Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

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02. NON-DESTRUCTIVE DETECTION OF INTERNAL DEFECTS IN APPLE FRUIT BY TIME-RESOLVED REFLECTANCE SPECTROSCOPY

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Abstract

In the present research Time-resolved Reflectance Spectroscopy (TRS) technique was tested to detect some internal defects in different apple cultivars. The absorption (μ_a) and the reduced scattering (μ_s') coefficients were measured at different wavelengths in 'Braeburn' (mealiness), 'Granny Smith' (internal browning) and 'Fuji' (watercore) apples. Afterwards, each fruit of 'Granny Smith' and 'Fuji' were cut and evaluated for internal defects. 'Braeburn' apples were submitted to sensory analysis (firm, crispy, mealy and juicy), percent juice and relative intercellular space volume (RISV) determinations. In addition, for 'Granny Smith', the colour of the pulp was measured. 'Granny Smith' sound fruits on average showed μ_a750=0.029 cm^{-1} and μ_s'750=12.2 cm^{-1}; with the development of internal browning, μ_a750 increased to values >0.04 cm^{-1} and μ_s'750 decreased to values <10 cm^{-1} in severely affected fruit. Non-mealy 'Braeburn' apples were characterized by significantly lower μ_s'790, μ_s'912 and higher μ_a912 than mealy ones. Sensory mealiness was positively related to μ_s'790 and μ_s'912 and negatively to μ_a912. The regions of 'Fuji' apples affected by watercore showed significantly higher μ_a790 and lower μ_s'790 values than the healthy regions. Results confirm the suitability of the TRS technique to inspect apples for internal defects, highlighting the need of determining the TRS threshold values as well as the number of measurement points specific to the cultivar and disorder.

Keywords: absorption coefficient, internal browning, mealiness, scattering coefficient, watercore

Introduction

Under specific conditions, apples (*Malus domestica* Borkh.) may develop internal physiological disorders which are only visible after cutting. Among them, internal browning may show symptoms at different locations, related to different factors, such as diffuse flesh browning, radial browning (related to senescent breakdown) and brown heart (related to CO_2 injury). Watercore develops in some cultivars at harvest, often on the sunny side of the fruit; the affected areas look glassy due to the presence of water instead of air in the intercellular spaces. The water-soaked tissue is usually located around the vascular bundles or the core area. Mealiness is an internal quality defect which develops during storage and shelf life, when fruit flesh softens more by the weakening of middle lamella than of cell wall, producing more cell separation than cell breakages. The intact cells are responsible for the dry feeling in the mouth during mastication associated to the mealy texture (Harker *et al.* 2002).

Time-resolved Reflectance Spectroscopy (TRS) is a non-destructive optical technique by which the optical parameters of absorption coefficient (μ_a, related to chemical composition) and reduced scattering coefficient (μ'_s, related to physical parameters) are estimated. The technique explores a banana-shaped volume of tissue at a depth of 1-2 cm depending on the distance between the injecting and collecting optical fibres (Cubeddu *et al.* 2001).

TRS has been successfully used to detect some internal defects in pears, kiwifruits and apples. In 'Conference' pears brown heart was detected by using μ_a690 and μ_a720, while the presence of translucent tissue, related to overripening and bruises, was detected by μ'_s690 (Eccher Zerbini *et al.* 2002). In kiwifruit, by measuring μ'_s630, it was possible to distinguish the sound region of fruit from that affected by *Botrytis*, the latter characterized by the higher translucency, which corresponded to a lower μ'_s630 (Eccher Zerbini *et al.* 2008). As for apples, Vanoli *et al.* (2007) found, for 'Jonagored', that μ'_s780 increased with increasing mealiness while firmness, per cent juice and sensory crispness were negatively correlated to μ'_s780. To identify mealiness in 'Golden Delicious' and 'Cox' apples, Valero *et al.* (2005) studied classification models based on TRS measurements at 672, 750, 818, 900 and 950 nm, with model performances ranging from 47 to 100% of correctly identified mealy versus non-mealy apples.

This work aims at applying TRS as a nondestructive method to inspect apples for internal defects, by studying the relationships between reduced scattering and absorption coefficients and mealiness in 'Braeburn', watercore in 'Fuji', and internal browning in 'Granny Smith' apple.

Material & Methods

Two batches of 'Granny Smith' apples from Laimburg (Bolzano province, Italy) with different sensitivity to internal browning were examined after storage at the end of May 2000. Twenty fruits per batch were used. On the equator (the largest transverse circumference) of each fruit, eight equidistant reference points (A–H) were marked. 'Braeburn' apples harvested on 20 September, 2000 in Toggenburg (Bolzano province, Italy) and stored in normal atmosphere (NA) and ultra-low oxygen (ULO) till the end of March. Thirty fruits stored in NA (mealy) and sixteen stored in ULO (non-mealy) were used. On the equator of each fruit two equidistant reference points were marked. 'Fuji' apples were harvested in Laimburg (Bolzano province, Italy) on 7 November, 2000 (corresponding to the late harvest date), transported to CRA-IAA in Milano. On the equator of 30 fruits four equidistant reference points (A-D) were marked.

The TRS measurements were carried out using the system described in Eccher Zerbini *et al.* (2002) at 670 nm ('Fuji'), 750 nm ('Granny Smith'), 790 nm ('Braeburn' and 'Fuji'), and 912 nm ('Braeburn'). For each of the reference point, four measurements were performed using two optical fibers along the axial direction with an acquisition time of 1 s and averaged. For all the apple cultivars, the average of all points/fruit were computed before submitting the optical data to ANOVA.

'Granny Smith' fruit were cut equatorially, the equatorial section of each fruit was photographed, and the presence and position of disorder in the internal tissue were recorded. Browned fruit were divided into: 'brown core' (BC) when internal browning affected only the core and the flesh was healthy, and 'brown pulp' (BP) when the disorder affected either only the pulp or both the pulp and the core. Colour of the pulp was measured on all the browned apples and on five out of the twenty healthy (H) ones (randomly chosen) at the positions correspondent to the maximum and minimum μ_a750 measured values within the same fruit at a distance of 15 mm from the skin, with a Minolta Chroma Meter CR-300. 'Braeburn' apples were submitted to sensory analysis using a panel of eight semi-trained judges evaluating the intensity of the firm, crispy, mealy, and juicy sensory attributes on an unstructured graphic scale with anchors near the extremes ("low", "high"), to measurement of percent juice (Eccher Zerbini *et al.* 1999) and of relative intercellular space volume (RISV, Baumann & Henze,1983). As for the 'Fuji', the equatorial section of each fruit was photographed and the presence (W: watercore) or absence (H: healthy pulp) of watercore for each measurement point was coupled to its own μ_a670, μ_a790 and μ'_s790; the presence of affected tissue close to the measurement point (HW) was also considered.

Statistical analyses were performed using the SAS/STAT (SAS Institute Inc., Cary, NC, 1999) software package. For all the cultivars, data of TRS measurements were submitted to analysis of variance (PROC GLM) considering the internal disorder as factor. Sensory, percent juice and RISV data of 'Braeburn' apples were submitted to analysis of variance (PROC GLM) considering mealiness as factor. Colour data of 'Granny Smith' apples were submitted to analysis of variance considering the browned region as a factor (PROC GLM). Means were compared by Tukey's test at $P \leq 0.05\%$. Correlations between optical and sensory data ('Braeburn') and optical and colour data ('Granny Smith') were studied using the PROC CORR procedure. Significance of *P-value* and *r* are indicated as follows: ns=not significant;* $P \leq 0.05$; ** $P \leq 0.01$; ***$P \leq 0.001$.

Results

Internal Browning

The μ_a750 increased with the development of internal browning, with H fruits showing the lowest values of μ_a750 and BP ones the highest. On average, μ_s'750 was higher in H and BC than in BP apples (Table 1). Considering the pulp colour, H fruit had the highest hue and the lowest a*, b* and C* values (Table 1).

Table 1. Mean values of μ_a750, μ'_s750 and colour parameters of the pulp in healthy (H) and browned (brown core, BC; brown pulp, BP) 'Granny Smith' apples.

	H	BC	BP	Sign.	Tukey
Optical properties					
μa750 (cm^{-1})	0.029	0.033	0.039	***	c b a
μ's750 (cm^{-1})	12.22	11.78	10.86	***	a a b
Colour parameters					
L*	80.95	79.47	77.21	**	a a b
a*	-4.90	-3.70	-2.13	***	c b a
b*	15.60	18.10	19.03	***	b a a
C*	16.38	18.49	19.15	***	b a a
Hue (degree)	107.48	101.43	96.33	***	a b c

The μ_a750 was positively correlated to a*, b* and C* and negatively correlated to hue and L*, while μ_s'750 was significantly correlated only to a* and L* (Table 2).

Table 2. 'Granny Smith' apples: correlation coefficients of μ_a750 and μ'_s750 with colour parameters.

	μ_a750	μ'_s750
L*	-0.659 ***	0.543 **
a*	0.867 ***	-0.573 **
b*	0.669 ***	-0.221
hue	-0.855 ***	0.502
C*	0.606 **	-0.158

The μ_a750 was able to distinguish H fruit from BP ones, as the former showed μ_a750 values below 0.030 cm^{-1} and the latter values above 0.033 cm^{-1}. When internal browning affected only the core region of the apple, μ_a750 ranged from 0.030 to 0.035 cm^{-1} (Fig 1).

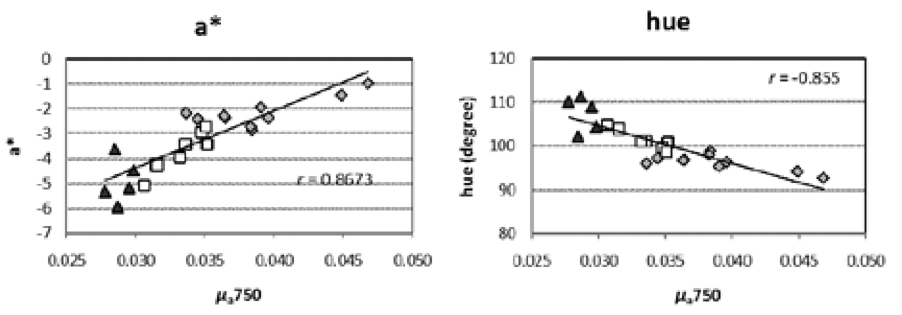

Fig 1. 'Granny Smith' apples: correlations of μ_a750 with a* (left) and hue (right) (H, triangle; BC, square; BP, diamond).

Examples of results of TRS measurements on the eight points around the equator of H, BC and BP fruit are reported in Fig. 2. Point F of μ_a graph for the BP panel has a low value due to an axial zone of pulp with no browning.

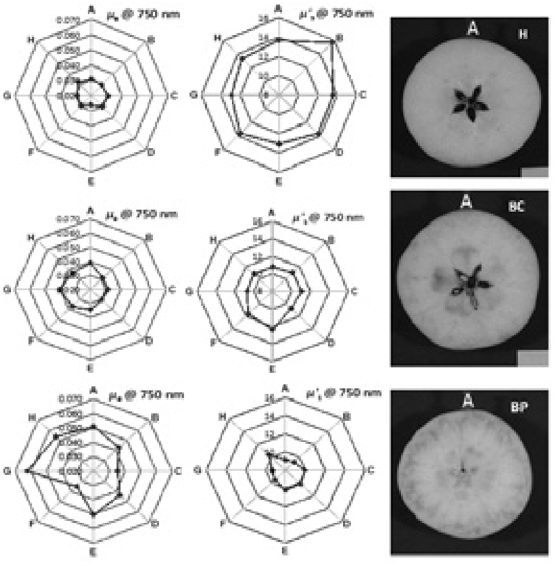

Fig 2. 'Granny Smith' apples: μ_a750 (left) and μ'_s750 (centre) as a function of the position around the equator in a healthy fruit (H), in a fruit affected by brown core (BC) and in a fruit affected by brown pulp (BP). The equatorial sections of fruit are shown in the panels (right). Units for absorption and scattering are cm^{-1}.

Mealiness

Non-mealy 'Braeburn' apples were characterized by significantly lower μ_s'790, μ_s'912 and higher μ_a912 than mealy ones (Table 3).

Table 3. Mean values of μ_a790, μ'_s790, μ_a912, μ'_s912, scores of sensory attributes per cent juice and RISV in non-mealy and mealy 'Braeburn' apples.

	non mealy	mealy	Sign
Optical properties			
μ_a790 (cm^{-1})	0.037	0.037	ns
μ_a912 (cm^{-1})	0.091	0.084	***
μ'_s790 (cm^{-1})	16.41	20.13	***
μ'_s912 (cm^{-1})	16.26	19.47	***
Sensory attributes			
firm	83.9	48.3	***
juicy	81.9	49.6	***
mealy	38.4	70.6	***
crispy	83.0	48.8	***
Quality parameters			
% juice	33.1	17.6	***
RISV(%)	20.7	17.1	***

Mealy apples had significantly lower percent juice and RISV than non-mealy ones, and were described at sensory analysis as less firm, less juicy, less crispy and more mealy than non-mealy fruit (Table 3).

The μ_a790 did not correlate with any of the sensory attributes and quality parameters, while μ_a912 was positively correlated to firmness, crispness, juiciness and percent juice and negatively correlated to mealiness and RISV. The μ_s'790 and μ_s'912 were positively correlated to mealiness and RISV, while they were negatively correlated to the other sensory attributes and percent juice (Table 4). For μ_s'790 and μ_s'912 values above 19 cm^{-1} and for μ_a912 values below 0.09 cm^{-1} mealy, non-crispy and non-juicy apples were found (Fig 3).

Table 4. 'Braeburn' apples: correlation coefficients of μ_a and μ'_s at 790 and 912 nm with sensory attributes and quality parameters (sensory attributes and %juice n=46; RISV n=20).

	μ_a790	μ_a912	μ'_s790	μ'_s912
firm	0.114	0.781	-0.750	-0.742
juicy	0.057	0.746	-0.758	-0.747
mealy	-0.042	-0.720	0.713	0.717
crispy	0.148	0.706	-0.732	-0.734
%juice	0.095	0.759	-0.833	-0.867
RISV	-0.087	-0.664	0.687	0.647

significance of *r*: grey:***; pale grey**

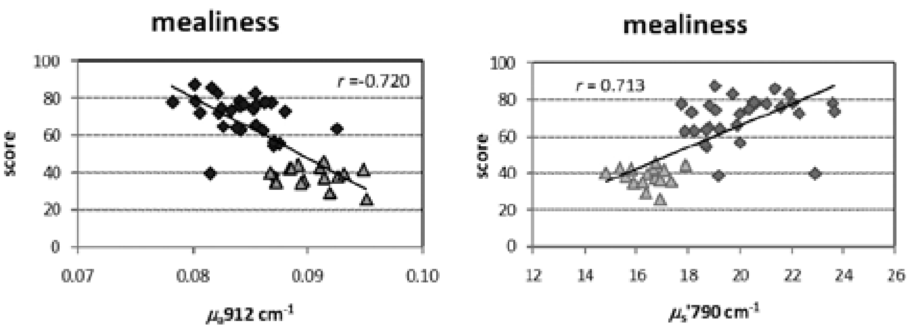

Fig 3. 'Braeburn' apples: correlations of μ_a912 (right) and of μ'_s790 (left) with mealiness (mealy, diamond; non-mealy, triangle).

Watercore

Healthy 'Fuji' pulp was characterized by significantly lower μ_a790 and higher μ_s'790 than zones affected by watercore. If the tissue affected by watercore was near the point of measurement of the optical properties, μ_a790 was on average slightly higher and μ_s'790 slightly lower than the healthy points (Table 5). Examples of TRS results on the four points around the equator of H, HW and W fruit are shown in Fig 4.

Table 5. Mean values of μ_a670, μ_a790 and μ'_s790 in 'Fuji' apples (H, healthy; HW, watercore near the position of measurement; W, watercore).

	H (n=42)	W (n=33)	HW (n=5)	sign	Tukey
μ_a670 (cm^{-1})	0.079	0.096	0.112	*	a a a
μ_a790 (cm^{-1})	0.049	0.059	0.053	***	b a ab
μ'_s790 (cm^{-1})	8.99	8.21	8.68	***	a b ab

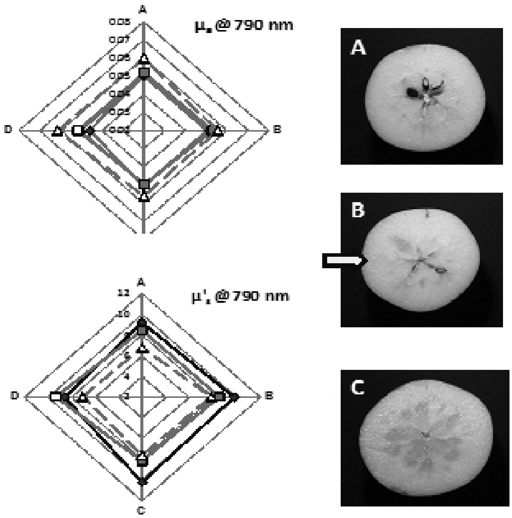

Fig 4. 'Fuji' apples: μ_a790 (top column left) and μ'_s790 (bottom column left) as a function of the position around the equator in an healthy fruit (diamond, H), in a fruit with watercore near the position of measurement (square, HW) and in a fruit affected by watercore (triangle, W). The equatorial sections of fruit are shown in the panels on the right column.

Discussion

Using TRS it was possible to detect some of the internal disorders of apples related to changes in colour (browning) or texture (mealiness, watercore) of the pulp. It was confirmed that internal browning is more related to the absorption coefficient as shown by the high correlation coefficients with colour parameters. In fact, using μ_a750, healthy fruit could be distinguished from those affected by internal browning both in the core and in the pulp, whereas using μ'_s750 fruit affected by internal browning in the core could be classified as healthy.

The positive correlation between μ_s' and mealiness, already found in 'Jonagored' apples (Vanoli *et al.* 2007) was confirmed. The threshold value above which only mealy 'Braeburn' apples are found is μ_s'790>19 cm^{-1} vs μ'_s780>11 cm^{-1} found by Vanoli *et al.* (2007) for 'Jonagored', underlining the need of determining the TRS threshold values specific to the cultivar. Moreover, in this work, high correlations were also found between mealiness and the optical properties measured at 912 nm, near the absorption peak of water.

Tissue affected by watercore can be distinguished by the healthy ones using both μ_a790 and μ_s'790. Differently from what found in pears (Eccher Zerbini *et al.* 2002) and kiwifruits (Eccher Zerbini *et al.* 2008), where using the absorption coefficient measured near the chlorophyll peak (690 and 630, respectively) it was possible to detect translucent tissue, in this experiment no relationships were found between μ_a670 and watercore.

Our results stressed that to make certain the detection of the defect, the number of measurement points has to be suited to the localization and distribution of the affected tissue. In fact, to detect internal defects involving all the pulp, such as mealiness, two measurement points were sufficient, while to detect defects involving only part of the pulp, such as watercore, four points at a distance of 90° were not enough.

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