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Non-destructive detection of potato tubers internal defects: critical insight on the use of time-resolved spectroscopy

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Abstract: Aiming at investigating the feasibility of time-resolved reflectance spectroscopy (TRS) for the non-destructive detection of internal brown spot (IBS) and other defects in 'El Beida' potatoes, 90 tubers were measured in 8 points by TRS for the absorption coefficient at 730 nm (μ_a730) and then transversally cut open for recording presence and position of internal defects and IBS severity. The μ_a730 was lower in healthy tissue than in defected ones and increased with increasing IBS severity with no difference between healthy and slightly IBS tissues. Tubers having at least one out of the eight μ_a730 measures ≥0.04262 cm^{-1} were considered "defected". Therefore, TRS tubers classification performance were: defected, 73.5%; healthy, 45.5%; slightly IBS, 57.1%; moderate IBS, 60%; and severe IBS, 100% of the cases. Misclassification could be due to the high variability in flesh color of 'El Beida' potatoes, as some healthy tubers showed *L, *b** and *C** color parameters very similar to that of defected ones, especially when IBS severity was slight or moderate, resulting in μ_a730 values not significantly different between healthy and IBS tissues. The feasibility of TRS in detecting internal disorders in potatoes must be investigated in other susceptible cultivar to see if flesh color can represent a real problem in the detection of defects linked to browning development.**

1. Introduction

Detecting internal defects (internal brown spot, hollow heart, heat necrosis, black heart) in potatoes (*Solanum tuberosum* L.) is an important challenge for food engineering as potato is one of the main consumed products in the world: potato occupies the fifth position in terms of production after sugarcane, maize, wheat and rice (FAO, 2019).So, it is crucial to ensure tuber quality along the potato supply chain.

The presence of internal defects is not visible until tubers are cut or peeled and determines economic losses in potato industry as growers are not able to separate healthy from defected potatoes, causing waste during processing, and negatively influences consumer confidence. Usually only a representative sample is cut and, if internal defects are present, the whole lot is removed without verifying the real incidence of affected tubers, so increasing food waste. This problem can be tackled by using non-destructive techniques which potentially allow to segregate raw potato tubers according to the actual presence of internal defects, before the product reaches the fresh market or is processed.

Many noninvasive techniques (spectroscopic techniques, computer vision systems, ultrasound methods) have been investigated to assess internal defects in potato tubers with various performance results depending on the type of defect, as reviewed by Rady and Guyer (2015).

Spectroscopic techniques can detect potato defects as changes in absorbance have been found comparing sound and damaged tissues. However, the similarity of absorption characteristics between skin and damaged tissue represents a limiting factor for the segregation of defected tubers. Rady and Guyer (2015) reported that the classification rate of defected tubers by using spectroscopic techniques ranged from 50 to 98%. Recently, a transmission spectrum system in the visible/near infrared region was able to classify blackheart potatoes with an overall classification rate of 96.5% by using six selected wavelengths (711, 817, 741, 839, 678, and 698 nm) (Zhou *et al.*, 2015). Very good performances were reached by using imaging techniques. Infrared Hyperspectral imaging was able to detect hollow heart in 'Agria' potatoes achieving an accuracy of 89.1% of correct classification (Dacal-Nieto *et al*., 2011). Visible-Near Infrared and Short Wave Infrared hyperspectral imaging coupled with PLS-DA (partial least square discriminant analysis) were successfully used to detect black spot in raw tubers of three potato cultivars achieving an overall correct classification rate of 95.5% and 98.6%, respectively (López-Maestresalas *et al*., 2016).

Internal brown spot (IBS) is a physiological disorder of potato tubers which has an important economic impact in Italy. IBS incidence up to 50% has been observed under inductive environmental conditions and in susceptible cultivars (Parisi *et al*., 2014; Pentangelo *et al*., 2017). IBS is characterized by the presence of punctiform and/or enlarged rust-colored necrosis in the parenchymal tissues of the tubers. Irregular-shaped spots already appear in the vascular ring during the tuber bulking growth stage; IBS symptoms increase from the end of tuber filling to the complete tuber maturity (Raimo *et al*., 2018). IBS can affect different areas of the tuber depending on variety. In some cultivar, such as 'Luminella', necrosis areas are localized in the apical position, while in other varieties, such as 'Ricciona di Napoli', the symptoms can affect large portions of the parenchymal tissues, compromising potato tuber appearance and taste and altering the processing features (specific gravity and frying quality) of the tubers (Pentangelo *et al*., 2017; Raimo *et al*., 2018). Positive correlations have been found between IBS incidence and severity with tuber size and skin roughness (Parisi *et al.*, 2014; Raimo *et al.,* 2018). Environmental conditions, soil properties and irrigation rate also strongly affect IBS development, making difficult the prevention, the prediction and the cure of this disorder (Parisi *et al*., 2014; Pentangelo *et al*., 2015, 2017; Raimo *et al*., 2018). In addition, potatoes affected by IBS do not show external symptoms and at present it is not possible to segregate healthy from IBS tubers during mechanical grading and packaging (Parisi *et al*., 2014; Raimo *et al*., 2018). Recently, Vanoli *et al*. (2012) investigated the possibility of using time-resolved reflectance spectroscopy (TRS) to non-destructively detect IBS in 'Luminella' potato tubers, a well-known susceptible genotype.

TRS is a non-destructive optical technique which, in combination with proper models of photon migration, explores the fruit tissue at a depth of 1-2 cm with no or limited influence from the skin, allowing the measurement of the absorption (μ_a) and reduced scattering coefficients (μ_s) (Cubeddu *et al*., 2001; Torricelli *et al*., 2008). The absorption properties are related to the chemical composition (water, pigments), whereas the scattering properties are related to the structure (intercellular spaces, cell size and shape, starch granules). TRS has been mainly applied in postharvest studies for estimating the internal fruit attributes related to maturity in apples, peaches, nectarines, plums, mangoes and pears, for discriminating fruit with different texture and sensory characteristics and for the detection of internal defects in fruits and vegetables (Rizzolo and Vanoli, 2016).

The development of internal disorders induces changes in the optical properties, as absorption increases with browning development (Vanoli *et al*., 2014) and scattering properties vary when a defect affects the fruit structure, as for mealiness, woolliness or watercore (Vanoli *et al*., 2010; Lurie *et al*., 2011; Vangdal *et al*., 2012; Rizzolo and Vanoli, 2016). Comparing healthy and browned fruit, the latter show higher μ_a values in the 670-940 nm range, as found in apples (internal browning), pears (brown heart), peaches (browning), plums (browning) and potatoes (IBS). The highest differences between the absorption spectra of browned and healthy tissues were found in the 670-780 nm range, and hence, these wavelengths were selected to distinguish healthy product from defected ones. High positive correlations were found among μ_a measured at 670 nm (μ_a670) and at 780 nm (μ_a780) and browning scores in plums (Vangdal *et al.,* 2012), nectarines (Lurie *et al.*, 2011) and in apples (Vanoli *et al*., 2014). It was possible to use μ_a750 to distinguish healthy 'Granny Smith' apples from those affected by internal browning with the former being characterized by μ_a750<0.030cm^{-1}; similarly, healthy 'Braeburn' apples had μ_a740<0.030 cm^{-1} while in 'Conference' pears, fruit with μ_a720≤0.034 cm^{-1} were not affected by brown hearth (Eccher Zerbini *et al*., 2002; Rizzolo and Vanoli, 2016). In potatoes, μ_a690 was used to segregate healthy from IBS tubers : tubers having μ_a690 values equal or higher than 0.039 cm^{-1} were considered as IBS and were correctly classified in the 81% of the cases. Healthy tubers showed μ_a690 values of 0.031±0.0032 cm^{-1} (mean±standard deviation) and were all correctly classified by TRS (Vanoli *et al*., 2012). These promising results were limited by the fact that the most part of the tubers had a small size and a round shape making quite easy the detection of IBS measuring each tuber by TRS in correspondence of four equidistant points around the equator.

The aim of this work was to detect IBS in 'El Beida', an oval shaped variety characterized by large size tubers with white flesh, in order to find the most suitable TRS set-up which allow to probe the whole bulk of each potato revealing also the presence of other defects such as necrosis, black spot and bruises.

2. Materials and Methods

Potatoes

Potato tubers cv. El Beida were supplied by a local grower in Bologna province (Italy) who found IBS in some potato samples. All potatoes were washed and dried with a paper towel. Defective samples showing bruises, rots, holes and greening were removed, and 120 tubers were selected for the experiment: 90 tubers were used for defect detection and 30 tubers for flesh color measurements. The tubers for defect determination were labeled, and morphological parameters including weight and diameters (x=longest axis, y= longest axis normal to x; z= longest axis normal to y) were measured. The geometric mean diameter (GMD) and the sphericity of each tuber were calculated according to Mohsenin (1986) as following:

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

$$\text{Sphericity} = \text{GMD}\ x^{-1}$$

Then, each tuber was measured by TRS and cut open for detecting IBS.

Time-resolved Reflectance Spectroscopy (TRS)

Each tuber was measured by TRS for the absorption coefficient at 730 nm (μ_a730), being this wavelength suitable for detecting IBS in potato (Vanoli *et al*., 2012). Considering both biological (large size of 'El Beida' tubers, IBS randomly distributed within the flesh), and the instrumenal characteristics (geometry of the TRS fibers), TRS measurements were performed in two regions of the tuber, at 15 mm distance from the sample center (on the right side-RING1 and on the left side-RING2), rotating the tuber 90° each time (0°, 90°, 180°, 270°) for a total of 8 measurement points.

A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Martinenghi *et al*., 2016) 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 originated 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 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 μ_a and μ_s at each wavelength.

IBS assessment

After TRS measurements, each tuber was transversally cut open in correspondence of the central part of the tuber, and at 15 mm from the center on the right and on the left side where the TRS fibers were positioned. Then, each equatorial section of each tuber was photographed, and the presence and position of internal defects and the IBS severity in correspondence of each TRS measurement point were recorded. Tuber without any visual IBS were considered healthy (H) while those affected by IBS at least in one section out the eight TRS measured sections were considered IBS (IBS).

IBS was also scored according to its severity as slight, moderate and severe considering the size of the tissue affected by IBS and the color intensity of the browned areas.

Flesh color

Thirty tubers were transversally cut open and color was measured on two opposite sides of the flesh 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 ($h°$) and chroma (C^*) were computed according to:

$h°$ = arctangent (b^*/a^*) × 360/(2×3.14) and $C^*=(a^{*2} + b^{*2})^{-2}$.

3. Results and Discussion

On average, 'El Beida' tubers had GMD = 67.2±0.6 mm (mean±SE) and sphericity=0.77±0.05 showing that potatoes studied in this experiment had medium-large size and cylindrical shape (Table 1), and were different from 'Luminella' potatoes used in the previous experiment (Vanoli *et al.*, 2012) which had GMD= 51.8±0.5 mm and sphericity= 0.91±0.01.

IBS was found in 26.7% of the tubers and the other defects were observed in 48.9% of the tubers, while 24.4% of tubers were healthy. Tubers affected by other defects showed small brown or grey spots under the skin and only in one case there was some internal necrosis area.

Table 1 - Morphological characteristics of potato tubers cv. El Beida

	Weight (g)	Diameter x (mm)	Diameter y (mm)	Diameter z (mm)	GMD (mm)	Sphericity
Mean	196.4	87.8	64.8	53.5	67.2	0.77
Min	96.7	65.4	52.1	44.2	54.0	0.67
Max	382.7	116.0	80.6	64.7	83.0	0.88
SD	55.5	11.0	6.3	4.9	6.1	0.04
SE	5.8	1.2	0.7	0.5	0.6	0.005

Table 2 - Absorption coefficient measured by TRS at 730 nm (μ_a730, cm-1) in healthy and in defected tubers and in relation to IBS severity

	Tissue type			IBS severity		
	Healthy	Other defects	IBS	Slight	Moderate	Severe
Mean	0.0375	0.0413	0.0420	0.0386	0.0404	0.0468
Min	0.0204	0.0228	0.0309	0.0309	0.0338	0.0338
Max	0.0542	0.0563	0.0651	0.0454	0.0517	0.0651
SD	0.0042	0.0053	0.0066	0.0033	0.0041	0.0082
SE	0.0002	0.0005	0.0007	0.0007	0.0007	0.0016

Considering the severity of IBS among IBS tubers, 29% showed slight severity, 42% moderate severity and 29% severe symptoms.

In a previous work on IBS detection in potatoes by TRS (Vanoli *et al.*, 2012), healthy and IBS affected tubers were measured in the 540-900 nm range and the highest relative percentage differences between the absorption coefficient values of these tissues were found in the 580-690 nm range, thus μ_a690 was chosen for IBS detection. However, also in correspondence of μ_a730 the relative percentage difference between healthy and IBS tissue was high. So, we choose to measure potato for the absorption coefficient at 730 nm. We select 730 nm also because in the current TRS set-up a higher power was available compared to 690 nm.

The μ_a730 was significantly lower in healthy tissue than in defected ones ($p \leq 0.05$), even if no difference was found between IBS affected tubers and those affected by other defects (Table 2).In IBS tubers, μ_a730 significantly increased with increasing IBS severity; however, no significant difference was found between healthy and slightly IBS tissues (Table 2).

Similarly, μ_a740 and μ_a750 measured in apples affected by internal browning, increased with the development of browning with healthy fruit showing the lowest μ_a740 and μ_a750 values and those affected by brown flesh the highest ones (Vanoli *et al.*, 2010, 2011). In addition, μ_a740 and μ_a750 values increased with increasing browning severity, even if this increase was significant only in fruit with moderate and severe browning while no difference was found between healthy flesh and that affected by slightly browning (Vanoli *et al.*, 2010, 2011).

In order to fix the threshold value of μ_a730 above which a potato tuber can be classified as affected by IBS or by other defects, the mean and the 95% Confidence Intervals of μ_a730 value of defected tissues were computed ($\mu_a730 = 0.04368 \pm 0.00105$ cm^{-1}). Hence, tubers having at least one out of the eight μ_a730 measures equal or higher than 0.04262 cm^{-1} were classified as affected by IBS or by other defects.

Figure1 shows the μ_a730 values for the eight measurement points for each of the 90 tubers under examination.

Only 73.5% of the tubers affected by defects was correctly classified by TRS measurements: 70.8% of tuber affected by IBS and 75.0% of tubers with other defects. Considering IBS potatoes, slightly affected

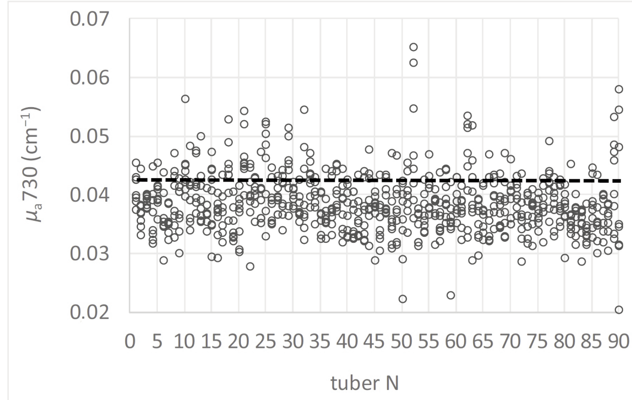

Fig. 1 - Values of the absorption coefficient measured by TRS at 730 nm in each tuber. The horizontal dashed line corresponds to the threshold value of μ_a730 for defect detection.

tubers were correctly classified in 57.1% of the case, moderate IBS in 60% of the cases, while 100% of severe IBS affected tubers were identified. Probably when IBS was slight and moderate, the single brown spots within the potato flesh and/or brown area with slightly brown color make the IBS detection by TRS difficult.

Figure 2 shows some examples of TRS measurements at 730 nm in correspondence of the 8 points (4 points for RING 1 and 4 points for RING 2) around the tuber in comparison with the actual localization of the defect within the flesh. Figures 2A and 2B show two IBS affected tubers correctly classified by TRS: when IBS spot are present, then the μ_a730 values were above the threshold values of 0.04262 cm^{-1} (see points 1, 3, 5 and 7 RING 1 and points 4 and 8 RING 2 for panel A; points 3, 5 and 7 RING 1 for panel B), while in healthy regions, the μ_a730 values were below the threshold values (see points 2 and 6 RING 2 for panel A; points 1 RING 1 and points 2, 4, 6 and 8 RING 2 for panel B). Figure 2C shows that TRS was able to reveal the presence of a bruise spot under the skin as only in point 4 RING 2 the value of μ_a730 was above the threshold of 0.04262 cm^{-1}. However, in panel D of Figure 2 is reported an example of an IBS affected tuber not correctly classified by TRS, as IBS spots are present in correspondence of the points 1 and 5 RING 1 and point 8 RING 2 but all the μ_a730 values are below the threshold of 0.04262 cm^{-1}.

It seems that the detection of defects in 'El Beida' potatoes did not depend on tuber size. Considering the distribution of potato tubers within 4 GMD classes (50-60 mm, 60-70 mm, 70-80 mm, 80-90 mm), 86.5% of the tubers belongs to 60-70 mm and 70-80

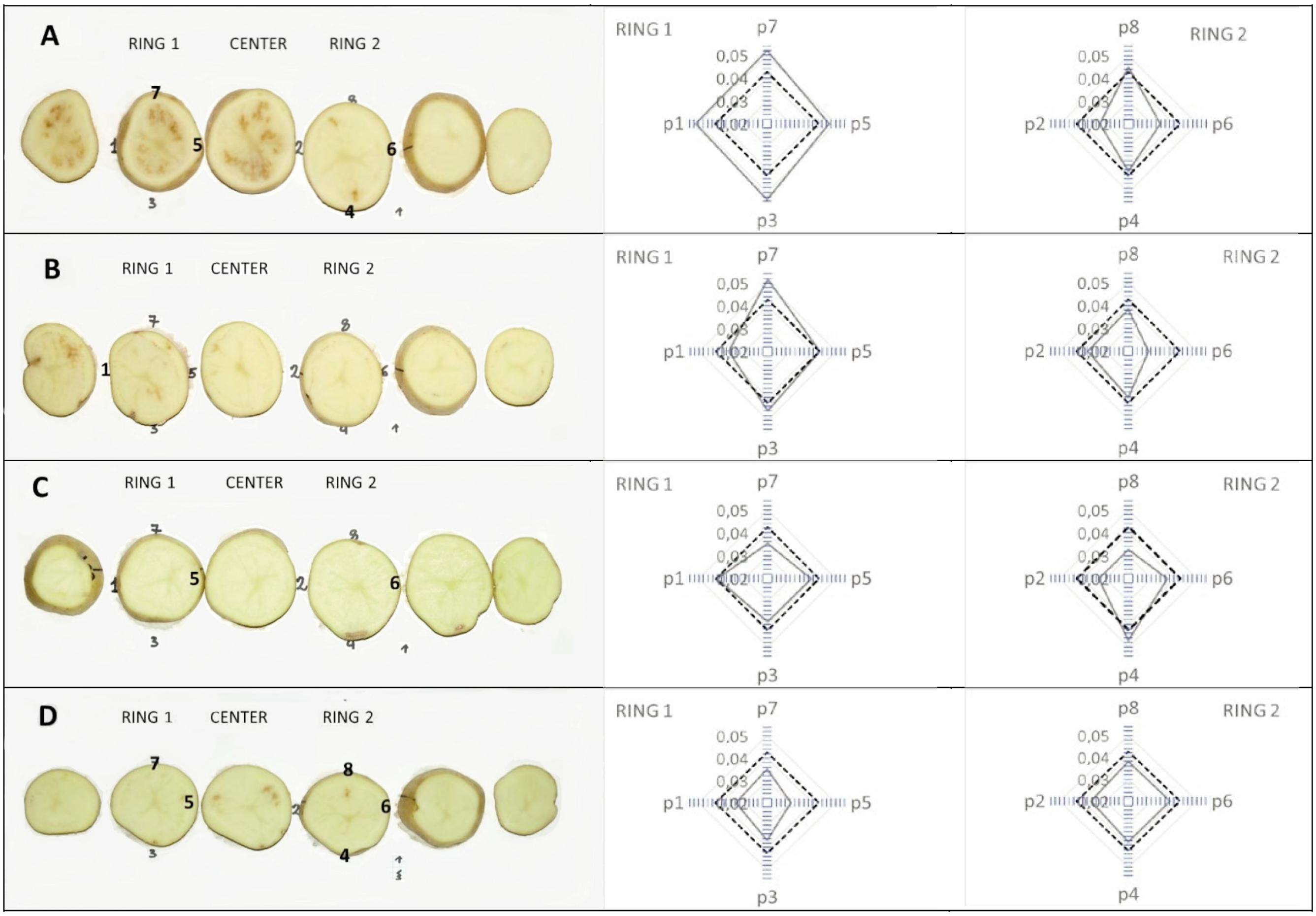

Fig. 2 - Comparison between localization of defects in potato tubers (left) and the corresponding TRS measurements at 730 nm (right). IBS tubers correctly classified by TRS (panels A and B); tuber with bruise correctly classified by TRS (panel C); IBS tuber not identified by TRS (panel D). The dashed lines correspond to the threshold value of μ_a730 for defect detection.

mm classes, where tubers correctly classified as defected by TRS are 70.0% and 75.0%, respectively (Fig. 3).

On the other hand, there were some problems in the identification of healthy tubers, as only 45.5% of healthy tubers was correctly classified by TRS. In this case it can be hypothesized a kind of relationship with tuber size, as the percentage of tuber correctly classified as healthy increased with tuber size, being correctly classified 0% of tuber for 50-60 mm class, 45.5% for 60-70 mm size and 71.1% for 70-80 mm size (Fig. 3). Healthy tubers considered by TRS as affected by defect were characterized by μ_a730 values ≥0.04262 cm^{-1} in at least 1 point out 8 measured ones by TRS (Fig. 4).

This misclassification could be due to differences in the flesh color of potato tubers. In fact, changes in the absorption coefficients measured by TRS in the

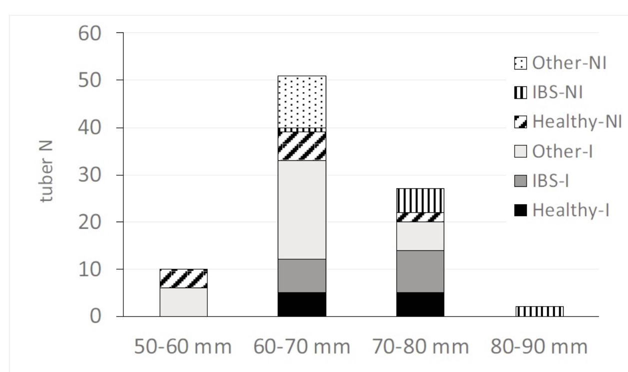

Fig. 3 - Healthy and defected tubers distribution according to 4 GMD classes correctly (I) or not correctly (NI) identified by TRS.

540-780 nm range reveal variations in the flesh color due to the presence of pigments (carotenoids, anthocyanins, chlorophylls) or to browning development.

Good correlations were obtained between TRS absorption spectra and total carotenoids content (R^2_{cv}=0.83 -0.93) and flesh color parameters (R^2_{cv}=0.78-0.96) in different mango cultivars (Vanoli *et al*., 2016).

In fruit affected by browning, high negative correlations were found between μ_a measured at 720 nm ('Conference' pears), 740 nm ('Braeburn' apples), 750 nm ('Granny Smith' apples) and *L** and *h*°, while positive correlations were observed for *a**, *b** and *L** (Eccher Zerbini *et al*., 2002; Vanoli *et al.,* 2010, 2011). Flesh color was significantly different between browned and healthy tissues, showing the former higher *a**, *b**, *C** and lower *L** and *h*° values than the healthy ones (Eccher Zerbini *et al*., 2002; Vanoli *et al.,* 2010, 2011). *L** and *h*° significantly decreased and *a** significantly increased with increasing browning severity, even if no clear distinction was found between healthy and slightly browned tissues.

In this experiment, 30 tubers not used for IBS detection were cut open and flesh color was measured. Six tubers showed IBS: in this case flesh color was measured on browned areas. 'El Beida' potatoes were characterized by a white flesh color (Table 3). As expected, tissue affected by IBS had lower values of *L** and *h*° and higher values of *a**, *b** and *C** than healthy tubers (Table 3), as previously observed in pears affected by brown heart (Eccher Zerbini *et al*., 2002) and in apples with internal browning (Vanoli *et al*., 2010, 2011). Considering healthy potatoes, a high variability in flesh color is observed, with *L**, *b** and *C** values close to those of browned tissues. This scenario could explain why healthy tubers with darker flesh color have been classified by TRS as affected by defects. In the previous work on 'Luminella' potatoes (Vanoli *et al*., 2012), in which all healthy tubers were correctly classified, flesh color was not measured, so it can only be hypothesized that flesh color of this cultivar showed less variation than in 'El Beida' and with values of healthy tissue not so close to slightly affected tubers, making easier the correct tubers classification.

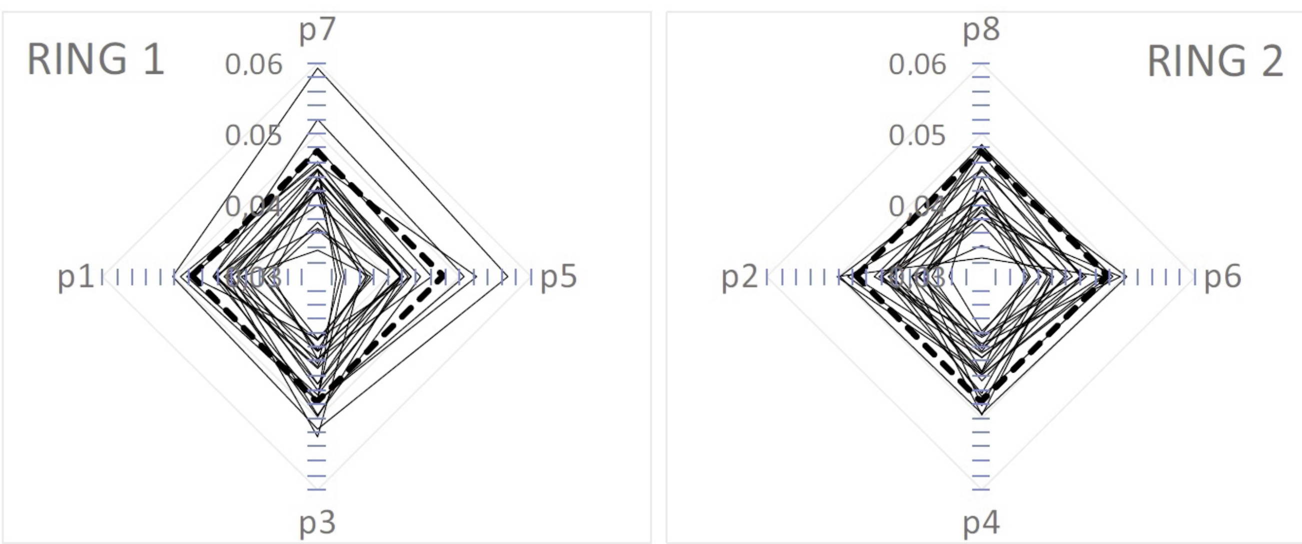

Fig. 4 - Values of the absorption coefficients measured at 730 nm in healthy tubers. The dashed lines correspond to the threshold value of μ_a730 for defect detection.

Table 3 - Color of healthy and IBS potato flesh

	Healthy flesh					IBS flesh				
	*L**	*a**	*b**	*C**	*h*°	*L**	*a**	*b**	*C**	*h*°
Mean	71.78	-2.21	15.80	15.95	97.93	62.96	0.21	19.23	19.27	89.74
Min	66.93	-2.85	14.38	14.49	96.90	56.30	-1.39	17.12	17.19	85.02
Max	74.80	-1.80	19.01	19.21	98.77	68.93	1.82	21.63	21.67	94.57
SD	1.91	0.24	1.04	1.05	0.63	4.28	1.19	1.99	1.98	3.53
SE	0.39	0.05	0.21	0.13	0.13	0.49	0.49	0.81	0.81	1.44

4. Conclusions

Time resolved reflectance spectroscopy has shown to be a feasible tool for detecting internal defects in potato tubers of medium-large size. However, there are some problems to be solved.
First at all, 'El Beida' potatoes showed high variability in flesh color, with some healthy tubers having color very similar to those affected by internal defects and so healthy tubers were misclassified by TRS measurements. On the other side, when IBS severity was slight or moderate, it was difficult to find significant differences between the absorption coefficients measured at 730 nm in healthy and IBS tissues, as flesh color was not so different. So, the feasibility of TRS in detecting internal disorders in potatoes must be investigated in other susceptible cultivar in order to see if flesh color can represent a real problem in the detection of internal defects linked to browning development.

In this study, eight measurement points were used to explore each tuber in a non-destructive way. This TRS set-up allowed to better explore the whole bulk of each potato: in fact, IBS detection did not depend on the tuber size. However, when IBS developed through some small and brown spots, the detection by TRS was very difficult. On the other hand, it's not possible to increase the regions explored by TRS, as when the fibers are positioned too close the tuber ends, the TRS signal is not reliable due to boundaries effect. At boundaries, photon can escape from the tissue and, if it not properly modelled, this might introduce overestimation of the absorption coefficient. The TRS set-up used in this study is based on the contact between the tubers and the optical fibers; the positions and the distance between the fibers determine the volume explored by TRS; perhaps, a non-contact system could allow to better localize the defect, if properly coupled to advanced modelling of the boundaries effect. It is worth noting that these effects can influence classical (not time resolved) NIR spectroscopy system since absorbance estimate are influenced not only by absorption and scattering properties of the tissue but also by the geometrical properties (size and shape).

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