

1 **Studies on classification models to discriminate ‘Braeburn’ apples affected by internal**
2 **browning using the optical properties measured by time-resolved reflectance spectroscopy**

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25 **Abstract**

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

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

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

43 In both years, μ_s' 780 was higher in healthy fruit than in IB ones, while μ_a 780 was higher in IB fruit
44 than in H ones, significantly increased with IB severity, and was higher in BP than in BC tissues.

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

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

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

66

67 **Keywords: max 6**

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

70

71 **1. Introduction**

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

80 The incidence and the severity of IB varies markedly from year to year and is affected by orchard
81 factors as well as by postharvest conditions. The incidence of IB was higher in late than in early-
82 harvested -fruit, in fruit on light than on heavily cropping trees and in apples with high K and P
83 content or with high K/Ca ratio (Lau, 1998; Elgar et al., 1999; Neuwald et al., 2008). IB is a CO₂-
84 related injury: its incidence is associated with high CO₂ partial pressure in the storage room and can
85 be exacerbated by decreased O₂ and can be reduced or eliminated by delayed controlled atmosphere
86 (CA) storage (Elgar et al., 1998; Lau, 1998; Saquet et al., 2003; Neuwald et al., 2008; Ho et al.,
87 2013).

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

97 dramatically altered tissue structure: there was a drastic ‘closing’ of the microstructure in the inner
98 and middle cortex due to the disappearance of the open intercellular air space, while the outer
99 cortex tissue, close to the apple skin, seems to remain largely unaffected. IB tissue appeared
100 extremely dense and can be further destroyed, leaving large cavities. All these microstructural
101 changes caused a further decrease in the local O₂ concentration and an increase in the CO₂
102 concentration altering fruit metabolism; as a consequence, cells cannot maintain membrane integrity
103 and leakage of the cell content occurs leading to flooding of the pores, possibly followed by further
104 collapse of the tissue structure and formation of cavities (Dražeta et al., 2004; Lee et al., 2012;
105 Herremanns et al., 2013; Vandendriessche et al., 2013).

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

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

123 Also time-resolved reflectance spectroscopy (TRS) showed interesting results in the detection of
124 internal disorders, as it nondestructively measures the internal properties of fruits (Torricelli et al.,
125 2008). In TRS a short pulse of monochromatic light is injected within a diffusive medium.
126 Following the injection of the light pulse, the temporal distribution of the re-emitted photons at a
127 distance ρ from the injection point will be delayed, broadened and attenuated. The delay is a
128 consequence of the finite time that light takes to travel the distance between source and detector;
129 broadening is mainly due to the many different paths that photons undergo because of multiple
130 scattering; attenuation appears because absorption reduces the probability of detecting a photon, and
131 diffusion into other directions within the medium decreases the number of detected photons in the
132 considered direction. By applying a proper theoretical model, the absorption coefficient μ_a (units
133 are typically cm^{-1}) and the reduced scattering coefficient μ_s' (cm^{-1}) can be accurately estimated.
134 Chemical constituents in the fruit such as pigments, water, soluble solids affect the μ_a , while fruit
135 density, cell size, middle-lamella, intra- and extracellular characteristics are likely to affect the μ_s' .
136 The volume probed by a TRS measurement is a 'banana-shaped' region connecting the injection
137 and collection points, thus the measured coefficients roughly correspond to the average of the
138 optical properties in this region. It is not easy to define the measurement volume since the photon
139 paths are more densely packed in the banana region, but can be distributed in the whole medium. A
140 series of measurements was performed on apples (Cubeddu et al., 2001b) and pears (Eccher Zerbini
141 et al., 2002) to determine the maximum depth in the tissue that yields a detectable contribution to
142 the TRS curve and it was concluded that for both fruits TRS measurement probes a depth of at least
143 2 cm in the pulp. Although this is not a direct estimation of the penetration depth of this technique,
144 it proves that TRS is not confined to the surface of the fruit. This was also confirmed by Saeys et al.
145 (2008) which compared the optical properties measured by NIR in the skin and in the flesh of three
146 apple cultivars with those obtained by TRS on intact fruit, highlighting how the optical properties
147 measured by TRS are dominated by the flesh characteristics. The penetration depth reached by TRS
148 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 μ_a and μ_s can be involved in the detection of browning disorders in apples and pears.
152 ‘Braeburn’ apples affected by brown heart had significantly higher μ_a values in the 740-1000 nm
153 spectral range than healthy ones (Vanoli et al., 2011b). The μ_a measured at 740 nm (μ_{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 $\mu_{a740} > 0.08 \text{ cm}^{-1}$ distinguished severely browned pulp
156 (Vanoli et al., 2011b). ‘Granny Smith’ apples showed an increase of μ_{a750} values with the
157 development of internal browning with healthy fruit having $\mu_{a750} < 0.030 \text{ cm}^{-1}$ and browned apples
158 $\mu_{a750} > 0.033 \text{ cm}^{-1}$; furthermore, severely affected fruit showed also a decrease of μ_s ’750 to values
159 $< 10 \text{ cm}^{-1}$ (Vanoli et al., 2010). The presence of brown heart in the pulp of ‘Conference’ pears
160 caused an increase in the μ_a values from 710 to 850 nm with brown tissue showing $\mu_{a720} > 0.04$
161 cm^{-1} while the μ_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, μ_{a780} was able to differentiate between healthy and fruit with either woolliness, internal
166 browning or internal bleeding. In ‘Jubileum’ plums, μ_{a670} and μ_{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*

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

176 In 2009 apples were picked on October 26th which was considered to be the optimal commercial
177 harvest date for long-term commercial storage for Belgium, as determined by the Flanders Centre of
178 Postharvest Technology (VCBT). Afterwards, apples were stored at 1°C under two types of
179 controlled atmospheres: browning inducing storage conditions (BAD storage: 1% O₂, 5% CO₂) and
180 optimal storage conditions (OPT storage: 2.5% O₂, 0.7% CO₂ with a 3-week delay of CA to prevent
181 IB development). After 3- and 6-month storage, at arrival in the laboratory of Politecnico in Milan,
182 sixty apples/storage atmosphere were measured by TRS at 670 nm and in the spectral range 740-
183 1100 nm on four equidistant points (0°, 90°, 180°, 270°) around the equator (the largest transverse
184 circumference).

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

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

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

206

207 *2.2 TRS measurements*

208 In 2009, TRS measurements were performed at 670 nm and in the spectral range 740-1040 nm (at
209 40 nm intervals). The broadband setup employed a white light laser (SC450, Fianium Ltd., UK) for
210 generation of light pulses (10 ps duration, 40 MHz repetition rate, 1 mW/nm average power), a
211 watercooled double microchannel plate photomultiplier (R1564U, Hamamatsu Photonics, Japan),
212 and a time-correlated single photon counting board (SPC-130, Becker & Hickl GmbH, Berlin,
213 Germany). The temporal resolution of the overall system, calculated as the FWHM of the
214 instrumental response function (IRF), was <90 ps. Details on the setup can be found in D'Andrea et
215 al. (2009).

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

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

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

231

232 *2.3 Fruit diameter*

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

234

235 *2.4 Flesh firmness*

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

240

241 *2.5 Statistical analysis*

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

247 In 2009, classification models were developed using TRS absorption coefficients in the 670-1040
248 nm spectral range and scattering coefficient measured at 780 nm as explanatory variables in the
249 Linear Discriminant Analysis (LDA) in order to discriminate between browned and healthy tissues.

250 Classification functions were estimated with a stepwise approach, selecting or removing each

251 variable in order to evaluate the contribution of the respective variable to the discriminatory power

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

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

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

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

267

268 **3. Results**

269 *3.1. Year 2009*

270 *3.1.1. Flesh firmness*

271 Flesh firmness was significantly higher in apples stored under BAD ($76.1 \pm 1.6\text{N}$, mean \pm standard
272 error) than under OPT conditions ($63.3 \pm 0.7\text{N}$) and in IB apples than in H ones but only after 6-
273 month storage (3-month: H= $71.8 \pm 1.3\text{N}$, IB= $71.7 \pm 3.1\text{N}$; 6-month: H= $63.2 \pm 1.4\text{N}$, IB= $71.3 \pm$
274 2.2N). Firmness significantly decreased with storage time only in H fruit.

275

276 *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 μ_a
294 values in the 820-1040nm range and IB presence affected the μ_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 μ_a values measured at 670, 780 and 980 nm and the values of μ_s '780
299 are reported. We have chosen to show the values of μ_a 670 and μ_a 980, as they correspond to the two
300 maxima of TRS spectra and the values of μ_a 780 and of μ_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).

303 On the average, μ_a670 and μ_a780 were significantly higher in BAD stored apples than in OPT ones
304 and in IB fruit comparing to H ones (Table 1). The μ_a980 was significantly higher in BAD stored
305 apples and decreased with storage time (Table 1), probably due to some water loss (Vanoli et al.,
306 2011a). The $\mu_s'780$ was significantly lower in BAD stored apples than in OPT ones and increased
307 with storage time; $\mu_s'780$ was also higher in H than in IB fruit after 6 months' storage; the opposite
308 was found after 3 month's storage (Table 1).

309 Optical properties changed also with the IB position and severity within fruit (Fig. 3). The μ_a780
310 showed the lowest values in H tissues, was higher in BP than in BC tissues and increased with
311 increasing IB severity. The simultaneous presence of cavities further increased μ_a780 values but
312 only when IB was localized in the core region. The μ_a670 showed similar values for H and
313 BC/BCCV tissues, it significantly increased in BP/BPCV tissues and in severe IB ones. The μ_a980
314 had the lowest values when IB affected pulp tissues and cavities were also present. The $\mu_s'780$ was
315 significantly lower in BC tissue than in H ones, and did not significantly differ for BP, BCCV and
316 BPCV tissues.

317

318 *3.1.4. Linear Discriminant Analysis and classification models*

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

322 By analyzing 591 H tissues and 357 IB ones, the best classification performance was obtained using
323 all the absorption coefficients plus the $\mu_s'780$. The obtained discriminant function (DF) (canonical
324 correlation of 0.465, $P < 0.00001$) had the highest standardized coefficient for μ_a780 (0.731),
325 followed by μ_a670 (-0.654), μ_a820 (0.502) and $\mu_s'780$ (-0.430), allowing to well-classify 75.1% of
326 apple tissues (95.4% of H but only 41.5% of IB ones). However, when this model was used to
327 classify apple fruit, H fruit were well-classified in 70.7% of the cases and IB ones in the 75.8% of
328 the cases (Table 2).

329 As μ_a780 showed the highest standardized coefficient in the DF and also $\mu_s'780$ was crucial in the
330 development of the tissue classification model, a new model was built by using as explanatory
331 variables μ_a780 and $\mu_s'780$. The obtained DF (canonical correlation of 0.390, $P<0.00001$) allowed
332 to correctly classify 71.9% of the apple tissues, actually 99.7% of H tissues but only 26% of IB
333 ones. Nevertheless, when this model was used to classify apple fruit, H and IB fruit were well-
334 classified in 70.7% of the cases (Table 2).

335 In order to better classify both H and IB fruit, two other models were developed considering the IB
336 position within the fruit (BC or BP). The first model was based on all the absorption coefficients
337 plus $\mu_s'780$ and analyzed 591 H, 266 BC and 91 BP tissues. Two DF were obtained: the first DF
338 (92.9% of the variance, with a significant [$P<0.0001$] canonical correlation of 0.633) had the
339 highest standardized coefficient for μ_a780 (0.608), followed by μ_a740 (0.581), μ_a670 (-0.371) and
340 $\mu_s'780$ (-0.252), whereas the second DF (7.1% of the variance, with a significant [$P<0.0001$]
341 canonical correlation of 0.221) had higher coefficients for μ_a740 (-1.090), μ_a670 (-0.868), μ_a820
342 (0.989) and $\mu_s'780$ (-0.589). H tissues were well classified in 96.3% of the cases, BC tissues in
343 22.6% of the cases and BP in 41.8% of the cases. Misclassified H tissues were considered BC;
344 misclassified BC tissues were considered H in 74.1% of the cases, whereas misclassified BP tissues
345 were considered H only in 38.5% of the cases. Applying this model to apples, H fruit were correctly
346 classified only in 56.4% of the cases, BC fruit were classified as IB in 80.2 % of the cases and BP
347 fruits were considered IB in 92.6% of the cases (Table 3).

348 A second model was built using μ_a780 and $\mu_s'780$ as explanatory variables. One DF was obtained
349 (canonical correlation of 0.587, $P<0.0000$) which well classified all H tissues, but only 1.5% of BC
350 and 39.1% of BP tissues. Applying this model to apples (Table 3), H fruit were well-classified only
351 in 44.3% of the cases, BC fruit were well-classified in 61.1% of the cases and BP fruit in 74.1% of
352 the cases. Misclassified H fruit were all considered BC, while BC and BP fruit were considered H
353 in 19.4% and 7.4% of the cases, respectively.

354

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 ($91.8 \pm 0.8\text{N}$) than OPT ones ($82.3 \pm 0.8\text{N}$). Firmness slightly decreased with storage
359 time (4 months: $89.4 \pm 0.8\text{N}$, 6 months: $84.6 \pm 1.0\text{N}$) and slightly increased with IB development (H
360 fruit: $85.0 \pm 1.3\text{N}$, IB fruit: $87.4 \pm 0.7\text{N}$).

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, μ_a670 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 μ_a670 values.

392 The μ_a780 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 μ_a670 and of μ_a780 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 μ_a780 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

407 analyzing 734 H tissues and 1186 IB ones. The obtained discriminant function (canonical
408 correlation of 0.484; $P < 0.0001$) allowed to well classify 76.5% of the fruit tissues (67.7% H; 82.0%
409 IB). However, when this model was used for fruit classification, IB fruit were well-classified in
410 96% of the cases, while H fruit only in 31% of the cases (Table 5).

411 To understand why this model did not well classify H fruit, a different model was built based on IB
412 position within the fruit (BC and BP), by analyzing 734 H, 578 BC and 603 BP. The obtained
413 discriminant function (canonical correlation of 0.695, $P < 0.0001$) correctly classified 90.5% of H,
414 24.7% of BC and 65.4 % of BP tissues. Misclassified H tissue was considered BC, misclassified BC
415 was considered in 67% of the cases H and misclassified BP was considered H in 12.3% of the cases.
416 Applying this model to fruit (Table 6), H fruit were correctly classified in 89.7% of the cases, BC in
417 42.7% and BP in 75% of the cases. Misclassified H fruit were considered BC; misclassified BC
418 fruit were considered H in 53.9% of the cases and misclassified BP fruit were considered H in 9.8%
419 of the cases.

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

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

434 **4. Discussion**

435 Some differences were found by comparing data of the two years. As for optical properties of
436 healthy fruit, for which there was no influence due to browning, apples produced in 2009 had lower
437 values of μ_a670 and μ_a780 and higher values of μ_s780 than fruit produced in 2010. In both years,
438 the μ_a670 values were very close to those found in previous studies on 'Braeburn' apples (Vanoli et
439 al., 2011b; Zanella et al., 2012, Vanoli et al., 2013). The lower μ_a670 values observed in 2009
440 indicated that these apples were more mature than those of 2010. The μ_a670 , in fact, can be
441 considered a maturity index for apples and for other fruit species such as nectarines, peaches, pears
442 and mangoes (Eccher Zerbini et al., 2002; Torricelli et al., 2008; Pereira et al. 2010; Rizzolo et al.,
443 2013): less mature fruit are characterized by high values of μ_a670 , while more mature fruit are
444 characterized by lower values of μ_a670 . In apples of different cultivars, the μ_a measured in the 630-
445 670 nm range, in correspondence to chlorophyll-*a* and chlorophyll-*b* absorption peaks, significantly
446 decreased delaying harvest date (Torricelli et al., 2008; Vanoli et al., 2013; Zanella et al., 2012).
447 'Braeburn' apples having high μ_a670 (less mature) showed higher firmness and were perceived
448 firmer and crisper than those having low μ_a670 values (more mature). 'Jonagored' apples classified
449 as more mature by TRS had lower fruit mass and less titratable acidity at harvest and more soluble
450 solids after storage and were also perceived sweeter, more aromatic and pleasant than the less
451 mature ones (Torricelli et al., 2008). Apples of different TRS maturity showed also a different
452 polyuronide content, with less mature fruit having a less advanced breakdown of insoluble
453 protopectines: μ_a670 and μ_a630 were negatively correlated to galacturonic acid content in the
454 residue insoluble pectin fraction, i.e. upon increasing maturity (decreasing μ_a670) pectin
455 solubilization occurred (Vanoli et al., 2009). In addition, a positive correlation between μ_a670 and
456 firmness was found when there was an high firmness variability coupled to a not too advanced
457 chlorophyll degradation, as observed in 'Braeburn' apples, even if this correlation, having $R^2=0.47$
458 (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 μ_a670 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, μ_a670
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 μ_a670 values for OPTfert
484 apples compared to SUBOPTfert ones, while under BAD storage no difference in μ_a670 was found

485 regarding the fertilization treatment. The μ_a780 was significantly higher in BAD storage in 2009,
486 whereas in 2010 BG and GB apples showed the highest μ_a780 values and BB ones the least. The
487 μ_s780 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 occurs (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 μ_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 μ_a780 significantly changed with IB
502 development and severity. Both in 2009 and 2010, μ_a780 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, μ_a780 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, μ_a780 was higher in IB fruit than in H ones, significantly increased with IB severity, and
509 was higher in BP than in BC.

510 These results were in agreement with previous results obtained for browned apples and for fruit of
511 other species even if at wavelengths slightly different. In 'Braeburn' apples, Vanoli et al. (2011b)
512 found that μ_a740 showed the lowest values in healthy fruits and the highest values in BP ones and it
513 was significantly higher in moderate and severe browned fruits compared to healthy ones. In
514 'Granny Smith' apples, μ_a750 increased with the development of internal browning, with H fruits
515 showing the lowest values of μ_a750 and BP ones the highest (Vanoli et al., 2010). In pears, Eccher
516 Zerbini et al. (2002) found that the presence of browned tissues caused an increase of μ_a720 , which
517 was significantly higher than in healthy tissues. Also in stone fruit significant changes in μ_a780
518 were found with chilling injuries development, especially with those related to browning/reddening
519 appearance in the fruit flesh; in nectarines μ_a780 discriminated healthy fruit from those affected by
520 bleeding and browning and fruit with reddening from those affected by browning (Lurie et al.,
521 2011), whereas in plums μ_a780 increased with browning development and browning severity,
522 showing lower values in healthy fruit and the highest in the severe affected ones (Vangdal et al.,
523 2012).

524 The behavior of the absorption coefficients measured at 720, 740 and 780 nm reflected the changes
525 in flesh color occurring with browning development. Both in apples and pears, pulp color was
526 significantly different between browned and healthy tissue, showing lower L^* and H° and higher
527 a^* , b^* and C^* in browning tissue, indicating a red-brown color (Eccher Zerbini et al., 2002; Vanoli
528 et al., 2010 and 2011b). Good correlations between μ_a740 and μ_a750 with pulp color were found by
529 Vanoli et al. (2010, 2011b). The μ_a740 and μ_a750 were positively correlated to a^* , b^* and C^* and
530 negatively to H° and L^* . In 'Granny Smith' apples by using the correlation between μ_a750 and the
531 parameter a^* of the pulp ($r=0.87$) it was possible to discriminate healthy fruits, showing μ_a750
532 values below 0.030 cm^{-1} from the browned pulp ones, showing μ_a750 values above 0.033 cm^{-1} .
533 Similarly, in 'Braeburn' apples the correlation between μ_a740 and pulp L^* ($r=-0.95$) showed that
534 μ_a740 values below 0.038 cm^{-1} indicate only healthy pulp, whereas for $\mu_a740 > 0.08 \text{ cm}^{-1}$ only
535 severely browned pulp can be found (Vanoli et al., 2011b).

536 In the present work it was found that also μ_a670 changed with browning development, as in 2009
537 and in 2010 μ_a670 was significantly higher in IB fruit than in H ones and increased with IB
538 severity. However, differently from μ_a780 , the μ_a670 was not able to clearly discriminate H fruit
539 from IB ones, considering both IB position within the fruit and IB severity. This finding could be
540 due to the fact that μ_a670 is mainly affected by the chlorophyll content in the pulp and, hence, the
541 absorption at 670 nm alone cannot have a unique interpretation, as a high value can be due either to
542 high chlorophyll content (less mature fruit) or to the presence of internal browning. Also Eccher
543 Zerbini et al. (2002) in pears found that μ_a690 increased in the presence of brown heart in affected
544 fruit and decreased with ripening in sound fruit due to chlorophyll degradation. Similarly Lurie et
545 al. (2011) found that μ_a670 in nectarines was able to discriminate healthy fruit from those
546 simultaneously affected by bleeding and browning, and was not able to distinguish healthy fruit
547 from those affected by either bleeding alone or browning alone, whereas μ_a780 clearly
548 discriminated between H fruit and those affected by chilling injuries. The μ_a670 and μ_a780 showed
549 a different kinetics during shelf life in nectarines soon after harvest or cold stored. The μ_a670 of
550 fruit at harvest decreased during shelf life, as fruit ripened and chlorophyll disappeared; in contrast,
551 μ_a670 of fruit stored at 4°C increased during shelf life as these fruit developed internal browning.
552 The μ_a780 of fruit at harvest did not change during shelf life, while it dramatically increased in cold
553 stored fruit, especially in those stored at 4°C which showed a severe incidence of chilling injury
554 symptoms. In agreement with what observed in this research were also the results obtained by
555 Hashim et al. (2013) for bananas affected by chilling injury considering backscattering profiles. In
556 fact these authors reported that backscattering profiles measured at 660 nm were mainly affected by
557 the ripening stage, and secondly, by chilling injury development, as they found strong correlations
558 for all backscattered parameters measured at 660 nm and chlorophyll-*a* and chlorophyll-*b* contents,
559 but not with visual assessment of browning severity or with water content, and concluded that the
560 laser-induced backscattering imaging at 660 nm cannot provide reasonable data when monitoring
561 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 μ_a '670 and
563 μ_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, T₂, 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, μ_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 μ_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 μ_s '780
580 than chilling injured plums, even if these differences were not significant and no correlation was
581 observed between μ_s '780 and internal disorders. Similarly, in 'Granny Smith' apples, μ_s '750 was
582 higher in H fruit than in BP ones and significant, even if weak, correlations were found between
583 μ_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, μ_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 μ_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 μ_s' 790 than H ones (Vanoli et al., 2010). In nectarines, Lurie et al. (2011) found that
591 μ_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: μ_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, μ_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 μ_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 μ_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 μ_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 μ_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 μ_s' 780 with that based on μ_a 780 plus μ_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

640 fruit detection (89% adding BC+BP) but worsened for H fruit detection (44%) probably due to the
641 fact that 4 measurements points were not enough to clearly distinguished the tissue type.
642 Our work also highlighted that the size of the fruit used in the experiment of 2010 (largest
643 equatorial diameter ranging from 63.3 to 88.3 mm) did not affect the detection of IB when it was
644 localized in the core region. Probably is it the asymmetrically distribution typical of the IB
645 developed by 'Braeburn' apples that makes the detection of this defect difficult, as also stated by
646 Clark et al. (2003), McGlone et al. (2005) and Vanoli et al. (2011b). In fact, also when TRS
647 measurements were made on 8 equidistant points, if IB affected only a small part of the core or of
648 the pulp there is the possibility that the defect is not revealed by TRS.

650 **5. Conclusion**

651 Our results showed that TRS was able to non destructively detect IB in intact 'Braeburn' apples as
652 both absorption and scattering coefficients measured at 780 nm significantly changed with
653 browning development. The μ_a 780 increased with the presence of internal browning allowing to
654 distinguish browned fruit from healthy ones, while the μ_s '780 showed the highest values in healthy
655 apples. Both coefficients are important to achieve a good classification of the fruit on the basis of IB
656 development, even if this classification was not always completely satisfactory. The best
657 classification was obtained in 2010 when it was possible to discriminate 71% of browned fruit and
658 90% of healthy ones, while in 2009 only 71% of healthy apples was correctly classified together
659 with the same percentage of browned fruit. The better result of 2010 is due to the increased number
660 of TRS measurement points that allowed to better exploring the fruit tissues. However, the
661 asymmetric nature of this disorder makes difficult its detection, especially when the disorder is
662 localized in the inner part of the fruit (core) or when it occurs in spots. A different TRS set-up
663 (position and distance of fibers, time resolution) should be studied in order to reach the deeper
664 tissue within the fruit, improving browning detection.

665

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670

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790 **Figure captions**

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

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

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

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

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

812 **Fig. 6** – Year 2010: absorption coefficients measured at 670 and at 780 and reduced scattering
813 coefficient measured at 780 nm in relation to healthy (H) and browned (IB) tissue, browning
814 position (BC: core; BP: pulp), presence of cavity (CV) or both (BCCV: BC and CV; BPCV: BP and
815 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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824 **TABLE 1** – Year 2009: means and standard errors of absorption coefficients measured at 670, 780 and 980
 825 nm and of reduced scattering coefficient measured at 780 nm in ‘Braeburn’ apples in relation to storage
 826 atmosphere, storage time and browning (significance of the *F*-ratio: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$;
 827 ns=not significant)

Storage Atmosphere	Months of storage	Browning	N_{obs}	$\mu_a 670$ (cm^{-1})	$\mu_a 780$ (cm^{-1})	$\mu_a 980$ (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
<i>Main effects</i>							
A storage atmosphere				***	***	***	***
B storage time				ns	ns	***	***
C internal browning				**	***	ns	ns
<i>Interactions</i>							
A x B				ns	ns	ns	*
A x C				ns	*	*	ns
B x C				ns	ns	ns	***

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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

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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}	μ_a^{670} (cm ⁻¹)	μ_a^{780} (cm ⁻¹)	μ_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
<i>Main effects</i>						
A fertilization				*	ns	ns
B storage atmosphere				***	***	***
C storage time				***	*	***
D internal browning				***	***	ns
<i>Interactions</i>						
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
μ_a780, μ_s780	H	39	30.8	69.2
	IB	201	4.5	95.5

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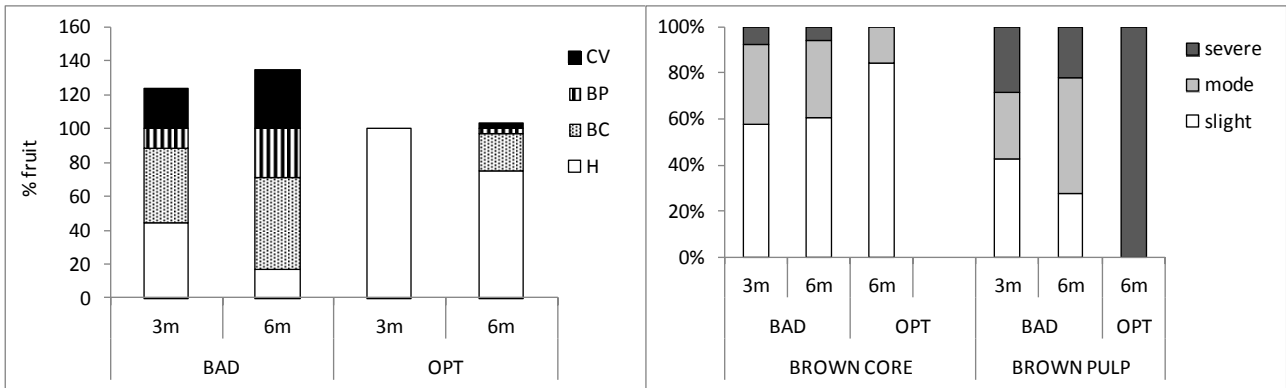
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850 **TABLE 6** – Year 2010: classification table of ‘Braeburn’ apples according to IB presence and position
851 (percentage of well-classified fruit in each class (bold): column: actual group, row: predicted class)

TRS variables	Classification table				
	Actual class	Group size	H	BC	BP
μ_a780, μ_s780	H	39	89.7	10.3	0.0
	BC	89	53.9	42.7	3.4
	BP	112	9.8	15.2	75.0

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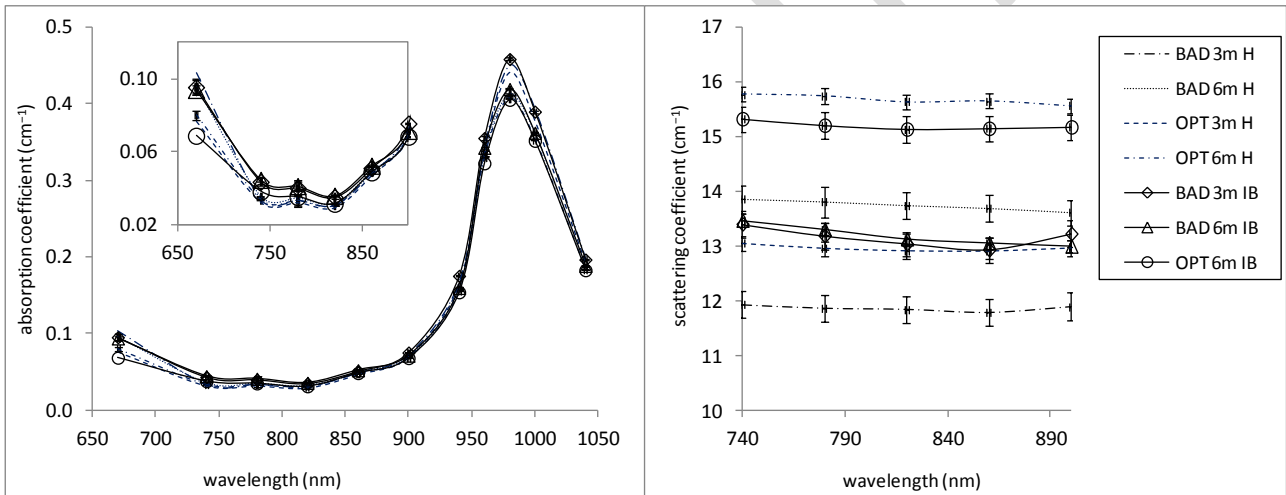
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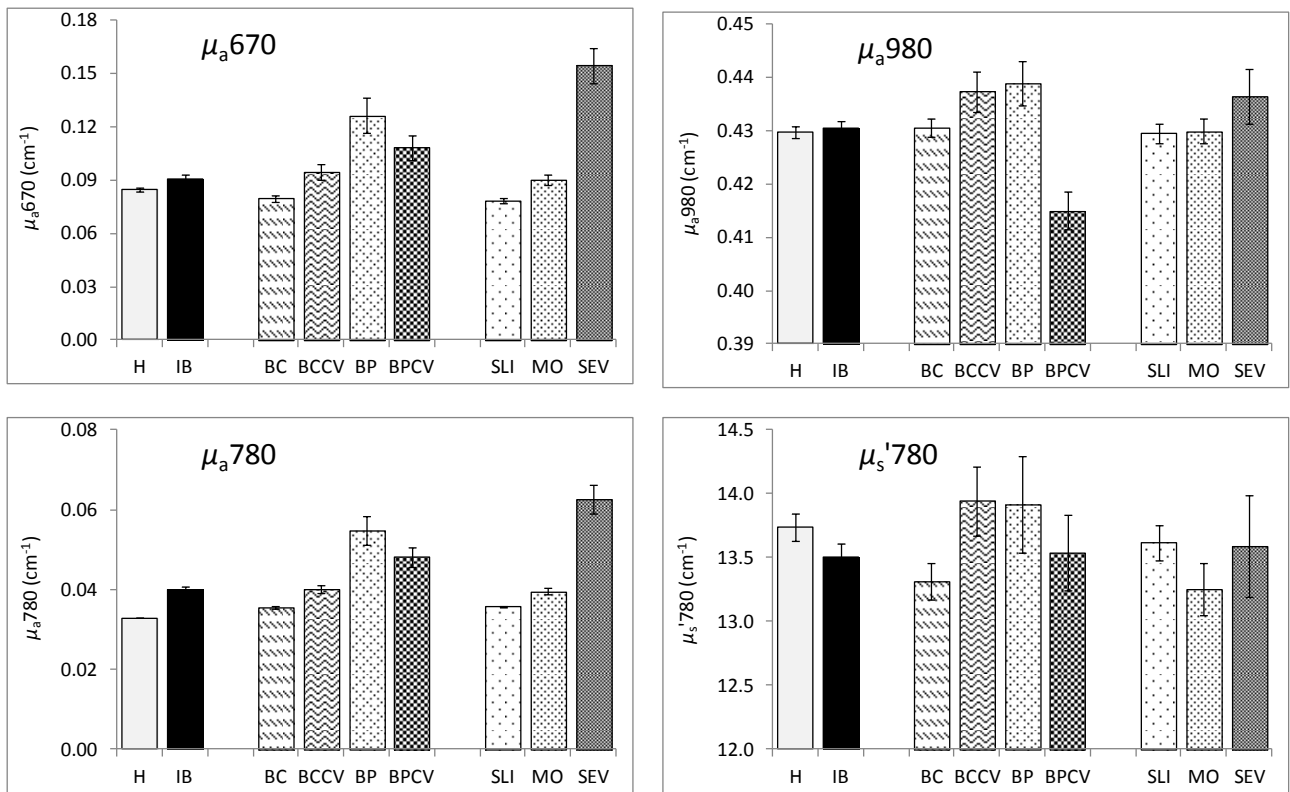
855 Fig. 1 – Year 2009: Incidence (left) and severity (right) of internal browning in ‘Braeburn’ apples
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 857 apples (H: healthy, BC: brown core, BP: brown pulp); the percent of fruit having also cavities (CV)
 858 is added in the incidence graph.

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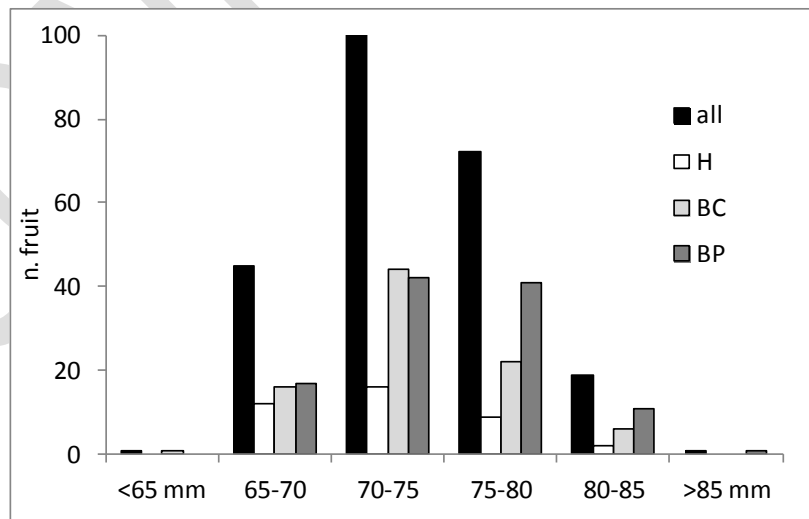
861 Fig. 2 – Year 2009: absorption (left) and scattering (right) spectra of healthy (H) and browned (IB)
 862 ‘Braeburn’ apples for 3-and 6- month storage under BAD and OPT conditions. Bars refer to
 863 standard errors
 864



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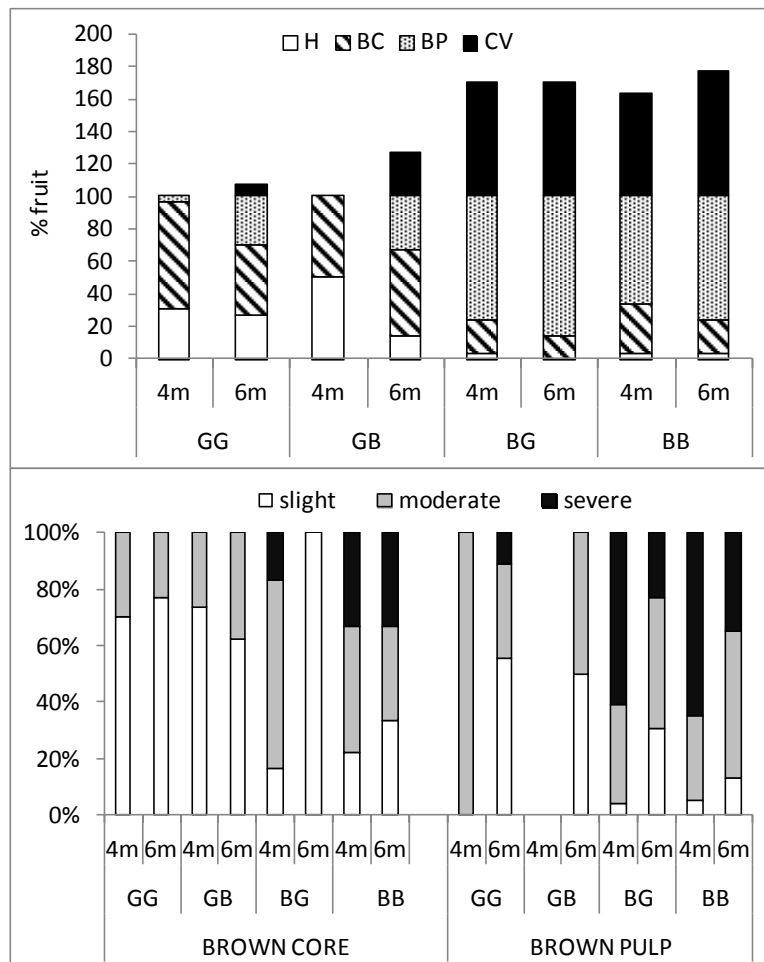
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874 Fig. 4 – Year 2010: fruit distribution among different diameter classes and according to browning
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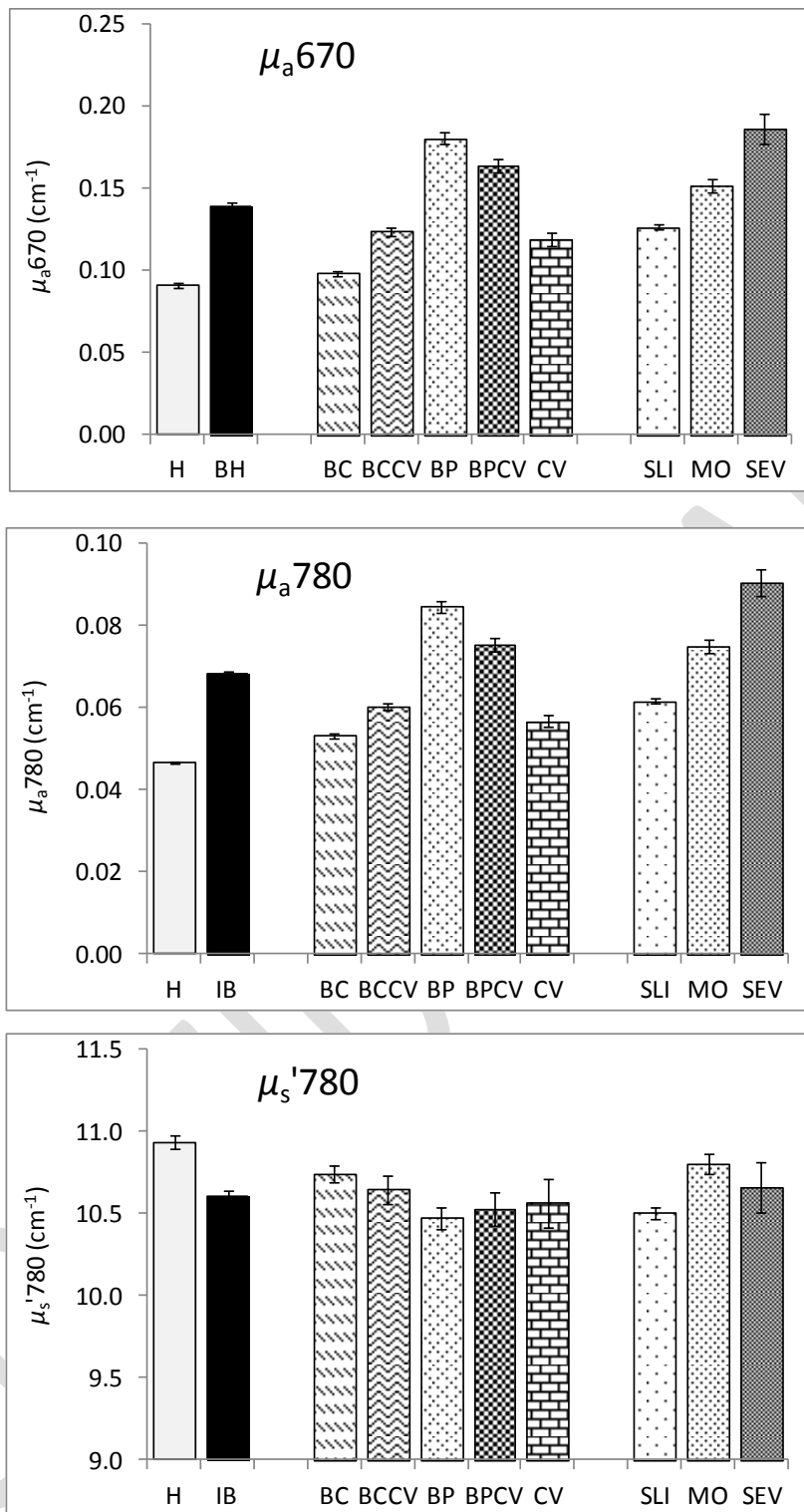
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878 Fig. 5– Year 2010: Incidence (top) and severity (bottom) of internal browning in ‘Braeburn’ apples
 879 submitted to optimal or suboptimal fertilization and stored for 4 and 6 months under optimal or
 880 browning inducing conditions in relation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

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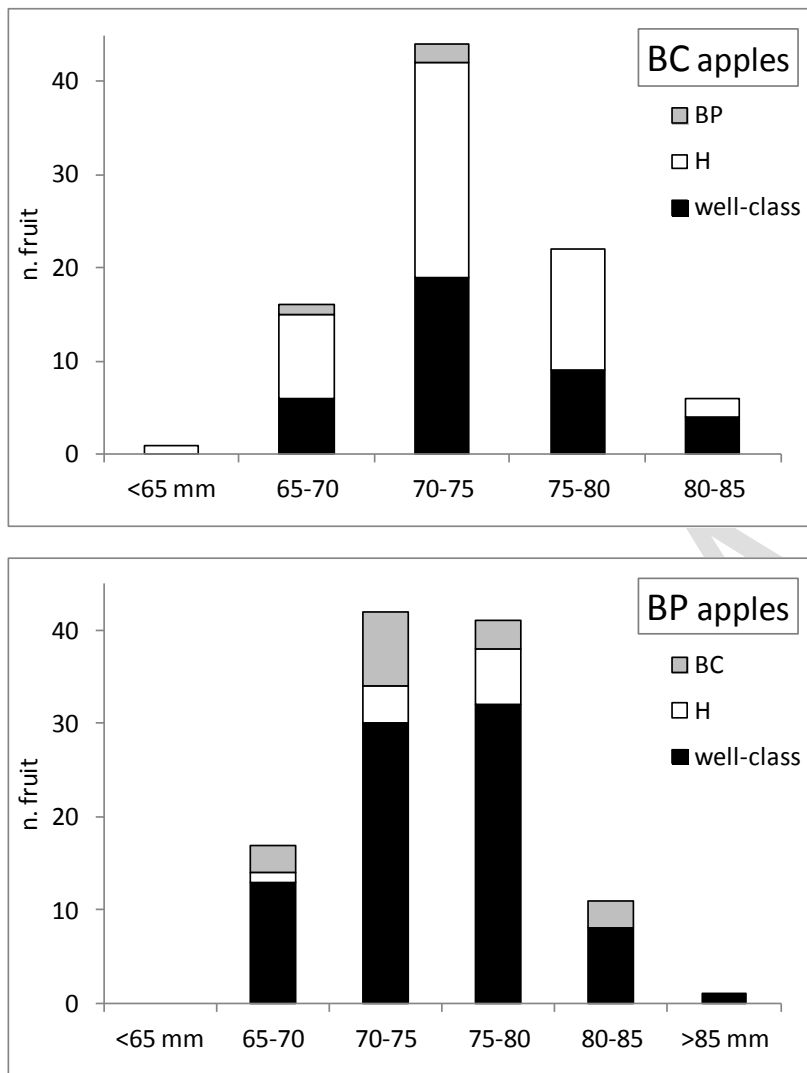


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 887 coefficient measured at 780 nm in relation to healthy (H) and browned (IB) tissue, browning
 888 position (BC: core; BP: pulp), presence of cavity (CV) or both (BCCV: BC and CV; BPCV: BP and
 889 CV), and browning severity (SLI: slight, MO: moderate, 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)

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894

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 896 diameter class. For classification data of BC apples see Table 6.

897