# Studies on classification models to discriminate 'Braeburn' apples affected by internal browning using the optical properties measured by time-resolved reflectance spectroscopy

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**Abstract**

During storage ‘Braeburn’ apples can develop Internal Browning (IB), a physiological disorder asymmetrically distributed within the fruit flesh, which is visible only when fruit are cut open. This work aimed at studying the optical properties non destructively measured by time-resolved reflectance spectroscopy (TRS) in intact ‘Braeburn’ apples in relation to the IB development, and at obtaining classification models based on absorption ($\mu_a$) and reduced scattering ($\mu_s$’) coefficients in order to discriminate healthy fruit from IB ones.

This research was carried out in 2009 and in 2010. In both years ‘Braeburn’ apples were picked at commercial harvest and stored up to 6 months in IB inducing (BAD ) and in optimal (OPT) storage atmospheres. In 2009, after 3 and 6 month’s storage, apples were measured by TRS at 670 nm and in the spectral range 740-1100 nm on four equidistant points around the equator, while in 2010, after 4 and 6 month’s storage, apples were measured by TRS at 670 nm and at 780 nm on eight equidistant points around the equator. In both years, flesh firmness was analyzed for each fruit and in 2010 also the largest equatorial diameter was measured. Apples were cut open equatorially; the presence, the position (BC-core; BP-pulp) and the severity of IB (H-healthy, slight, moderate, severe) in correspondence of each TRS measurement point were recorded.

In 2009 IB development significantly affected the $\mu_a$ values in the 670-940 nm range, while its effect on scattering spectra was opposite at 3 and 6 months of storage.

In both years, $\mu_s$’780 was higher in healthy fruit than in IB ones, while $\mu_a$780 was higher in IB fruit than in H ones, significantly increased with IB severity, and was higher in BP than in BC tissues.

The $\mu_a$780 was also higher in 2010 than in 2009, and in BAD stored apples than in OPT ones due to the higher incidence and severity of IB in both these cases.

The $\mu_a$670 also changed with IB development, but it was not able to clearly discriminate H fruit from IB ones because its value was also influenced by the chlorophyll content of the pulp, reflecting the maturity degree of the fruit, which was more advanced in 2009 when $\mu_a$670 and firmness were lower than in 2010.

The absorption and reduced scattering coefficients were used as explanatory variables in the Linear Discriminant Analysis in order to classify each apple tissue as H or IB and then to use the obtained model for fruit classification. The best classification performance was obtained in 2010 when 8 TRS measurements at 780 nm were carried out considering the IB position within the fruit: 90% of H fruit and 71% of IB fruit (adding BC+BP fruit) were correctly classified. In 2009 by using all the absorption coefficients plus the $\mu_s$'780 it was possible to enhance IB fruit classification (76%) but H fruit were well-classified only in 71% of the cases, while the model based only on the optical properties at 780 nm correctly classified H and IB fruit in 71% of the cases. IB detection was not affected by the fruit size. Probably is it the asymmetrical distribution typical of the IB developed by 'Braeburn' apples that makes the detection of this defect difficult. Eight TRS measurements carried out around the fruit equator allowed to better exploring the fruit flesh compared to the 4 points. However, 8 points could be not enough if the disorder is localized in the inner part of the fruit (core) or when it occurs in spots. A different TRS set-up (position and distance of fibers, time resolution) should be studied in order to reach the deeper tissue within the fruit in order to improve browning detection.

**Keywords: max 6**

Internal browning, absorption coefficient, reduced scattering coefficient, apple, models, non destructive technique

## 1. Introduction

During storage 'Braeburn' apples can develop Internal Browning (IB), a physiological disorder affecting the fruit flesh. IB is characterized by browned areas extending out from the core into the cortex often in an asymmetric spatial distribution which can also be accompanied by cavities (Elgar et al., 1998). Initially browning areas tend to be concentrated in the calyx end of the fruit and in the mid cortex, and in severely affected fruit they are visible throughout the cortical tissues. Cavities may be present within the brown tissue regions of either the core or cortical areas of the fruit, being generally dry when cut, and presumably forming when the brown tissues become dehydrated (Elgar et al., 1998).

The incidence and the severity of IB varies markedly from year to year and is affected by orchard factors as well as by postharvest conditions. The incidence of IB was higher in late than in early-harvested -fruit, in fruit on light than on heavily cropping trees and in apples with high K and P content or with high K/Ca ratio (Lau, 1998; Elgar et al., 1999; Neuwald et al., 2008). IB is a $CO_2$-related injury: its incidence is associated with high $CO_2$ partial pressure in the storage room and can be exacerbated by decreased $O_2$ and can be reduced or eliminated by delayed controlled atmosphere (CA) storage (Elgar et al., 1998; Lau, 1998; Saquet et al., 2003; Neuwald et al., 2008; Ho et al., 2013).

The susceptibility of 'Braeburn' apples to IB is related to their structural characteristics, as they have a relative dense and firm tissue, poor flesh gas diffusivity and low skin gas permeance (Dražeta et al., 2004; Schotsmans, 2004; Mendoza et al., 2007; Defraeye et al., 2013). Herremanns et al. (2013) studying the microstructure of the inner, middle and outer cortex of 'Braeburn' apples by X-ray micro-tomography, found differences in relation to storage conditions and to IB development. In a healthy 'Braeburn' apple, the overall porosity and the pore connectivity of the inner cortex was lower than that of the middle and outer cortex, hindering gas exchanges. During optimal storage, as a consequence of the loss of cell-to-cell adhesion typical of ageing process, previously present pores are connected so forming larger pores. On the contrary, IB development

dramatically altered tissue structure: there was a drastic 'closing' of the microstructure in the inner and middle cortex due to the disappearance of the open intercellular air space, while the outer cortex tissue, close to the apple skin, seems to remain largely unaffected. IB tissue appeared extremely dense and can be further destroyed, leaving large cavities. All these microstructural changes caused a further decrease in the local $O_2$ concentration and an increase in the $CO_2$ concentration altering fruit metabolism; as a consequence, cells cannot maintain membrane integrity and leakage of the cell content occurs leading to flooding of the pores, possibly followed by further collapse of the tissue structure and formation of cavities (Dražeta et al., 2004; Lee et al., 2012; Herremanns et al., 2013; Vandendriessche et al., 2013).

The unpleasant nature of IB is not acceptable to consumers and causes economic losses. Unfortunately, external symptoms are not evident, except when fruit are very badly affected. Consequently, a reliable non-destructive method for detecting and removing fruit with internal browning would be readily accepted by apple industry.

Vis/NIR spectroscopy has been shown great potential in inspecting brown heart in apples and pears (Clark et al., 2003; McGlone et al., 2005; Han et al., 2006; Fu et al., 2007). Fu et al. (2007) compared transmission and reflectance modes of Vis/NIR spectroscopy for detecting brown heart in 'Xueqing' pears, concluding that transmission mode was more suitable than reflectance mode for classifying fruit with brown heart from sound ones as light must pass right through the fruit to detect hidden internal defects. Clark et al. (2003), examining 'Braeburn' apples affected or not affected by brown hearth by using transmission NIR spectroscopy, found that sample orientation and degree of browning were significant factors in the design of online detection systems: the best model was obtained by averaging spectra from opposite sides of the fruit where the stem-calyx axis was horizontal and the light source and detector were located at right angles to one another at the equator. Two prototype on-line NIR transmission systems were constructed and tested by McGlone et al. (2005) demonstrating that an accurate measurements of the percentage of IB tissue in 'Braeburn' apples can be obtained moving at realistic grading speed (500 mm $s^{-1}$).

Also time-resolved reflectance spectroscopy (TRS) showed interesting results in the detection of internal disorders, as it nondestructively measures the internal properties of fruits (Torricelli et al., 2008). In TRS a short pulse of monochromatic light is injected within a diffusive medium. Following the injection of the light pulse, the temporal distribution of the re-emitted photons at a distance ρ from the injection point will be delayed, broadened and attenuated. The delay is a consequence of the finite time that light takes to travel the distance between source and detector; broadening is mainly due to the many different paths that photons undergo because of multiple scattering; attenuation appears because absorption reduces the probability of detecting a photon, and diffusion into other directions within the medium decreases the number of detected photons in the considered direction. By applying a proper theoretical model, the absorption coefficient $\mu_a$ (units are typically $cm^{-1}$) and the reduced scattering coefficient $\mu_s$' ($cm^{-1}$) can be accurately estimated. Chemical constituents in the fruit such as pigments, water, soluble solids affect the $\mu_a$, while fruit density, cell size, middle-lamella, intra- and extracellular characteristics are likely to affect the $\mu_s$'. The volume probed by a TRS measurement is a 'banana-shaped' region connecting the injection and collection points, thus the measured coefficients roughly correspond to the average of the optical properties in this region. It is not easy to define the measurement volume since the photon paths are more densely packed in the banana region, but can be distributed in the whole medium. A series of measurements was performed on apples (Cubeddu et al., 2001b) and pears (Eccher Zerbini et al., 2002) to determine the maximum depth in the tissue that yields a detectable contribution to the TRS curve and it was concluded that for both fruits TRS measurement probes a depth of at least 2 cm in the pulp. Although this is not a direct estimation of the penetration depth of this technique, it proves that TRS is not confined to the surface of the fruit. This was also confirmed by Saeys et al. (2008) which compared the optical properties measured by NIR in the skin and in the flesh of three apple cultivars with those obtained by TRS on intact fruit, highlighting how the optical properties measured by TRS are dominated by the flesh characteristics. The penetration depth reached by TRS depends on the optical properties of the fruits, as we expect deeper penetration where absorption

149 and/or scattering are lower, but also on the source-detector distance (Cubeddu et al., 2001b;
150 Torricelli et al., 2008).
151 Both $\mu_a$ and $\mu_s'$ can be involved in the detection of browning disorders in apples and pears.
152 'Braeburn' apples affected by brown heart had significantly higher $\mu_a$ values in the 740-1000 nm
153 spectral range than healthy ones (Vanoli et al., 2011b). The $\mu_a$ measured at 740 nm ($\mu_a740$) showed
154 increasing values with decreasing $L^*$ values in the pulp (i.e. increasing browning): $\mu_a740$ <0.038
155 $cm^{-1}$ indicated healthy pulp, whereas $\mu_a740$>0.08 $cm^{-1}$ distinguished severely browned pulp
156 (Vanoli et al., 2011b). 'Granny Smith' apples showed an increase of $\mu_a750$ values with the
157 development of internal browning with healthy fruit having $\mu_a750$<0.030 $cm^{-1}$ and browned apples
158 $\mu_a750$>0.033 $cm^{-1}$; furthermore, severely affected fruit showed also a decrease of $\mu_s'750$ to values
159 <10 $cm^{-1}$ (Vanoli et al., 2010). The presence of brown heart in the pulp of 'Conference' pears
160 caused an increase in the $\mu_a$ values from 710 to 850 nm with brown tissue showing $\mu_a720$>0.04
161 $cm^{-1}$ while the $\mu_s'720$ significantly changed with the presence of bruises in the pulp tissue (Eccher
162 Zerbini et al., 2002).
163 Similarly to what found in apples and pears, TRS has been successfully used in the detection of
164 chilling injuries in nectarines and plums (Lurie et al., 2011; Vangdal et al., 2012). In 'Morsiani 90'
165 nectarines, $\mu_a780$ was able to differentiate between healthy and fruit with either woolliness, internal
166 browning or internal bleeding. In 'Jubileum' plums, $\mu_a670$ and $\mu_a780$ increased with the
167 development of jellying and browning, allowing to distinguish healthy fruit from those affected by
168 internal disorders and the slightly browned fruit from those with medium and severe browning.
169 This work aimed at studying the optical properties measured by TRS in intact 'Braeburn' apples in
170 relation to the IB development, and at obtaining classification models based on absorption and
171 scattering coefficients in order to discriminate healthy fruit from IB ones.
172

173 **2. Materials and methods**

174 *2.1. Apple fruit*

Apples (*Malus x domestica* Borkh.) cv. 'Braeburn' were used.

In 2009 apples were picked on October 26$^{th}$ which was considered to be the optimal commercial harvest date for long-term commercial storage for Belgium, as determined by the Flanders Centre of Postharvest Technology (VCBT). Afterwards, apples were stored at 1°C under two types of controlled atmospheres: browning inducing storage conditions (BAD storage: 1% $O_2$, 5% $CO_2$) and optimal storage conditions (OPT storage: 2.5% $O_2$, 0.7% $CO_2$ with a 3-week delay of CA to prevent IB development). After 3- and 6-month storage, at arrival in the laboratory of Politecnico in Milan, sixty apples/storage atmosphere were measured by TRS at 670 nm and in the spectral range 740-1100 nm on four equidistant points (0°, 90°, 180°, 270°) around the equator (the largest transverse circumference).

In 2010, two cultivation treatments were applied: optimal fertilization (OPT fert: 30 kg/ha calcium nitrate, 20 kg/ha phosphorus, no potassium) and suboptimal fertilization (BAD fert: 30 kg/ha ammonium nitrate, 20 kg/ha phosphorus, 80 kg/ha potassium). The fertilization was applied on March 24$^{th}$ 2010. The apples, picked on October 27$^{th}$, were stored at 1°C under BAD and OPT atmosphere using the same gas composition of 2009. These pre- and postharvest treatments resulted in four batches of apples, which were labeled GG, BB, GB and BG: the first letter indicates the storage conditions and the second one indicates the fertilization type, where G is used for optimal (OPT) conditions and B for suboptimal (BAD) conditions. After 4- and 6-month storage, at arrival in the laboratory of Politecnico in Milan, thirty apples/treatment were measured by TRS at 670 nm and at 780 nm on eight equidistant points (0°, 45°, 90°, 135°, 180°, 225°, 270°, 315°) around the equator.

In both years, after the TRS measurements flesh firmness was analyzed for each fruit and in 2010 also the largest equatorial diameter was measured. Then, apples were cut open equatorially; the equatorial section of each fruit was photographed, and the presence, the position and the severity of IB in correspondence of each TRS measurement point were recorded. Considering IB position within fruit, browned fruit were divided into: brown core (BC), when at least in one section out of

the four (2009) or eight (2010) TRS measured sections IB affected only the core and the flesh was healthy; brown pulp (BP), when the disorder affected either only the pulp or both the pulp and the core at least in one section out of the four (2009) or eight (2010) TRS measured sections. IB was also scored according to its severity as: healthy, slight, moderate and severe. Also the presence of cavities alone (CV) or associated to IB in the core (BCCV) or in the pulp (BPCV) was considered.

*2.2 TRS measurements*

In 2009, TRS measurements were performed at 670 nm and in the spectral range 740-1040 nm (at 40 nm intervals). The broadband setup employed a white light laser (SC450, Fianium Ltd., UK) for generation of light pulses (10 ps duration, 40 MHz repetition rate, 1 mW/nm average power), a watercooled double microchannel plate photomultiplier (R1564U, Hamamatsu Photonics, Japan), and a time-correlated single photon counting board (SPC-130, Becker & Hickl GmbH, Berlin, Germany). The temporal resolution of the overall system, calculated as the FWHM of the instrumental response function (IRF), was <90 ps. Details on the setup can be found in D'Andrea et al. (2009).

In 2010, measurements were performed by a portable prototype for TRS measurements at discrete wavelengths. The light source was a pulsed laser diode (model PDL800, PicoQuant GmbH, Germany) working at 780 nm, with 80 MHz repetition frequency, 100 ps duration, and 1 mW average power. A compact photomultiplier (model R5900U-L16, Hamamatsu Photonics, Japan) and an integrated PC board for time-correlated single photon counting (model SPC130, Becker & Hickl GmbH, Germany) were used to detect TRS data. Typical acquisition time was 1 s per point. A couple of 1 mm plastic fibers (model ESKA GK4001, Mitsubishi, Japan) delivered light into the sample and collected the emitted photons. Overall, the IRF duration was <180 ps. A detailed description of the system can be found in Cubeddu et al. (2001a,b).

For both systems a home-built holder allowed the fibers to be positioned 1.5 cm apart, parallel to each other, normal to and in contact with the sample surface. A model for photon diffusion in turbid

media was used to analyse TRS data to assess the bulk optical properties (absorption coefficient, $\mu_a$, and reduced scattering coefficient, $\mu_s'$) of samples at each wavelength (Martelli et al. 2009). Convolution of the photon diffusion model with the IRF is performed before fitting the experimental data (Cubeddu et al. 1996).

*2.3 Fruit diameter*

The largest equatorial diameter of each fruit was measured by a digital caliper.

*2.4 Flesh firmness*

Firmness was measured with a 11 mm diameter plunger mounted on an Instron Universal Testing Machine Model 4301 (Instron Ltd, High Wycombe, UK) with crosshead speed of 480 mm/min to a depth of 8 mm. Two measurements were recorded per fruit, on two peeled areas on opposite sides of the equatorial region of the apple and the average value was considered.

*2.5 Statistical analysis*

TRS optical properties and firmness data were submitted to analysis of variance (ANOVA) considering fertilizer treatment, storage atmosphere, storage time and fruit type (healthy or browned) as factors and means were compared by Tukey's test at $P \leq 0.05$. TRS optical properties of each fruit section were submitted to ANOVA considering IB position and severity as factors and means were compared by Bonferroni's test at $P \leq 0.05$.

In 2009, classification models were developed using TRS absorption coefficients in the 670-1040 nm spectral range and scattering coefficient measured at 780 nm as explanatory variables in the Linear Discriminant Analysis (LDA) in order to discriminate between browned and healthy tissues. Classification functions were estimated with a stepwise approach, selecting or removing each variable in order to evaluate the contribution of the respective variable to the discriminatory power

of the model. The discriminatory ability of the models was evaluated by comparing the percentage of well-classified samples obtained with every model.

In 2010, classification models were developed using the absorption and scattering coefficients measured at 780 nm.

In both years, in a first approach fruit tissues were classified into two classes according to the tissue type: healthy (H) or browned (IB). Then, in order to understand whether the optical properties measured by TRS can discriminate fruit having different browning positions, tissue were also classified into three classes: healthy (H), brown core (BC) and brown pulp (BP). Then, the models developed on tissues were applied for fruit classification. To classify fruit as H/IB or as H/BC/BP, in order to have more probability to find IB, the highest $\mu_a$780 value (and the corresponding $\mu_a$ values measured at the other wavelengths in 2009 and the corresponding $\mu_s$'780 in both years) out of the four (2009) or the eight (2010) sectors measured by TRS was considered, as $\mu_a$780 was the absorption coefficient that well discriminated between healthy and brown tissues (see Results).

All statistical analyses were performed by Statgraphics version 7 (Manugistic Inc., Rockville MD, USA).

## 3. Results

### *3.1. Year 2009*

#### *3.1.1. Flesh firmness*

Flesh firmness was significantly higher in apples stored under BAD (76.1 ± 1.6N, mean ± standard error) than under OPT conditions (63.3 ± 0.7N) and in IB apples than in H ones but only after 6-month storage (3-month: H=71.8 ± 1.3N, IB=71.7 ± 3.1N; 6-month: H=63.2 ± 1.4N, IB=71.3 ± 2.2N). Firmness significantly decreased with storage time only in H fruit.

#### *3.1.2. Incidence of Internal Browning*

277 Storage atmosphere strongly affected IB incidence (Fig. 1, left). As expected, the incidence of IB
278 was higher in BAD stored apples, where dramatically increased with storage time, reaching 85% of
279 browned apples after 6 months' storage. IB was mainly localized in the core region showing a slight
280 and moderate severity; with storage time brown pulp incidence and severity increased.
281 In contrast, OPT apples did not developed IB at 3-month storage; after 6-month storage they
282 showed only 25% of IB apples and IB was mainly localized in the core region with a slight severity
283 (Fig. 1, right).
284 BAD apples showed also cavities, which were associated to IB, already after 3-month storage and
285 the incidence increased with storage time. OPT apples showed CV only after 6-month storage and
286 with a very low incidence (3% of apples affected) (Fig. 1, left).
287
288 *3.1.3 TRS spectra*
289 Overall, the absorption spectra (Fig. 2, left) showed two maxima: the first one at 670 nm,
290 corresponding to chlorophyll-*a* absorption and the second one at 980 nm, corresponding to water
291 (Cubeddu et al., 2001a); the reduced scattering coefficient spectra in the 740-900 nm (where there
292 was no influence due to pigments or water) are rather flat (Fig. 2, right). All the absorption
293 coefficients were significantly affected by storage atmosphere, while storage time influenced the $\mu_a$
294 values in the 820-1040nm range and IB presence affected the $\mu_a$ values measured in the 670-940nm
295 range. The scattering coefficients measured at 740, 780, 820, 860 and 900 nm were significantly
296 influenced by storage conditions and by storage time, while browning development affected in a
297 different way the scattering spectra according to storage time (Fig. 2, right).
298 In Table 1 and in Fig. 3, the $\mu_a$ values measured at 670, 780 and 980 nm and the values of $\mu_s$'780
299 are reported. We have chosen to show the values of $\mu_a$670 and $\mu_a$980, as they correspond to the two
300 maxima of TRS spectra and the values of $\mu_a$780 and of $\mu_s$'780, as at this wavelength both these
301 optical coefficients are involved in browning development (Eccher Zerbini et al., 2002; Lurie et al.,
302 2011; Vanoli et al., 2010 and 2011b; Vangdal et al., 2012).

On the average, $\mu_a$670 and $\mu_a$780 were significantly higher in BAD stored apples than in OPT ones and in IB fruit comparing to H ones (Table 1). The $\mu_a$980 was significantly higher in BAD stored apples and decreased with storage time (Table 1), probably due to some water loss (Vanoli et al., 2011a). The $\mu_s$'780 was significantly lower in BAD stored apples than in OPT ones and increased with storage time; $\mu_s$'780 was also higher in H than in IB fruit after 6 months' storage; the opposite was found after 3 month's storage (Table 1).

Optical properties changed also with the IB position and severity within fruit (Fig. 3). The $\mu_a$780 showed the lowest values in H tissues, was higher in BP than in BC tissues and increased with increasing IB severity. The simultaneous presence of cavities further increased $\mu_a$780 values but only when IB was localized in the core region. The $\mu_a$670 showed similar values for H and BC/BCCV tissues, it significantly increased in BP/BPCV tissues and in severe IB ones. The $\mu_a$980 had the lowest values when IB affected pulp tissues and cavities were also present. The $\mu_s$'780 was significantly lower in BC tissue than in H ones, and did not significantly differ for BP, BCCV and BPCV tissues.

*3.1.4. Linear Discriminant Analysis and classification models*

The absorption coefficients measured at 670 nm and in the 740-1040 nm range and the $\mu_s$'780 were used as explanatory variables in the Linear Discriminant Analysis in order to classify each apple tissue as healthy or browned and then to use the obtained model for fruit classification.

By analyzing 591 H tissues and 357 IB ones, the best classification performance was obtained using all the absorption coefficients plus the $\mu_s$'780. The obtained discriminant function (DF) (canonical correlation of 0.465, $P$<0.00001) had the highest standardized coefficient for $\mu_a$780 (0.731), followed by $\mu_a$670 (−0.654), $\mu_a$820 (0.502) and $\mu_s$'780 (−0.430), allowing to well-classify 75.1% of apple tissues (95.4% of H but only 41.5% of IB ones). However, when this model was used to classify apple fruit, H fruit were well-classified in 70.7% of the cases and IB ones in the 75.8% of the cases (Table 2).

As $\mu_a$780 showed the highest standardized coefficient in the DF and also $\mu_s$'780 was crucial in the development of the tissue classification model, a new model was built by using as explanatory variables $\mu_a$780 and $\mu_s$'780. The obtained DF (canonical correlation of 0.390, $P$<0.00001) allowed to correctly classify 71.9% of the apple tissues, actually 99.7% of H tissues but only 26% of IB ones. Nevertheless, when this model was used to classify apple fruit, H and IB fruit were well-classified in 70.7% of the cases (Table 2).

In order to better classify both H and IB fruit, two other models were developed considering the IB position within the fruit (BC or BP). The first model was based on all the absorption coefficients plus $\mu_s$'780 and analyzed 591 H, 266 BC and 91 BP tissues. Two DF were obtained: the first DF (92.9% of the variance, with a significant [$P$<0.0001] canonical correlation of 0.633) had the highest standardized coefficient for $\mu_a$780 (0.608), followed by $\mu_a$740 (0.581), $\mu_a$670 (−0.371) and $\mu_s$'780 (−0.252), whereas the second DF (7.1% of the variance, with a significant [$P$<0.0001] canonical correlation of 0.221) had higher coefficients for $\mu_a$740 (−1.090), $\mu_a$670 (−0.868), $\mu_a$820 (0.989) and $\mu_s$'780 (−0.589). H tissues were well classified in 96.3% of the cases, BC tissues in 22.6% of the cases and BP in 41.8% of the cases. Misclassified H tissues were considered BC; misclassified BC tissues were considered H in 74.1% of the cases, whereas misclassified BP tissues were considered H only in 38.5% of the cases. Applying this model to apples, H fruit were correctly classified only in 56.4% of the cases, BC fruit were classified as IB in 80.2 % of the cases and BP fruits were considered IB in 92.6% of the cases (Table 3).

A second model was built using $\mu_a$780 and $\mu_s$'780 as explanatory variables. One DF was obtained (canonical correlation of 0.587, $P$<0.0000) which well classified all H tissues, but only 1.5% of BC and 39.1% of BP tissues. Applying this model to apples (Table 3), H fruit were well-classified only in 44.3% of the cases, BC fruit were well-classified in 61.1% of the cases and BP fruit in 74.1% of the cases. Misclassified H fruit were all considered BC, while BC and BP fruit were considered H in 19.4% and 7.4% of the cases, respectively.

355 *3.2 Year 2010*

356 *3.2.1. Flesh firmness*

357 In 2010, flesh firmness was significantly affected only by storage atmosphere being BAD stored
358 apples firmer (91.8 ± 0.8N) than OPT ones (82.3 ± 0.8N). Firmness slightly decreased with storage
359 time (4 months: 89.4 ± 0.8N, 6 months: 84.6 ± 1.0N) and slightly increased with IB development (H
360 fruit: 85.0 ± 1.3N, IB fruit: 87.4 ± 0.7N).

361

362 *3.2.2. Fruit diameter*

363 Maximum equatorial diameter (MED) was 74.0 ± 0.3 mm (mean±standard error); the lowest value
364 was 63.3 mm and the highest 88.3 mm. MED was not affected by fertilization treatments, storage
365 conditions (atmosphere, time) and browning development, but it significantly changed considering
366 IB position, showing the lowest values in H fruit and the highest in BP ones (H=72.5 ± 0.6 mm;
367 BC=73.5 ± 0.4 mm; 74.9 ± 0.4 mm).
368 Six diameter classes were considered: <65 mm, 65-70 mm, 70.75 mm, 75-80 mm, 80-85 mm, >85
369 mm. Considering the fruit distribution among the different diameter classes (Fig. 4) regardless IB
370 presence, one fruit had diameter <65 mm and one fruit diameter >85 mm; about 73% of apples
371 belonged to the 70-75 mm and 75-80 mm classes, about 19% to the 65-70 mm class and about 8%
372 to the 80-85 mm class. If IB position was considered, H, BC and BP fruit were equally distributed
373 in the 65-70 mm class, BC and BP fruit were about three times as much H fruit in the 70-75 mm
374 class, while BC and BP fruit were about 2 and 5 times as much H fruit, respectively, in the 75-80
375 mm and in the 80-85 mm classes.

376

377 *3.2.3. Incidence of storage disorders*

378 Storage conditions strongly affected the IB incidence (Fig. 5). Under BAD storage, 97-100% of
379 fruit was affected by IB regardless fertilization treatment, showing also cavities in 70% of the cases.
380 Under OPT storage, after 4 months' storage 70% of OPT fertilized apples were browned *versus*

381 50% of BAD fertilized ones; however, after 6 months the percentage of IB fruit did not change for
382 OPT fertilized ones, while it increased for BAD fertilized treatment together with the presence of
383 cavities (Fig. 5). Considering the IB position, under BAD storage about 80% of the fruit showed
384 BP, while for OPT storage the BP incidence was about 40%. For both storage conditions, there was
385 an increase in BP incidence with storage time, mainly under OPT storage. BAD stored apples
386 showed higher IB severity than OPT stored ones, both for BC and BP (Fig. 5).
387
388 *3.2.4. TRS optical properties*
389 On the average, $\mu_a$670 was significantly higher in BAD fertilized apples, under BAD storage
390 atmosphere and increased with storage time and with IB development (Table 4). Browned BG and
391 BB apples after 6 months' storage showed the highest $\mu_a$670 values.
392 The $\mu_a$780 was significantly higher in BAD stored apples and increased with storage time and with
393 IB development: the highest values were observed in BB apples after 4 and 6 months' storage and
394 in BG apples after 6 months' storage (Table 4).
395 The $\mu_s$'780 was significantly lower in BAD stored apples and after 6 months' storage and higher in
396 H apples than in IB ones stored under OPT atmosphere (Table 4).
397 TRS optical properties measured at 670 and 780 nm significantly changed also in relation to the
398 position and severity of IB (Fig. 6). The absorption coefficients measured at 670 and at 780 nm
399 were significantly higher in BP than in BC tissues and gradually increased with IB severity. The
400 presence of cavities further increased the values of $\mu_a$670 and of $\mu_a$780 in BC tissues, while it
401 decreased the values of both coefficients in BP tissues. The $\mu_s$'780 was lower in BP tissue than in H
402 ones, but it did not significantly change with IB severity or when cavities were also present.
403
404 *3.2.5. Linear Discriminant Analysis*
405 The values of $\mu_a$780 and $\mu_s$'780 extracted from each TRS measurement were used as explanatory
406 variables in the Linear Discriminant Analysis in order to classify each apple tissue as H or IB by

analyzing 734 H tissues and 1186 IB ones. The obtained discriminant function (canonical correlation of 0.484; $P<0.0001$) allowed to well classify 76.5% of the fruit tissues (67.7% H; 82.0% IB). However, when this model was used for fruit classification, IB fruit were well-classified in 96% of the cases, while H fruit only in 31% of the cases (Table 5).

To understand why this model did not well classify H fruit, a different model was built based on IB position within the fruit (BC and BP), by analyzing 734 H, 578 BC and 603 BP. The obtained discriminant function (canonical correlation of 0.695, $P<0.0001$) correctly classified 90.5% of H, 24.7% of BC and 65.4 % of BP tissues. Misclassified H tissue was considered BC, misclassified BC was considered in 67% of the cases H and misclassified BP was considered H in 12.3% of the cases. Applying this model to fruit (Table 6), H fruit were correctly classified in 89.7% of the cases, BC in 42.7% and BP in 75% of the cases. Misclassified H fruit were considered BC; misclassified BC fruit were considered H in 53.9% of the cases and misclassified BP fruit were considered H in 9.8% of the cases.

To investigate why BC and BP fruit were classified as H, considering the fact that TRS explores the fruit pulp to a maximum depth of 2 cm, we tested the hypothesis that this misclassification could be due to a large fruit dimension. Hence, BC and BP apples (both well-classified and misclassified) were distributed within the six diameter classes (<65 mm; 65-70 mm; 70-75 mm; 75-80 mm; 80-85 mm and >85 mm) and the proportion of misclassified fruit with respect to well-classified ones in each diameter class was considered. Results reported in Fig. 7 showed that this proportion did not change with the increase of the diameter class for BC apples, while for BP fruit it was easier to find misclassified H fruit in the 70-75 and 75-80 mm classes.

In order to see if there was a relation between the misclassification of IB fruits as H and the extension of the IB in the fruit, the number of browned sectors out of the eight measured by TRS was considered. It was found that 52% of BC fruit classified H had 1-3 IB sectors, while only 15% had 7-8 IB sectors. As for BP fruit considered H, 39% had 1 IB sector and only 11% had 7-8 IB sectors.

## 4. Discussion

Some differences were found by comparing data of the two years. As for optical properties of healthy fruit, for which there was no influence due to browning, apples produced in 2009 had lower values of $\mu_a$670 and $\mu_a$780 and higher values of $\mu_s$'780 than fruit produced in 2010. In both years, the $\mu_a$670 values were very close to those found in previous studies on 'Braeburn' apples (Vanoli et al., 2011b; Zanella et al., 2012, Vanoli et al., 2013). The lower $\mu_a$670 values observed in 2009 indicated that these apples were more mature than those of 2010. The $\mu_a$670, in fact, can be considered a maturity index for apples and for other fruit species such as nectarines, peaches, pears and mangoes (Eccher Zerbini et al., 2002; Torricelli et al., 2008; Pereira et al. 2010; Rizzolo et al., 2013): less mature fruit are characterized by high values of $\mu_a$670, while more mature fruit are characterized by lower values of $\mu_a$670. In apples of different cultivars, the $\mu_a$ measured in the 630-670 nm range, in correspondence to chlorophyll-*a* and chlorophyll-*b* absorption peaks, significantly decreased delaying harvest date (Torricelli et al., 2008; Vanoli et al., 2013; Zanella et al., 2012). 'Braeburn' apples having high $\mu_a$670 (less mature) showed higher firmness and were perceived firmer and crisper than those having low $\mu_a$670 values (more mature). 'Jonagored' apples classified as more mature by TRS had lower fruit mass and less titratable acidity at harvest and more soluble solids after storage and were also perceived sweeter, more aromatic and pleasant than the less mature ones (Torricelli et al., 2008). Apples of different TRS maturity showed also a different polyuronide content, with less mature fruit having a less advanced breakdown of insoluble protopectines: $\mu_a$670 and $\mu_a$630 were negatively correlated to galacturonic acid content in the residue insoluble pectin fraction, i.e. upon increasing maturity (decreasing $\mu_a$670) pectin solubilization occurred (Vanoli et al., 2009). In addition, a positive correlation between $\mu_a$670 and firmness was found when there was an high firmness variability coupled to a not too advanced chlorophyll degradation, as observed in 'Braeburn' apples, even if this correlation, having $R^2$=0.47 (Zanella et al., 2012), could not be useful for a reliable nondestructive firmness estimation. As for

459 other optical indices based on chlorophyll-*a* absorption, such as $I_{AD}$ (index of the absorption
460 difference between 670 and 720 nm) or NDVI, studied in apples, Nyasordzi et al. (2013) found that
461 $I_{AD}$ significantly decreased from 10 days before harvest up to 10 days after harvest and it was well
462 correlated with firmness, starch and total soluble solids, Kuckenberg et al. (2008) reported a linear
463 relationship between NDVI and firmness (r=0.70) in 'Jonagold' and 'Golden Delicious,' while
464 Rutkowski et al. (2008) concluded that NDVI showed poor usefulness for firmness estimation of
465 'Golden Delicious' apples during ripening. These differences in the performance of firmness
466 estimation could be due to the fact that TRS measures the chlorophyll content of the pulp, while $I_{AD}$
467 and NDVI assess the chlorophyll content of the skin or of the outer mesocarp.
468 In 2009 apples were also characterized by lower firmness and higher $\mu_s$'780 than those of 2010,
469 confirming the more advanced maturity degree observed by $\mu_a$670 data. In fact it was found that
470 $\mu_s$'780 increased with softening and with pectin solubilisation and it was negatively correlated to
471 sensory and mechanical firmness, and to crispness (Vanoli et al,. 2009; Rizzolo et al., 2010; Vanoli
472 et al., 2013).
473 Taking into account that apples in 2009 and in 2010 were stored in the same atmospheres, a further
474 source of variation between data from the two years could be the fertilization treatment carried out
475 in 2010. Both storage atmosphere and orchard management could affect fruit quality and optical
476 characteristics. Considering the healthy apples, actually fertilization treatment significantly and
477 clearly influenced only $\mu_s$'780, which was higher in OPTfert fruit than in SUBOPTfert ones, while
478 the effects on the absorption coefficients depended also on storage atmosphere. In both years, $\mu_a$670
479 was significantly higher in BAD stored apples than in GOOD ones, probably due to the lower $O_2$
480 and higher $CO_2$ levels used in BAD storage that kept apples in a less advanced maturity, as
481 confirmed by firmness data and similarly to what found by Rizzolo et al. (2010) and Vanoli et al.
482 (2009) who compared the TRS optical properties of apples stored in normal and in controlled
483 atmospheres. However, in 2010 GOOD stored apples showed lower $\mu_a$670 values for OPTfert
484 apples compared to SUBOPTfert ones, while under BAD storage no difference in $\mu_a$670 was found

485 regarding the fertilization treatment. The $\mu_a$780 was significantly higher in BAD storage in 2009,
486 whereas in 2010 BG and GB apples showed the highest $\mu_a$780 values and BB ones the least. The
487 $\mu_s$'780 in 2009 was significantly lower in BAD stored apples than in OPT ones, while in 2010 no
488 difference between storage atmospheres was observed.
489 The absorption at 780 nm was related to browning development, as at this wavelength no pigment
490 (chlorophyll, carotenoids, anthocyanins) absorption occurrs (Torricelli et al., 2008). Hashim et al.
491 (2013) found high correlation for backscattering parameters measured at 785 nm with visual
492 assessment of chilling injury in bananas; Clark et al. (2003) and McGlone et al. (2005) found that
493 'Braeburn' apples strongly affected by brown heart had much higher absorbance in the red/near-red
494 region of the spectrum (650-840 nm) and lower absorbance above 840 nm for the samples less
495 affected by browning, attributing these changes in spectral appearance to the presence of browned
496 flesh. Similarly Han et al. (2006) found higher absorbance between 640 and 860 nm in brown core
497 pears than in healthy ones, with significant differences at 710 and 750 nm, and increasing values
498 with increasing browning severity.
499 In agreement with these authors, in this research it was found that IB apples had higher values of $\mu_a$
500 in the 670-940 nm range, confirming also our previous results on apples and pears (Eccher Zerbini
501 et al., 2002; Vanoli et al., 2011b). It was also confirmed that $\mu_a$780 significantly changed with IB
502 development and severity. Both in 2009 and 2010, $\mu_a$780 was significantly higher in fruit stored in
503 BAD conditions than in those stored in OPT atmosphere due to the higher incidence of IB in BAD
504 than in OPT conditions. Moreover, $\mu_a$780 was higher in 2010 than in 2009 in agreement with the
505 fact that in 2010 the incidences of IB and BP fruits were much higher (IB: 2010, 83.8%, 2009,
506 41.4%; BP: 2010, 56%, 2009, 27%) with percentages of slightly, moderately and severely browning
507 affected fruit quite similar, and in 2009 severe browning was observed only in 12% of the fruit. In
508 both years, $\mu_a$780 was higher in IB fruit than in H ones, significantly increased with IB severity, and
509 was higher in BP than in BC.

These results were in agreement with previous results obtained for browned apples and for fruit of other species even if at wavelengths slightly different. In 'Braeburn' apples, Vanoli et al. (2011b) found that $\mu_a$740 showed the lowest values in healthy fruits and the highest values in BP ones and it was significantly higher in moderate and severe browned fruits compared to healthy ones. In 'Granny Smith' apples, $\mu_a$750 increased with the development of internal browning, with H fruits showing the lowest values of $\mu_a$750 and BP ones the highest (Vanoli et al., 2010). In pears, Eccher Zerbini et al. (2002) found that the presence of browned tissues caused an increase of $\mu_a$720, which was significantly higher than in healthy tissues. Also in stone fruit significant changes in $\mu_a$780 were found with chilling injuries development, especially with those related to browning/reddening appearance in the fruit flesh; in nectarines $\mu_a$780 discriminated healthy fruit from those affected by bleeding and browning and fruit with reddening from those affected by browning (Lurie et al., 2011), whereas in plums $\mu_a$780 increased with browning development and browning severity, showing lower values in healthy fruit and the highest in the severe affected ones (Vangdal et al., 2012).

The behavior of the absorption coefficients measured at 720, 740 and 780 nm reflected the changes in flesh color occurring with browning development. Both in apples and pears, pulp color was significantly different between browned and healthy tissue, showing lower L* and H° and higher a*, b* and C* in browning tissue, indicating a red-brown color (Eccher Zerbini et al., 2002; Vanoli et al., 2010 and 2011b). Good correlations between $\mu_a$740 and $\mu_a$750 with pulp color were found by Vanoli et al. (2010, 2011b). The $\mu_a$740 and $\mu_a$750 were positively correlated to a*, b* and C* and negatively to H° and L*. In 'Granny Smith' apples by using the correlation between $\mu_a$750 and the parameter a* of the pulp (r=0.87) it was possible to discriminate healthy fruits, showing $\mu_a$750 values below 0.030 $cm^{-1}$ from the browned pulp ones, showing $\mu_a$750 values above 0.033 $cm^{-1}$. Similarly, in 'Braeburn' apples the correlation between $\mu_a$740 and pulp L* (r=−0.95) showed that $\mu_a$740 values below 0.038 $cm^{-1}$ indicate only healthy pulp, whereas for $\mu_a$740>0.08 $cm^{-1}$ only severely browned pulp can be found (Vanoli et al., 2011b).

In the present work it was found that also $\mu_a$670 changed with browning development, as in 2009 and in 2010 $\mu_a$670 was significantly higher in IB fruit than in H ones and increased with IB severity. However, differently form $\mu_a$780, the $\mu_a$670 was not able to clearly discriminate H fruit from IB ones, considering both IB position within the fruit and IB severity. This finding could be due to the fact that $\mu_a$670 is mainly affected by the chlorophyll content in the pulp and, hence, the absorption at 670 nm alone cannot have a unique interpretation, as a high value can be due either to high chlorophyll content (less mature fruit) or to the presence of internal browning. Also Eccher Zerbini et al. (2002) in pears found that $\mu_a$690 increased in the presence of brown heart in affected fruit and decreased with ripening in sound fruit due to chlorophyll degradation. Similarly Lurie et al. (2011) found that $\mu_a$670 in nectarines was able to discriminate healthy fruit from those simultaneously affected by bleeding and browning, and was not able to distinguish healthy fruit from those affected by either bleeding alone or browning alone, whereas $\mu_a$780 clearly discriminated between H fruit and those affected by chilling injuries. The $\mu_a$670 and $\mu_a$780 showed a different kinetics during shelf life in nectarines soon after harvest or cold stored. The $\mu_a$670 of fruit at harvest decreased during shelf life, as fruit ripened and chlorophyll disappeared; in contrast, $\mu_a$670 of fruit stored at 4°C increased during shelf life as these fruit developed internal browning. The $\mu_a$780 of fruit at harvest did not change during shelf life, while it dramatically increased in cold stored fruit, especially in those stored at 4°C which showed a severe incidence of chilling injury symptoms. In agreement with what observed in this research were also the results obtained by Hashim et al. (2013) for bananas affected by chilling injury considering backscattering profiles. In fact these authors reported that backscattering profiles measured at 660 nm were mainly affected by the ripening stage, and secondly, by chilling injury development, as they found strong correlations for all backscattered parameters measured at 660 nm and chlorophyll-*a* and chlorophyll-*b* contents, but not with visual assessment of browning severity or with water content, and concluded that the laser-induced backscattering imaging at 660 nm cannot provide reasonable data when monitoring chilling injury in ripe fruit, while backscattering profiles measured at 785 nm were mainly affected

562 by the chilling treatment. In contrast, in plums Vangdal et al. (2012) found that both $\mu_a$670 and
563 $\mu_a$780 were able to distinguish healthy fruit from those affected by internal disorders and both
564 coefficients showed a similar correlation with the browning area.
565 We expected some effects of IB also on scattering coefficient, as with IB development some
566 changes in fruit structure occurred. Herremanns et al. (2013) found a dramatically altered tissue
567 structure during IB development in 'Braeburn' apples: intercellular air space disappeared already
568 after 49 days of storage under IB inducing conditions and, later on in the season, the affected tissue
569 was further destroyed, leaving large cavities, the connectivity among pores dropped due to flooding
570 of the intercellular space caused by the breakdown of cell membranes, with leakage of cell content.
571 Also Defraeye et al. (2013) found some variations in MRI parameters (PD, $T_2$, DC) due to IB
572 development in 'Braeburn' apples caused by the partial either destruction or degradation of the
573 cellular structure by which water migrates to other regions in the fruit, leading to less water
574 availability and mobility and, consequently, to the formation and presence of cavities.
575 In our work, $\mu_s$'780 was higher in healthy fruit than in IB ones in both years, even if this difference
576 was significant only in 2010, as in 2009 an opposite behavior was observed at 3 and 5 months of
577 storage. In 2009 $\mu_s$'780 was significantly lower in BC tissues than in healthy ones, while in 2010 it
578 was lower in BP tissue than in H ones, but no significant differences were found in all the other
579 cases in both years. Also Vangdal et al. (2012) found that healthy fruit had higher values of $\mu_s$'780
580 than chilling injured plums, even if these differences were not significant and no correlation was
581 observed between $\mu_s$'780 and internal disorders. Similarly, in 'Granny Smith' apples, $\mu_s$'750 was
582 higher in H fruit than in BP ones and significant, even if weak, correlations were found between
583 $\mu_s$'750 and L* (r=0.543) and a* (r=−0.573) (Vanoli et al., 2010) indicating that browning presence
584 caused a decrease in the scattering properties. In pears, $\mu'_s$720 did not change with brown heart
585 development, but decreased in water-soaked tissue as observed in over-ripe fruit (where the tissue
586 becomes soft and juicy) and in bruised regions (where cell rupture and cellular content escape into
587 the intercellular space) (Eccher Zerbini et al., 2002).

588 In ‘Braeburn’ apples $\mu'_s$790 allowed to discriminate between mealy and not mealy fruit showing
589 increasing values with increasing sensory mealiness scores and ‘Fuji’ apples affected by watercore
590 showed lower $\mu'_s$790 than H ones (Vanoli et al., 2010). In nectarines, Lurie et al. (2011) found that
591 $\mu_s$’780 did not change with browning development but some correlations were observed with gel
592 breakdown and with woolliness as measured by expressible juice: $\mu_s$’780 showed lower values in
593 nectarines characterized by a less severe incidence of chilling injury symptoms, with a positive,
594 even if weak, correlation with expressible juice; on the other hand, when chilling injury symptoms
595 were severe, $\mu_s$’780 did not show any correlation with woolliness development, probably due to the
596 influence of the high absorption values related to internal browning presence which could have
597 affected the estimation of scattering properties. In bananas, chilling injury was accompanied by
598 changes in water content due to cellular breakdown and deterioration of membrane integrity: a
599 significant difference in the water content between chilling and control temperature was observed
600 and water content showed correlation ($R^2$=0.336) with backscattering parameters measured at 785
601 nm (Hashim et al., 2013). A significant and positive correlation was also found between $\mu'_s$790 and
602 percent relative internal space volume (RISV) in ‘Braeburn’ apples showing lower RISV in mealy
603 fruit than in non mealy ones (Vanoli et al., 2010). When ‘Braeburn’ apples were affected by IB,
604 RISV was significantly higher when IB was scored as severe or moderate, and when cavities were
605 also present; in this case it was supposed a negative correlation between IB development and RISV
606 as $\mu_s$’780 showed the lowest values in browned fruit (Vanoli et al., 2011b).
607 Nevertheless, the difficulty to find a clear relationship between scattering coefficients and browning
608 is probably due to the fact that scattering, according to the Mie theory (Cubeddu et al., 2001a),
609 depends on both the size and the density of the scattering centers that can be affected in a different
610 way by the interplay of various phenomena occurring during fruit storage: starch hydrolysis, flesh
611 softening, water loss and increase in RISV. These phenomena can lead to a decrease in the density
612 of the scattering particles, the cells in the pulp tissue became smaller with more air filled pores and
613 the size of the scatterers decreased but scattering increased as there was an higher refractive index

614 mismatch leading to more and stronger scattering events. So $\mu_s'$ can increase or decrease, depending
615 on which phenomenon dominates in that moment, complicating the relationships between scattering
616 properties and structural characteristics of the fruit tissue. However, when absorption and scattering
617 coefficients were combined, a better prediction of fruit structure was obtained (Valero et al., 2004;
618 Lu, 2009; Rizzolo et al., 2010; Vanoli et al., 2011a).
619 In this research it was found that the best classification performance in the Linear Discriminant
620 Analysis was obtained using all the absorption coefficients plus the $\mu_s$'780, confirming that
621 scattering is crucial both in discriminating healthy apples from those affected by IB, and in
622 distinguishing H fruit from BC and BP ones.
623 In 2009 comparing the model based on all the absorption coefficients (670 nm and in the range 740-
624 1040 nm) plus $\mu_s$'780 with that based on $\mu_a$780 plus $\mu_s$'780, the effectiveness in discriminating
625 healthy fruit from IB ones was about the same: both models correctly classified H fruit in about
626 71% of the cases, and IB ones were correctly classified in 76% of the cases by using all the
627 spectrum and in 71% of the cases when using only the measurement at 780 nm. In 2010, when TRS
628 measurements were made only at 780 nm, the model allowed to better classify IB fruit (96%) but,
629 contrary to our expectations, H fruit were correctly classified only in 31% of the cases. In fact, we
630 expected a better performance of the model in 2010 due to the fact that each apple was measured in
631 eight equidistant points around the equator *vs* the four equidistant points measured in 2009,
632 considering that IB is an asymmetrical disorder and eight measurements points should be sufficient
633 to explore most of the fruit pulp. Actually the classification model of 2010 well classified IB fruit
634 while poorly revealed healthy fruit. This could be due to the fact that in 2010 there were only 38%
635 of healthy tissues and only 16% of healthy fruits, while in 2009 there were 62% of healthy tissues
636 and 59% of healthy apples. The classification model developed in 2010 classified better healthy
637 fruit if the position of IB within the fruit was considered: in this case about 90% of healthy fruit and
638 71% of IB fruit (adding BC+BP fruit) were recognized. In contrast, in 2009 by using the
639 classification model based on H, BC and BP tissues the performance of the model improved for IB

fruit detection (89% adding BC+BP) but worsened for H fruit detection (44%) probably due to the fact that 4 measurements points were not enough to clearly distinguished the tissue type.

Our work also highlighted that the size of the fruit used in the experiment of 2010 (largest equatorial diameter ranging from 63.3 to 88.3 mm) did not affect the detection of IB when it was localized in the core region. Probably is it the asymmetrically distribution typical of the IB developed by 'Braeburn' apples that makes the detection of this defect difficult, as also stated by Clark et al. (2003), McGlone et al. (2005) and Vanoli et al. (2011b). In fact, also when TRS measurements were made on 8 equidistant points, if IB affected only a small part of the core or of the pulp there is the possibility that the defect is not revealed by TRS.

## 5. Conclusion

Our results showed that TRS was able to non destructively detect IB in intact 'Braeburn' apples as both absorption and scattering coefficients measured at 780 nm significantly changed with browning development. The $\mu_a$780 increased with the presence of internal browning allowing to distinguish browned fruit from healthy ones, while the $\mu_s$'780 showed the highest values in healthy apples. Both coefficients are important to achieve a good classification of the fruit on the basis of IB development, even if this classification was not always completely satisfactory. The best classification was obtained in 2010 when it was possible to discriminate 71% of browned fruit and 90% of healthy ones, while in 2009 only 71% of healthy apples was correctly classified together with the same percentage of browned fruit. The better result of 2010 is due to the increased number of TRS measurement points that allowed to better exploring the fruit tissues. However, the asymmetric nature of this disorder makes difficult its detection, especially when the disorder is localized in the inner part of the fruit (core) or when it occurs in spots. A different TRS set-up (position and distance of fibers, time resolution) should be studied in order to reach the deeper tissue within the fruit, improving browning detection.

**Aknowledgements**

This publication has been produced with the financial support of the European Union (project FP7-226783 - InsideFood). The opinions expressed in this document do by no means reflect the official opinion of the European Union or its representatives.

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789

790 **Figure captions**

**Fig. 1** – Year 2009: incidence (left) and severity (right) of internal browning in 'Braeburn' apples stored for 3 and 6 months under BAD and OPT conditions in relation to browning position within apples (H: healthy, BC: brown core, BP: brown pulp); the percent of fruit having also cavities (CV) is added in the incidence graph.

**Fig. 2** – Year 2009: absorption (left) and scattering (right) spectra of healthy (H) and browned (IB) 'Braeburn' apples for 3-and 6- month storage under BAD and OPT conditions. Bars refer to standard errors.

**Fig. 3** – Year 2009: absorption coefficients measured at 670, 780 and 980 nm and reduced scattering coefficient measured at 780 nm in relation to browning presence (H: healthy, IB: browned), browning position (BC: core, BP: pulp), cavity (BCCV: brown core plus cavities, BPCV: brown pulp plus cavities) and browning severity (SLI: slight, MO: moderate, SEV: severe). Bars refer to standard errors. ($N_{obs}$: H=591, IB=357, BC=221, BCCV=49, BP=41, BPCV=46, SLI=204, MO=112, SEV=41)

**Fig. 4** – Year 2010: fruit distribution among different diameter classes and according to browning position (H=healthy, BC=brown core, BP=brown pulp).

**Fig. 5** – Year 2010: Incidence (top) and severity (bottom) of internal browning in 'Braeburn' apples submitted to optimal or suboptimal fertilization and stored for 4 and 6 months under optimal or browning inducing conditions in relation to browning position within apples (H: healthy, BC: brown core, BP: brown pulp); the percent of fruit having also cavities (CV) is added in the incidence graph. Samples captions: first letter refers to storage condition, second letter to fertilization, G, optimal conditions, B, bad conditions.

**Fig. 6** – Year 2010: absorption coefficients measured at 670 and at 780 and reduced scattering coefficient measured at 780 nm in relation to healthy (H) and browned (IB) tissue, browning position (BC: core; BP: pulp), presence of cavity (CV) or both (BCCV: BC and CV; BPCV: BP and CV), and browning severity (SLI: slight, MO: moderate, SEV: severe). Bars refer to standard error

816 of the mean. ($N_{obs}$: H=734, IB=1186, BC=436, BCCV=142, BP=459, BPCV=96, CV=53, SLI=688,

817 MO=327, SEV=118)

818 **Fig. 7** – Year 2010: Distribution of well-classified and misclassified BC (left) and BP apples (right)

819 according to diameter class. For classification data of BC apples see Table 6.

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**TABLE 1** – Year 2009: means and standard errors of absorption coefficients measured at 670, 780 and 980 nm and of reduced scattering coefficient measured at 780 nm in 'Braeburn' apples in relation to storage atmosphere, storage time and browning (significance of the *F*-ratio: ***, $P<0.001$; **, $P<0.01$;*, $P<0.05$; ns=not significant)

| Storage Atmosphere | Months of storage | Browning | $N_{obs}$ | $\mu_a670$ ($cm^{-1}$) | $\mu_a780$ ($cm^{-1}$) | $\mu_a980$ ($cm^{-1}$) | $\mu_s'780$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| BAD | 3 | healthy | 114 | 0.104±0.004 | 0.034±0.003 | 0.450±0.002 | 11.92±0.25 |
| BAD | 6 | healthy | 56 | 0.096±0.004 | 0.035±0.005 | 0.413±0.002 | 13.94±0.23 |
| OPT | 3 | healthy | 235 | 0.077±0.002 | 0.032±0.002 | 0.441±0.001 | 12.96±0.14 |
| OPT | 6 | healthy | 186 | 0.080±0.003 | 0.033±0.002 | 0.407±0.001 | 15.76±0.14 |
| BAD | 3 | browned | 120 | 0.095±0.004 | 0.040±0.004 | 0.458±0.002 | 13.27±0.24 |
| BAD | 6 | browned | 188 | 0.094±0.003 | 0.041±0.003 | 0.419±0.001 | 13.24±0.11 |
| OPT | 6 | browned | 50 | 0.069±0.004 | 0.035±0.005 | 0.405±0.002 | 15.05±0.26 |
| *Main effects* | | | | | | | |
| A storage atmosphere | | | | *** | *** | *** | *** |
| B storage time | | | | ns | ns | *** | *** |
| C internal browning | | | | ** | *** | ns | ns |
| *Interactions* | | | | | | | |
| A x B | | | | ns | ns | ns | * |
| A x C | | | | ns | * | * | ns |
| B x C | | | | ns | ns | ns | *** |

829

830 **TABLE 2** – Year 2009: classification table of 'Braeburn' apples according to IB presence (percentage of

831 well-classified fruit in each class (bold): column: actual group, row: predicted class)

| TRS variables | Classification table | | | |
|---|---|---|---|---|
| | Actual class | Group size | H | IB |
| $\mu_a$670, $\mu_a$ 740-1040, $\mu_s$'780 | H | 140 | **70.7** | 29.3 |
| | IB | 99 | 24.2 | **75.8** |
| $\mu_a$780, $\mu_s$'780 | H | 140 | **70.7** | 29.3 |
| | IB | 99 | 29.3 | **70.7** |

832

833

834 **TABLE 3** – Year 2009: classification table of 'Braeburn' apples according to IB presence and position

835 (percentage of well-classified fruit in each class (bold): column: actual group, row: predicted class)

| TRS variables | Classification table | | | | |
|---|---|---|---|---|---|
| | Actual class | Group size | H | BC | BP |
| $\mu_a$670, $\mu_a$ 740-1040, $\mu_s$'780 | H | 140 | **56.4** | 43.6 | 0.0 |
| | BC | 72 | 20.8 | **66.7** | 12.5 |
| | BP | 27 | 7.4 | 18.5 | **74.1** |
| $\mu_a$780, $\mu_s$'780 | H | 140 | **44.3** | 55.7 | 0.0 |
| | BC | 72 | 19.4 | **61.1** | 19.4 |
| | BP | 27 | 7.4 | 18.5 | **74.1** |

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838 **TABLE 4** – Year 2010: means and standard errors of absorption coefficients measured at 670 nm and at 780
839 nm and of reduced scattering coefficients measured at 780 nm on 'Braeburn' apples in relation to
840 fertilization treatment, storage atmosphere, storage time and browning presence (significance of the *F*-ratio:
841 ***,*P*<0.001; **,*P*<0.01;*,*P*<0.05; ns=not significant). Sample captions: first letter refers to storage
842 condition, second letter to fertilization, G, optimal condition, B, bad conditions.

| Sample | Months of storage | Browning | $N_{obs}$ | $\mu_a$670 ($cm^{-1}$) | $\mu_a$780 ($cm^{-1}$) | $\mu_s$'780 ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| GG | 4 | healthy | 169 | 0.074±0.001 | 0.048±0.001 | 10.93±0.07 |
| | 6 | healthy | 157 | 0.085±0.003 | 0.044±0.001 | 11.40±0.09 |
| GB | 4 | healthy | 191 | 0.093±0.002 | 0.047±0.001 | 11.09±0.07 |
| | 6 | healthy | 108 | 0.093±0.002 | 0.045±0.001 | 10.59±0.12 |
| BG | 4 | healthy | 27 | 0.118±0.009 | 0.050±0.001 | 10.56±0.21 |
| | 6 | healthy | 22 | 0.116±0.005 | 0.058±0.002 | 10.05±0.25 |
| BB | 4 | healthy | 30 | 0.100±0.005 | 0.047±0.001 | 10.75±0.16 |
| | 6 | healthy | 30 | 0.156±0.010 | 0.048±0.001 | 9.99±0.18 |
| GG | 4 | browned | 71 | 0.082±0.002 | 0.050±0.001 | 10.81±0.09 |
| | 6 | browned | 83 | 0.110±0.005 | 0.055±0.002 | 10.76±0.13 |
| GB | 4 | browned | 49 | 0.085±0.002 | 0.049±0.001 | 10.57±0.10 |
| | 6 | browned | 132 | 0.103±0.004 | 0.053±0.001 | 10.44±0.10 |
| BG | 4 | browned | 213 | 0.131±0.004 | 0.067±0.001 | 10.67±0.07 |
| | 6 | browned | 218 | 0.173±0.005 | 0.078±0.002 | 10.40±0.08 |
| BB | 4 | browned | 210 | 0.149±0.005 | 0.077±0.002 | 11.00±0.09 |
| | 6 | browned | 210 | 0.171±0.004 | 0.075±0.001 | 10.32±0.08 |
| *Main effects* | | | | | | |
| A fertilization | | | | * | *ns* | *ns* |
| B storage atmosphere | | | | *** | *** | *** |
| C storage time | | | | *** | * | *** |
| D internal browning | | | | *** | *** | *ns* |
| *Interactions* | | | | | | |
| A x B | | | | *ns* | *ns* | ** |
| A x C | | | | *ns* | * | ** |
| A x D | | | | *ns* | * | *ns* |
| B x C | | | | * | *ns* | *** |
| B x D | | | | *** | *** | *** |
| C x D | | | | *ns* | *ns* | *ns* |
| A x B x C | | | | * | * | *ns* |
| A x B x D | | | | *ns* | ** | *ns* |
| A x C x D | | | | ** | *ns* | *ns* |
| B x C x D | | | | *ns* | *ns* | *ns* |
| A x B x C x D | | | | ** | *ns* | *ns* |



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845 **TABLE 5 –** Year 2010: classification table of 'Braeburn' apples according to IB presence (percentage of

846 well-classified fruit in each class (bold): column: actual group, row: predicted class)

| TRS variables | Classification table | | | |
|---|---|---|---|---|
| | Actual class | Group size | H | IB |
| $\mu_a$780, $\mu_s$'780 | H | 39 | **30.8** | 69.2 |
| | IB | 201 | 4.5 | **95.5** |

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849

850 **TABLE 6** – Year 2010: classification table of 'Braeburn' apples according to IB presence and position

851 (percentage of well-classified fruit in each class (bold): column: actual group, row: predicted class)

| TRS variables | Classification table | | | | |
|---|---|---|---|---|---|
| | Actual class | Group size | H | BC | BP |
| $\mu_a$780, $\mu_s$'780 | H | 39 | **89.7** | 10.3 | 0.0 |
| | BC | 89 | 53.9 | **42.7** | 3.4 |
| | BP | 112 | 9.8 | 15.2 | **75.0** |

852

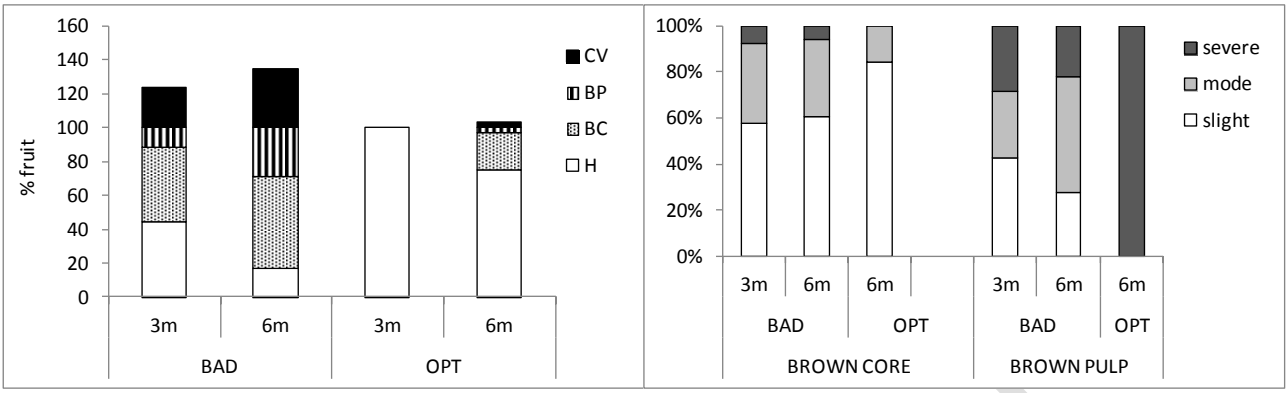



Fig. 1 – Year 2009: Incidence (left) and severity (right) of internal browning in 'Braeburn' apples stored for 3 and 6 months under BAD and OPT conditions in relation to browning position within apples (H: healthy, BC: brown core, BP: brown pulp); the percent of fruit having also cavities (CV) is added in the incidence graph.

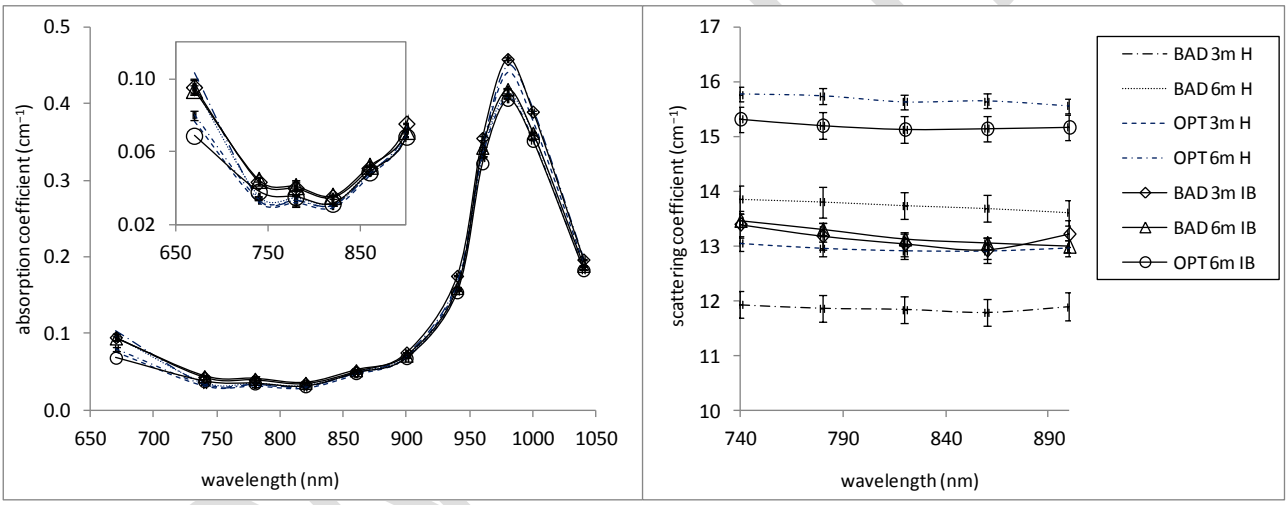
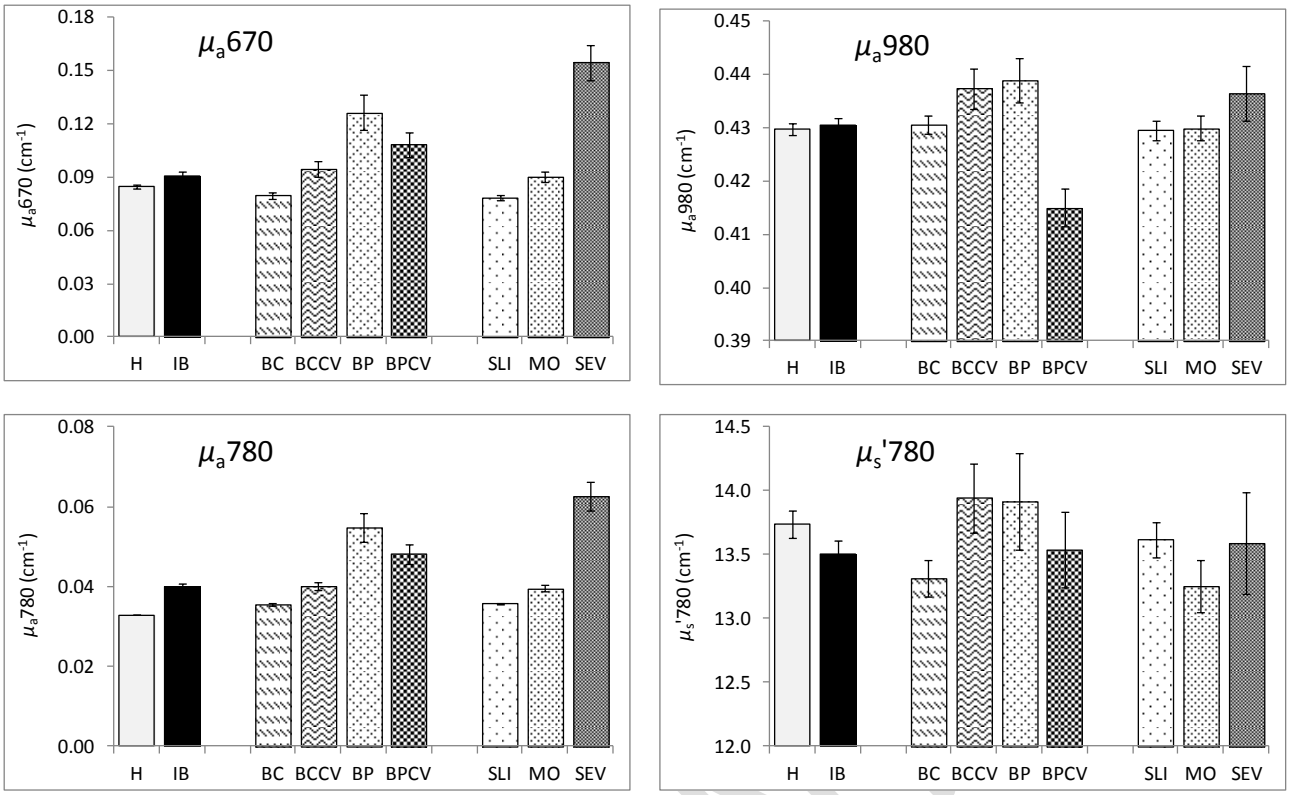


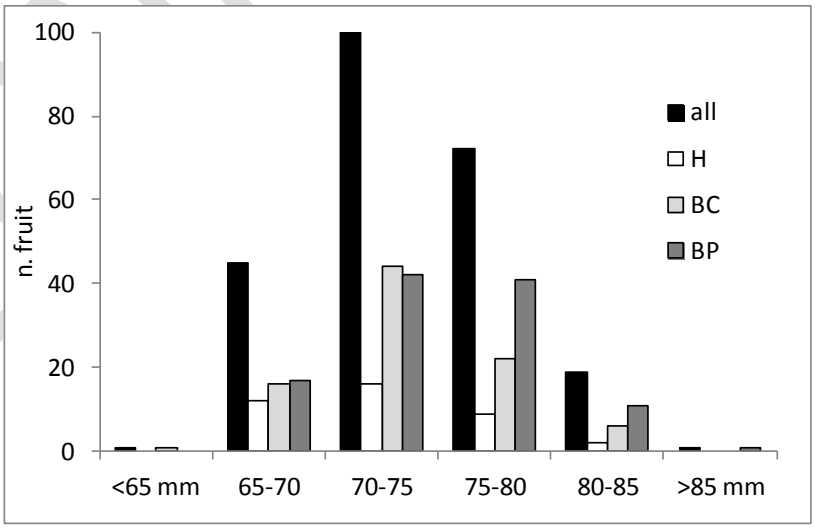

Fig. 2 – Year 2009: absorption (left) and scattering (right) spectra of healthy (H) and browned IB) 'Braeburn' apples for 3-and 6- month storage under BAD and OPT conditions. Bars refer to standard errors




865

866 Fig. 3 – Year 2009: absorption coefficients measured at 670, 780 and 980 nm and reduced
867 scattering coefficient measured at 780 nm in relation to browning presence (H: healthy, IB:
868 browned), browning position (BC: core, BP: pulp), cavity (BCCV: brown core plus cavities, BPCV:
869 brown pulp plus cavities) and browning severity (SLI: slight, MO: moderate, SEV: severe). Bars
870 refer to standard errors. ($N_{obs}$: H=591, IB=357, BC=221, BCCV=49, BP=41, BPCV=46, SLI=204,
871 MO=112, SEV=41)

872




873

874 Fig. 4 – Year 2010: fruit distribution among different diameter classes and according to browning
875 position (H=healthy, BC=brown core, BP=brown pulp).

876

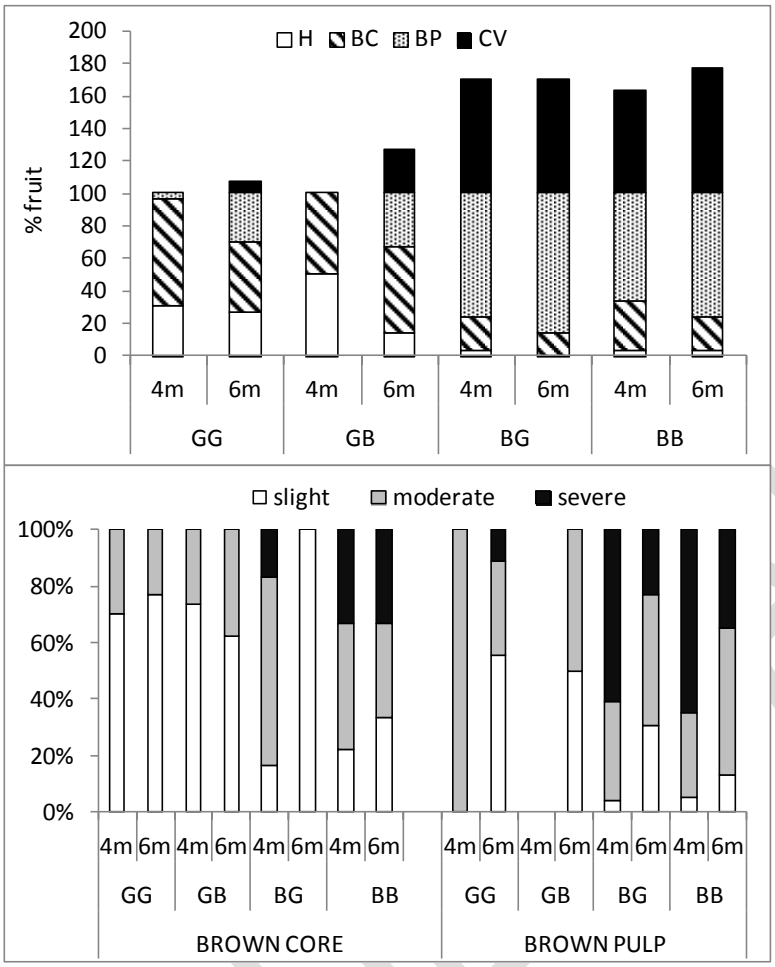
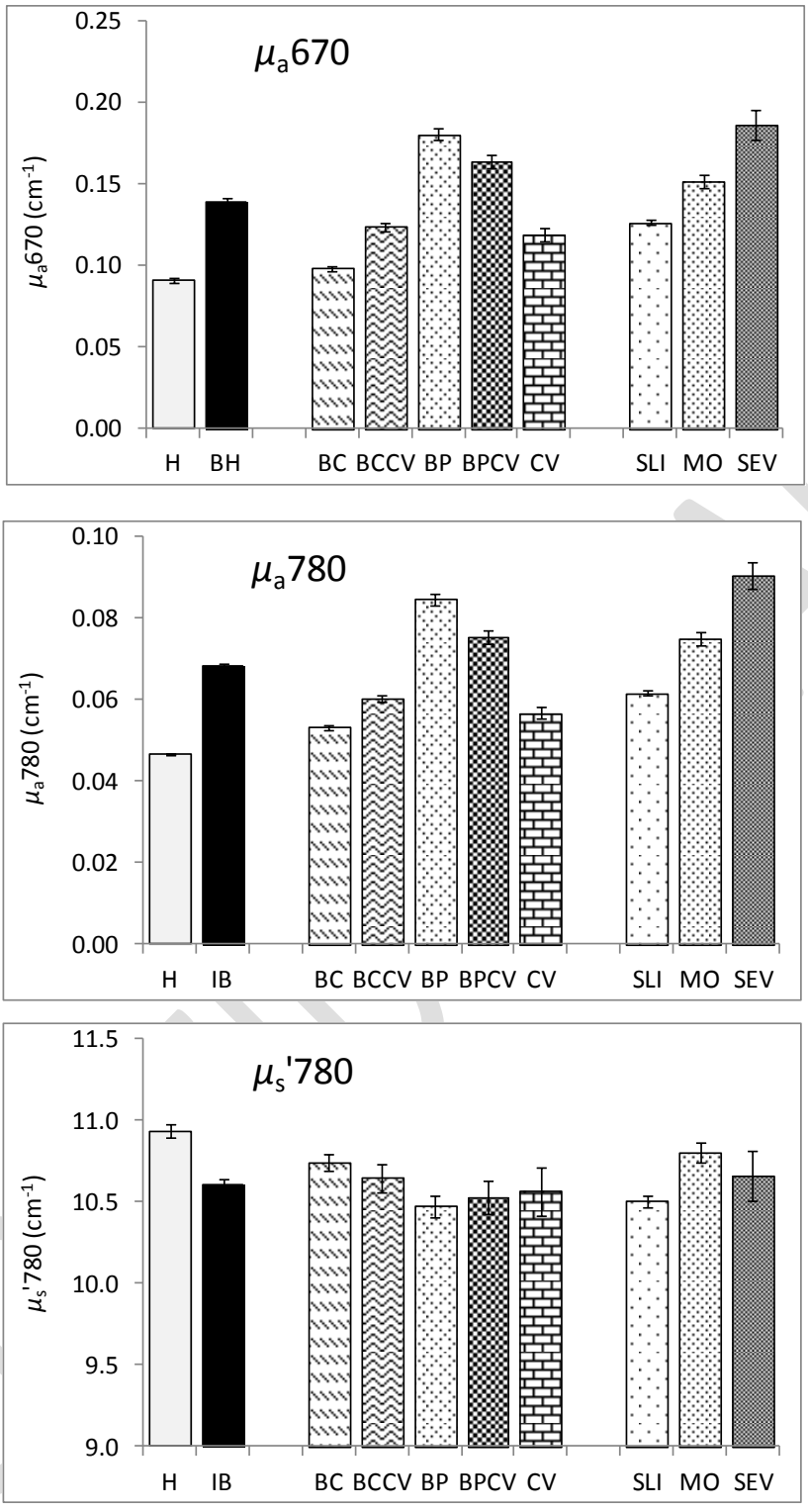


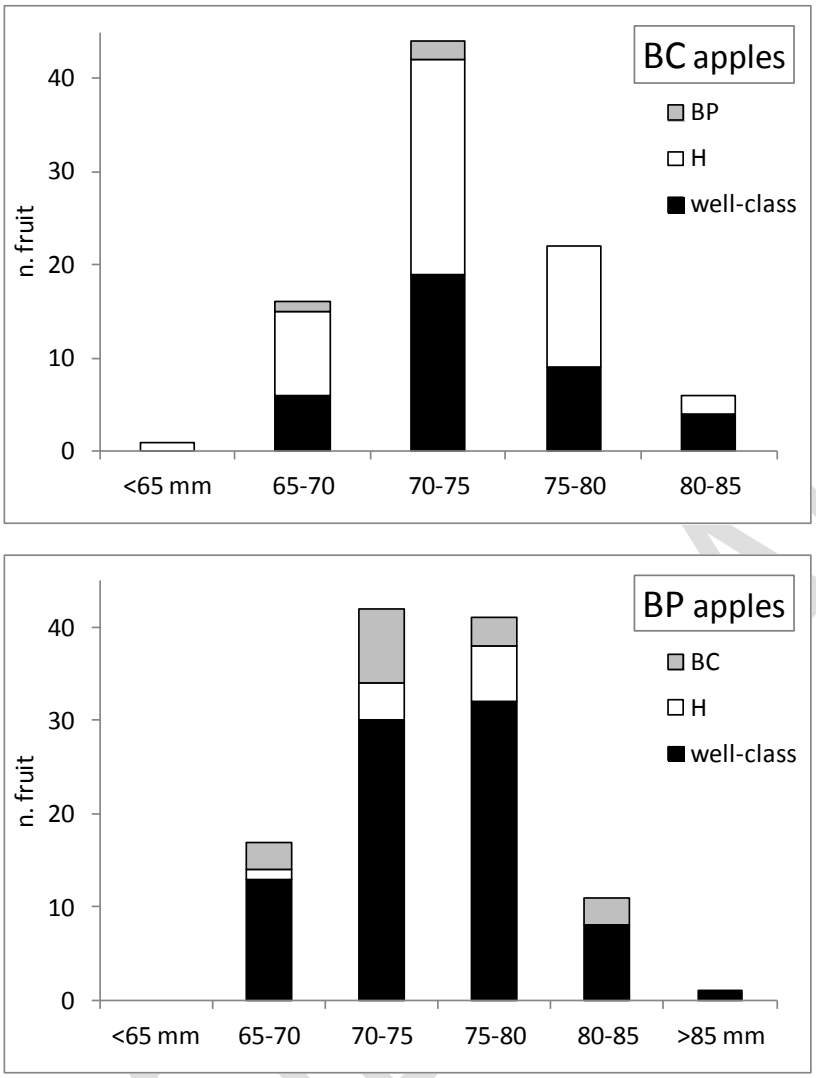

Fig. 5– Year 2010: Incidence (top) and severity (bottom) of internal browning in 'Braeburn' apples submitted to optimal or suboptimal fertilization and stored for 4 and 6 months under optimal or browning inducing conditions in relation to browning position within apples (H: healthy, BC: brown core, BP: brown pulp); the percent of fruit having also cavities (CV) is added in the incidence graph.. Samples captions: first letter refers to storage condition, second letter to fertilization, G, optimal conditions, B, sub-optimal conditions.




885

886 Fig. 6 – Year 2010: absorption coefficients measured at 670 and at 780 and reduced scattering
887 coefficient measured at 780 nm in relation to healthy (H) and browned (IB) tissue, browning
888 position (BC: core; BP: pulp), presence of cavity (CV) or both (BCCV: BC and CV; BPCV: BP and
889 CV), and browning severity (SLI: slight, MO: moderate, SEV: severe). Bars refer to standard error
890 of the mean. ($N_{obs}$: H=734, IB=1186, BC=436, BCCV=142, BP=459, BPCV=96, CV=53, SLI=688,
891 MO=327, SEV=118)
892

893




894

895 Fig. 7 – Year 2010: Distribution of well-classified and misclassified BC and BP apples according to
896 diameter class. For classification data of BC apples see Table 6.

897