2013 - *Beneficial phytochemicals in potato - a review*. - Food Res. Int., 50: 487-496.

FERNANDEZ-OROZCO R., GALLARDO-GUERRERO L., HORNERO-MÉNDEZ D., 2013 - *Carotenoid profiling in tubers of different potato (*Solanum *sp) cultivars: Accumulation of carotenoids mediated by xanthophyll esterification*. - Food Chem., 141: 2864-2872.

GIUSTI M., WROLSTAD R.E., 2001 - *Characterization and measurement of anthocyanins by UV-visible spectroscopy*. - Current Protocols in Analytical Food Chemistry, F1.2.1-F1.2.13.

HEJTMANKOVÁ K., KOTIKOVÁ Z., HAMOUZ K., PIVEC V., VACEK J., LACHMAN J., 2013 - *Influence of flesh colour, year and growing area on carotenoid and anthocyanin content in potato tubers.* - J. Food Comp. Anal., 32: 20-27.

KASPAR K.L., PARK J.S., BROWN C.R., WELLER K., ROSS C.F., MATHISON B.D., CHEW B.P., 2013 - *Sensory evaluation of pigmented flesh potatoes (*Solanum tuberosum *L.).* - Food Nutr. Sci., 4: 77-81.

KITA A., BAKOWSKA-BARCZAK A., HAMOUZ K., KUŁAKOWSKA K., LISINSKA G., 2013 - *The effect of frying on anthocyanin stability and antioxidant activity of crisps from red- and purple-fleshed potatoes (*Solanum tuberosum *L.)*. - J. Food Comp. Anal., 32: 169-175.

KOTÍKOVÁ Z., ŠULC M, LACHMAN J., PIVEC V., ORSÁK M., HAMOUZ K., 2016 - *Carotenoid profile and retention in yellow-, purple- and red-fleshed potatoes after thermal processing*. - Food Chem., 197: 992-1001.

LACHMAN J., HAMOUZ K., MUSILOVÁ J., HEJTMÁNKOVÁ K., KOTÍKOVÁ Z., PAZDERU K., DOMKÁROVÁ J., PIVEC V., CIMR J., 2013 - *Effect of peeling and three cooking methods on the content of selected phytochemicals in potato tubers with various colour of flesh*. - Food Chem., 138: 1189-1197.

LACHMAN J., HAMOUZ K., ORSÁK M., PIVEC V., HEJTMÁNKOVÁ K., PAZDERU K., DVORÁK P., CEPL L., 2012 - *Impact of selected factors - Cultivar, storage, cooking and baking on the content of anthocyanins in coloured-flesh potatoes.* - Food Chem., 133: 1107-1116.

LACHMAN J., HAMOUZ K., ŠULC M., ORSÁK M., PIVEC V., HEJTMÁNKOVÁ A., DVORÁK P., CEPL J., 2009 - *Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity*. - Food Chem., 114: 836-843.

LAKSMIANI N.P.L., VIDYA PARAMITA N.L.P., WIRASUTA I M.A.G., 2016 - In vitro *and in silico antioxidant activity of purified fractions from purple sweet potato ethanolic extract*. - Int. J. Pharm. Pharm. Sci., 8: 177-181.

LIU Y., SUN Y., XIE A., YU H., YIN Y., LI X., DUAN X., 2017 - *Potential of hyperspectral imaging for rapid prediction of anthocyanin content of purple-fleshed sweet potato slices during drying process*. - Food Anal. Methods, 10: 3836-3846.

LÓPEZ A., ARAZURI S., GARCÍA I., MANGADO J., JARÉN C., 2013 - *A review of the application of near-infrared spectroscopy for the analysis of potatoes*. - J. Agric. Food Chem., 61: 5413-5424.

LÓPEZ A., JARÉN C., ARAZURI S., MANGADO J., 2014 - *Estimation of the total phenolic content in potatoes by NIRS*. - Proceedings International Conference of Agricultural Engineering, Zurich, 6 October.

LU W., HAYNES K., WILEY E., CLEVIDENCE B., 2001 - *Carotenoid content and color in diploid potatoes*. - J. Amer. Soc. Hort. Sci., 126: 722-726.

MARTELLI F., DEL BIANCO S., ISMAELLI A., ZACCANTI G., 2009 - *Light propagation through biological tissue and other diffusive media: Theory, solution, and software*. - SPIE Press, Washington, DC, USA, pp. 298.

MAZUREK M., SZOSTAK R., KITA A., KUCHARSKA A.Z., SOKÓŁ-ŁĘTOWSKA A., HAMOUZ K., 2017 - *Determination of antioxidant activity and polyphenols content in chips by Raman and IR spectroscopy*. - Food Anal. Methods, 10: 3964-3971.

MOHSENIN N.N., 1986 - *Physical properties of plant and animal materials*. - Gordon and Breach Science Publishers, New York, USA.

MURNIECE I., KRUMA Z., SKRABULE I., VAIVODE A., 2013 - *Carotenoids and phenols of organically and conventionally cultivated potato varieties.* - Int. J. Chem. Eng. Appl., 4: 342-348.

NAYAK B., DE J., BERRIOS E., POWERS J.R., TANG J., JI Y., 2011 - *Colored potatoes (*Solanum tuberosum *L.) dried for antioxidant-rich value-added foods*. - J. Food Proc. Pres., 35: 571-580.

NICOLAI B.M., DEFRAEYE T., DE KETELAERE B., HERREMANS E., HERTOG M.L.A.T.M., SAEYS W., TORRICELLI A., VANDENDRIESSCHE T., VERBOVEN P., 2014 - *Nondestructive measurement of fruit and vegetable quality*. - Ann. Rev. Food Sci. Technol., 5: 285-312.

NODA N., YOSHIOKA S., KISHIMOTO S., NAKAYAMA M., 2017 - *Generation of blue chrysanthemums by anthocyanin B-ring hydroxylation and glucosylation and its coloration mechanism*. - Sci. Adv., 3: e1602785.

PICCHI V., MIGLIORI C., LO SCALZO R., CAMPANELLI G., FERRARI V., DI CESARE L.F., 2012 - *Phytochemical content in organic and conventionally grown Italian cauliflower*. - Food Chem., 130: 501-509.

RIZZOLO A., VANOLI M., 2016 - *Time-resolved technique for measuring optical properties and quality of food,*

pp. 187-224. - In: LU R. (ed.) *Light scattering technology for food property, quality and safety assessment*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, pp. 439.

RIZZOLO A., VANOLI M., SPINELLI L., TORRICELLI A., 2016 - *Non-destructive assessment of flesh colour in mangoes by time-resolved reflectance spectroscopy: problems and solutions*. - Acta Horticulturae, 1119: 147-154.

SEBBEN J.A., DA SILVEIRA ESPINDOLA J., RANZAN L., DE MOURA N.F., TRIERWEILER L.F., TRIERWEILER J.O., 2018 - *Development of a quantitative approach using Raman spectroscopy for carotenoids determination in processed sweet potato*. - Food Chem., 245: 1224-1231.

SHIROMA-KIAN C., TAY D., MANRIQUE I., GIUSTI M.M., RODRIGUEZ-SAONA L.E., 2008 - *Improving the screening process for the selection of potato breeding lines with enhanced polyphenolics content*. - J. Agric. Food Chem., 56: 9835-9842.

TIERNO R., HORNERO-MENDEZ D., GALLARDO-GUERRERO L., LOPEZ-PARDO R., RUIZ DE GALARRETA J.I., 2015 - *Effect of boiling on the total phenolic, anthocyanin and carotenoid concentrations of potato tubers from selected cultivars and introgressed breeding lines from native potato species*. - J. Food Comp. Anal. 41: 58-65.

TIERNO R., LÓPEZ A., RIGA P., ARAZURI S., JARÉN C., BENEDICTO L., RUIZ DE GALARRETA J., 2016 - *Phytochemicals determination and classification in purple and red fleshed potato tubers by analytical methods and near infrared spectroscopy*. - J. Sci. Food Agric., 96: 1888-1899.

TORRICELLI A., CONTINI D., DALLA MORA A, MARTINENGHI E., TAMBORINI D., VILLA F., TOSI A., SPINELLI L., 2015 - *Recent advances in time-resolved NIR spectroscopy for nondestructive assessment of fruit quality.* - Chemical Engineering Transactions, 44:43-48.

VANOLI M., GRASSI M., SPINELLI L., TORRICELLI A., RIZZOLO A., 2018 - *Quality and nutraceutical properties of mango fruit: influence of cultivar and biological age assessed by Time-resolved Reflectance Spectroscopy*. - Adv. Hort. Sci., 32: 407-420.

VANOLI M., RIZZOLO A., SPINELLI L., AZZOLLINI S., TORRICELLI A., 2016 - *Carotenoid content and flesh colour non-destructively measured by time-resolved reflectance spectroscopy in different cultivars of Brazilian mangoes*. - Acta Horticulturae, 1119: 305-312.

ZAHEER K., AKHTAR M.H., 2016 - *Potato Production, Usage, and Nutrition - A Review*. - Crit. Rev. Food Sci. Nut., 56(5): 711-721.