1   **Time- and spatially-resolved spectroscopy to determine the bulk optical**
2   **properties of ‘Braeburn’ apples after ripening in shelf life**

3

4   Maristella Vanoli^{a,*1}, Robbe Van Beers^{b*1}, Nadja Sadar^c, Anna Rizzolo^a, Marina Buccheri^a
5   Maurizio Grassi^a, Fabio Lovati^a, Bart Nicolaï^{b,d}, Ben Aernouts^{b,e} Rodrigo Watté^b, Alessandro
6   Torricelli^{f,g}, Lorenzo Spinelli^g, Wouter Saeys^b, Angelo Zanella^c

7

8   ^aCREA Research Centre for Engineering and Agro-Food Processing, Via Venezian 26, I-20133,
9   Milano, Italy

10   ^bKU Leuven Department of Biosystems, MeBioS, Kasteelpark Arenberg 30, 3001 Leuven,
11   Belgium

12   ^cResearch Centre Laimburg, Laimburg 6, 39040 Ora (BZ), Italy

13   ^dVCBT - Flanders Centre of Postharvest Technology, Willem de Croylaan 42, 3001 Leuven,
14   Belgium

15   ^eKU Leuven, Department of Biosystems, Cluster for Biosystems Engineering Technology,
16   Campus Geel, Kleinhoefstraat 4, 2440 Geel, Belgium

17   ^fPolitecnico di Milano, Dipartimento di Fisica, Piazza L. da Vinci, 32, 20133 Milano, Italy.

18   ^gIstituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche (IFN-CNR), Piazza L.
19   da Vinci, 32, 20133 Milano, Italy.

20

21

22   *Corresponding author:

23   E-mail address: maristella.vanoli@crea.gov.it (M. Vanoli)

24

25   ¹ These two authors contributed equally to this work.

26

27 Abstract

28 Bulk optical properties, in terms of absorption (μ_a) and reduced scattering coefficients (μ'_s), can be
29 used for the non-destructive monitoring of fruit quality during ripening. In this study, the
30 performance of time-resolved (TRS) and spatially-resolved (SRS) spectroscopy were compared by
31 analyzing 'Braeburn' apples over a 21 d period of ripening. Nine batches of 20 apples each were
32 measured on the blush side by TRS (540-1064 nm) and SRS (550-1000 nm). Every fruit was
33 analyzed for skin color, texture characteristics, relative internal space volume (RISV), total solid
34 soluble and titratable acidity contents. TRS absorption spectra showed two maxima, the highest at
35 980 nm (water) and the second at 670 nm (chlorophyll), while in SRS spectra the main peak was
36 measured at 550 nm (anthocyanins) followed by that at 670 nm. The values of $\mu_{a580\text{SRS}}$ and of
37 $\mu_{a670\text{SRS}}$ were much higher than those measured at the same wavelengths by TRS suggesting that
38 TRS and SRS actually explore the apple tissue (skin and/or flesh) in a different way. The values of
39 $\mu_{a980\text{TRS}}$ were higher than those of $\mu_{a980\text{SRS}}$, probably due to the fact that water content was lower
40 in the skin (mostly probed by SRS) than in the flesh (mostly probed by TRS). No significant
41 correlations were found between $\mu_{a580\text{SRS}}$ and $\mu_{a580\text{TRS}}$ and between $\mu_{a980\text{SRS}}$ and $\mu_{a980\text{TRS}}$ but a
42 low positive relationship was observed between $\mu_{a670\text{TRS}}$ and $\mu_{a670\text{SRS}}$. On the contrary, high
43 correlations were found between $\mu_{a670\text{SRS}}$ and the spectral index I_{AD} (index of absorbance
44 difference) related to chlorophyll in the skin and between $\mu_{a580\text{SRS}}$ and the spectral index ARI
45 (anthocyanin reflectance index), related to anthocyanin content in the peel, suggesting that $\mu_{a580\text{SRS}}$
46 is linked to the development of the red color in the peel. Both $\mu_{a670\text{TRS}}$ and $\mu_{a670\text{SRS}}$ decreased
47 during fruit ripening, indicating a decline in chlorophyll in the flesh and skin, respectively. During
48 the shelf life period, apples became soft and mealy, as mechanical and acoustic parameters
49 decreased and RISV increased. Fruit softening was accompanied by increasing values of both μ'_{sTRS}
50 and μ'_{sSRS} . The μ'_{sTRS} and μ'_{sSRS} were positively related to each other, were positively correlated to
51 RISV and negatively related to mechanical and acoustic parameters. Both the TRS and SRS
52 technique were able to follow ripening processes in 'Braeburn' apples during the shelf life period,

53 as absorption phenomena were related to changes in pigments present in the fruit flesh and skin,
54 while scattering events mirrored changes in the flesh texture.

55

56 **Keywords**

57 Absorption, scattering, apple, non-destructive techniques, quality, texture, TRS, SRS

58

59

60 **1. Introduction**

61 Bulk optical properties (BOP) of fruit can be used for the non-destructive monitoring of their
62 internal quality. During fruit development, maturation and ripening at harvest and after storage,
63 changes in chemical composition (synthesis/degradation of pigments, starch degradation, sugar,
64 water, pectins, ...) and in structural traits (cell size and shape, presence of middle lamella between
65 individual cells, cell wall, intercellular spaces) affect the BOP of fruit (Lu et al., 2020). Thus, the
66 BOP of fruit can be used to monitor physiological changes related to maturation, ripening and fruit
67 quality.

68 A fruit is visually opaque and can be modelled as a diffusive medium, where light distribution is
69 determined by the interplay between scattering and absorption phenomena. When photons travel
70 through fruit tissue they encounter the cellular structures which cause changes in light propagation
71 (scattering), and the molecules which are responsible for the photon absorption. The separation of
72 scattering and absorption information should lead to a better quantification of the fruits' chemical
73 and structural characteristics. Scattering can be a disturbing factor when determining chemical
74 compounds. Zude et al. (2011) showed that the analysis of cherry pigments was enhanced by taking
75 the influence of scattering events in account. Combining Vis/NIR spectroscopy with the distribution
76 of time-of-flight resolved readings, they achieved a dramatic reduction in bias and higher
77 coefficients of determination. Two approaches can be followed to separate the effect of scattering
78 from that of absorption. The first approach is to employ spectral indices normalized by readings at

79 specific wavelengths (780-850 nm) where no absorption occurs and only scattering is present (Ziosi
80 et al., 2008; Vanoli and Bucchini, 2012; Seifert et al., 2014). The second approach is to apply data
81 pre-processing on the acquired spectra, such as multiplicative scatter correction, standard normal
82 variate and derivatives (Naes et al., 2002). However, these techniques/methods try to reduce the
83 spectral variation due to light scattering, but do not actually measure absorption and scattering
84 separately in the fruit tissues. Scattering information can also be valuable as it relates to the physical
85 properties of fruit that change during maturation and ripening. In the last twenty years advanced
86 optical techniques have been developed, mainly in the biomedical field, with the aim to measure
87 light absorption and scattering in biological tissues. These methods have been introduced in the
88 postharvest sector (Lu et al., 2020). Time- and spatially-resolved reflectance spectroscopy (TRS and
89 SRS, respectively) and spatial-frequency domain imaging are already used for the nondestructive
90 determination of the absorption and scattering properties of fruit and vegetables (Ying et al., 2016;
91 Lu et al., 2020).

92 The fundamental optical parameters that characterize light propagation in biological tissue are the
93 bulk absorption coefficient μ_a , the bulk scattering coefficient μ_s and the anisotropy factor g . The
94 reduced (transport) scattering coefficient μ'_s is often used instead of μ_s , with $\mu'_s = \mu_s(1-g)$ (Martelli
95 et al., 2009). The absorption coefficient μ_a is the probability of a photon being absorbed (per unit
96 distance travelled) by plant pigments (e.g. chlorophylls, carotenoids, anthocyanins) and/or by other
97 chemical constituents in the tissue (e.g. water, sugars). The scattering coefficient μ_s is the probability
98 of a photon changing direction (per unit distance travelled) due to refractive index mismatches in
99 the tissue microstructures (Ying et al., 2016). Special attention has been paid to the optical properties
100 of apples because of the importance of this crop worldwide (Mendoza et al., 2014; Rowe et al.,
101 2014; Van Beers et al., 2015, 2017; Zhang et al., 2017). The absorption and the reduced scattering
102 coefficients were investigated in apple tissues by using TRS (Cubeddu et al., 2001; Rizzolo et al.,
103 2010; Seifert et al., 2014; Vanoli et al., 2014, 2018; Rizzolo and Vanoli, 2016) and SRS (Cen et al.,
104 2013; Nguyen Do Trong et al., 2014; Qin et al., 2009; Qin and Lu, 2008; Wang et al., 2020). SRS

105 and TRS combine multiple conventional Vis/NIR spectroscopic measurements at different source-
106 detector distances (resolved in the spatial domain) or record photon paths in the tissue (resolved in
107 the time domain) to separate the spectral information from fruit tissue samples into scattering and
108 absorption components by means of light propagation models (Tuchin, 2007; Lu et al., 2020).
109 TRS relies on the ability to measure the distribution of photon time-of-flight (DTOF) in a diffusive
110 medium (Torricelli et al., 2013). Following the injection of a light pulse within a turbid medium,
111 the DTOF measured at a fixed distance from the injection point (typically in the range 1-4 cm) will
112 be delayed, broadened and attenuated. The delay is a consequence of the finite time that light takes
113 to travel the distance between the source and the detector. Broadening is mainly due to the different
114 paths that photons undergo because of multiple scattering. Attenuation appears because absorption
115 reduces the probability of detecting a photon, and diffusion into other directions within the medium
116 decreases the number of detected photons in the considered direction. Fitting the experimental
117 DTOF with a solution of the diffusion approximation to the radiative transfer equation yields the
118 reduced scattering coefficient μ'_s and the absorption coefficient μ_a . A complete description of
119 available solutions for different geometries (e.g. semi-infinite, slab, parallelepiped, layered medium
120 or sphere) can be found in Martelli et al. (2009). In the range of measured values of the optical
121 coefficients, with the set-up and the theoretical model used, the uncertainty on the estimates for both
122 μ_a and μ'_s is usually below 10%. However, the error made in the assessment of the absorption line
123 shape is definitely smaller (<2%) (Torricelli et al., 2013). In the VIS/NIR range, TRS is able to
124 probe the tissue of most fruit to a depth of 1–2 cm with no or limited influence from the skin as the
125 spectra acquired on whole fruit for both absorption and scattering were shown to be very similar to
126 the spectra of the same fruit when peeled (Cubeddu et al., 2001; Eccher Zerbini et al., 2002; Saeys
127 et al., 2008; Torricelli et al., 2008; Spinelli et al., 2012; Rizzolo and Vanoli, 2016). TRS has been
128 applied mainly in postharvest studies on fruit and vegetables to estimate the internal attributes
129 related to maturity and ripeness, to detect internal defects and to discriminate fruit with different
130 texture and sensory characteristics (Rizzolo and Vanoli, 2016). The absorption coefficient measured

131 by TRS at 670 nm (μ_a670) is linked to the biological age of the fruit and represents an effective
132 maturity index for different fruit. The μ_a670 allowed the softening rate in nectarines and mangoes
133 to be predicted (Tjiskens et al., 2007; Eccher Zerbini et al., 2015). In apples, μ_a670 decreased during
134 fruit growth (Seifert et al., 2014) and ripening (Vanoli et al., 2005, 2015; Rizzolo et al., 2010;
135 Zanella et al., 2013) as well as during cold storage (Vanoli et al., 2011, 2015), more in air than in
136 controlled atmosphere (Rizzolo et al., 2010), and during shelf life (Zanella et al., 2013). Less ripe
137 apples (high μ_a670 values) showed a firm and crispy texture, while fruit having low μ_a670 values
138 (riper) were softer and often showed a mealy texture (Rizzolo et al., 2014; Vanoli et al., 2005, 2015).
139 As for scattering, the μ'_s changed with advancing maturity and during storage depending on the
140 apple cultivars and allowed apples with different texture characteristics to be distinguished (Rizzolo
141 et al., 2014, Vanoli et al., 2015, 2018; Rizzolo and Vanoli, 2016).

142 In SRS, instead of collecting a reflectance signal at one location as a function of time like in TRS,
143 the reflectance is collected at multiple distances from a point illumination using a steady-state point
144 source. The acquired signal is the diffuse reflection as a function of the distance from the point of
145 illumination (Farrell et al., 1992). The photons collected at a larger distance will have travelled a
146 longer path through the tissue and as a result will have interacted with a larger volume of the sample.
147 The acquired diffuse reflectance profile is affected by the interconnected effects of scattering and
148 absorption, such that an inverse modelling technique is required to estimate the BOP from these
149 measurements. As with TRS, the diffusion theory has been widely used to estimate the BOP from
150 SRS data. Although the assumption that light propagation in biological tissues is dominated by
151 scattering is often violated, many researchers have used this approach to determine the BOP of agro-
152 food products, such as cucumbers (Lu et al., 2011), apples (Askoura et al., 2016), citrus fruit
153 (Lorente et al., 2015), peaches, pears, plums and tomatoes (Qin and Lu, 2008, Mollazade et al.,
154 2012). Besides this approach, an inverse Monte Carlo technique has been used because of its higher
155 accuracy in describing reflectance at short illumination-detector distances (Baranyai and Zude,
156 2009, Mollazade et al., 2012). However, this method is less practical due to the long computation

157 times needed. Different measurement configurations involving different types of illumination,
158 detectors and operating conditions have been proposed for SRS. Configurations using optical fiber
159 arrays or reflectance imagery are most commonly used (Lu et al., 2020). Fibers require good contact
160 with the sample, which is often inconvenient for solid food products. A probe SRS setup was used
161 to investigate the microstructure-texture relationships for the quality assessment of ‘Braeburn’
162 apples during storage (Nguyen Do Trong et al., 2014), while a contact-setup with 30 detecting fibers
163 was used for the assessment of tomato firmness (Huang et al., 2018a), SSC and pH (Huang et al.,
164 2018b). These authors showed that the SRS method achieved better predictions of the quality
165 parameters compared to single-point spectroscopy (Huang et al., 2018b). On the other hand, system
166 configurations using an imaging approach (Van Beers et al., 2015) can be used in a contactless way,
167 which is often advantageous for food products. Contactless systems using white light illumination
168 and a post-dispersive imaging strategies have been used on several fruit and vegetables, including
169 apple, pear, peach, kiwifruit, sugar beet, potato, tomato and cucumber (Rady et al., 2015, Adebayo
170 et al., 2016). Monochrome light in combination with a camera system for contactless measurement
171 of the diffuse reflectance has been used on kiwifruit (Baranyai and Zude, 2009), citrus fruit (Lorente
172 et al., 2015) and for the quality determination of apples (Qing et al., 2007, Mollazade and Arefi,
173 2017, Wang et al., 2020). A good correlation between μ_s' and apple porosity ($R^2 = 0.89$) was recently
174 shown by Wang et al. (2020).

175 In summary, both TRS and SRS have shown potential for the quality determination of agro-food
176 products. However, these techniques have not yet been compared on the same fruit. Therefore, the
177 objectives of this study were (i) to compare the TRS and SRS techniques in relation to the acquisition
178 of the optical properties (absorption and reduced scattering coefficients) on the same apple samples,
179 highlighting similarities and differences; (ii) to study the performance of the TRS and SRS
180 techniques in the postharvest quality determination of ‘Braeburn’ apples in terms of physical-
181 chemical characteristics (skin color, total soluble and acidity contents, mechanical and acoustic
182 properties). To our knowledge, this is the first time that the performance of the TRS and SRS

183 techniques have been studied on the same samples allowing a direct and reliable comparison of
184 these techniques in the context of fruit quality assessment.

185

186 **2. Materials and methods**

187 *2.1 Apple fruit*

188 Apples cv ‘Braeburn’ from trees grafted on M9 EMLA rootstock were harvested in 2016 from trees
189 of uniform vigor and crop loads grown in a GAP-certified orchard in Laimburg (Bolzano, Italy) at
190 220 m above sea level (a.s.l). Trees were grown in a single row system with a planting density of
191 3000 trees/ha and trained as super spindle. To obtain a wide range of firmness levels, apples with a
192 diameter between 75-80 mm were harvested earlier than optimal harvest date for long term storage
193 and showed the following standard maturity parameters (measured on 45 apples; mean $\pm$ standard
194 deviation): starch pattern index SPI (1-5) = 2.1 ± 0.3 ; firmness = 87.3 ± 5.6 N; titratable acidity = 6.1
195 ± 0.25 g malic acid kg^{-1} ; soluble solids contents = 9.5 ± 0.54 %. A total of 180 apples were used in
196 order to have 9 batches of 20 apples each with different quality characteristics. For this purpose, one
197 batch was treated with 1000 nL L^{-1} 1-MCP (SmartFresh, AgroFresh Co., Spring House, PA, USA)
198 soon after harvest in order to have apples with quality characteristics closed to that of just picked
199 fruit. This batch, together with a second batch of untreated fruit, was stored at 1.3 °C, 70 % R.H. for
200 1 month. The remaining seven batches were put in shelf life at 20 °C, 70 % R.H and each batch was
201 dedicated to a specific shelf life removal time, viz after 2, 4, 6, 8, 10, 14 and 21 d. As this work aims
202 at comparing the performance of the TRS and SRS techniques by measuring the same apple samples,
203 and being TRS at Milan (Italy) and SRS at Leuven (Belgium), in order to guarantee that optical
204 measurements could be done about at the same time, after each specific shelf life period apples were
205 stored at 1.3 °C, 70 % R.H. for a defined period as reported in Table 1. In this way, the quality
206 characteristics of apples belonging to a defined shelf life period were maintained by storing fruit at
207 1.3 °C until analysis.

208

Table 1- Description of apple samples

sample	1-MCP	days at 20 °C	days at 1.3 °C
d0-MCP	yes	0	30
d0	no	0	30
d2	no	2	28
d4	no	4	26
d6	no	6	24
d8	no	8	22
d10	no	10	20
d14	no	14	16
d21	no	21	9

After 1 month all the 9 apple batches were packed in one-layer cardboard boxes with rigid plastic insert trays for mechanical protection of individual fruit and transported in a temperature-controlled truck to Leuven (Belgium) for SRS measurements and then to Milan (Italy) for TRS and quality measurements. This meant the apples with different shelf life periods could be measured at exactly the same time. Per shelf life condition, skin color and optical measurements by SRS and TRS were performed on the sun exposed side (blush side) of the 20 apples, as this side would also be encountered in field conditions. Then, every fruit was analyzed for texture characteristics by measuring mechanical-acoustic parameters, immediately deep frozen, and kept at -20°C until analysis of the relative internal space volume (RISV), the total soluble solids content (SSC) and titratable acidity (TA).

2.2 Quality analysis

2.2.1 Skin color and spectral indices

Skin color and spectral indices were measured on the blush side with a Spectrophotometer CM-2600d (Minolta Co, Japan) in correspondence of the TRS and SRS measurement point. Skin color was measured in the L^* , a^* , b^* color space using the primary illuminant D65 and 2° observer; from a^* and b^* values, hue (H°) and chroma (C^*) were computed according to $H^{\circ} = \arctan(b^*/a^*) \times 360 / (2 \times 3.14)$ and $C^* = (a^{*2} + b^{*2})^{-2}$. Concomitantly to skin color, spectral indices related to chlorophyll (I_{AD}), carotenoid (I_{CAROT}) and anthocyanin (ARI) contents were

231 computed from local spectral measurements according to Rizzolo et al. (2015) and Gitelson et al.
232 (2001). Reflectance spectra from 360 to 740 nm at 10 nm intervals were acquired on the blush side
233 of fruit. The index of absorbance difference $I_{AD} = A_{670} - A_{720}$ was computed according to Ziosi et al.
234 (2008) after converting the reflectance data at 670 and 720 nm into absorbance using $A = \log(100/R)$;
235 the carotenoid index $I_{CAROT} = R_{700} (1/R_{480} - 1/R_{680})$ was calculated according to Merzlyak et al.
236 (2003) as modified by Herrero-Langreo et al. (2012), while the anthocyanin reflectance index ARI
237 $= 1/R_{550} - 1/R_{700}$ was computed according to Gitelson et al. (2001).

238

239 2.2.2 Texture properties

240 Texture properties were measured through mechanical and acoustic tests by using a TA-XT plus
241 Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with an acoustic emission
242 detector (AED, Stable Microsystems) in order to simultaneously profile the mechanical force
243 displacement together with the corresponding acoustic response. The measurements were carried
244 out on two peeled areas of each fruit, in correspondence of the TRS and SRS measurement point
245 and of the opposite one. Then the two data were averaged. The mechanical profile was obtained
246 using an 11 mm diameter cylindrical plunger at the cross-head speed of 3.33 mm/s to a depth of 8
247 mm; acoustic response was profiled by the AED device with a frequency cut off at 3.125 kHz,
248 placing the microphone at about 10 mm far from the apple in correspondence of mid-height of the
249 sample.

250 Table 2 – Mechanical and acoustic parameters extracted from the mechanical-acoustic profile

251

<u>Typology</u>	<u>Name</u>	<u>Label</u>	<u>Description</u>
<u>Mechanical</u>	Maximum force (N)	<u>Fmax</u>	<u>Maximum force value within 8 mm</u>
	Stiffness (N/mm)	<u>St</u>	<u>Slope measured from 0 to 2 mm</u>
	Work (N×mm)	<u>W</u>	<u>Energy related to flesh penetration up to 8mm</u>
<u>Acoustic</u>	Average sound (mV)	<u>MEAN</u>	<u>Average sound value</u>
	Linear distance (mV×s)	<u>LD</u>	<u>Length of the acoustic profile</u>
	No. of peaks	<u>PK</u>	<u>Total number of acoustic peaks >0.003 V</u>
	Maximum value of acoustic peaks (mV)	<u>acMAX</u>	<u>Maximum value among the acoustic peaks</u>
	Value of acoustic peak at Fmax (mV)	<u>acFmax</u>	<u>Value of the acoustic peak corresponding to Fmax</u>

252

Following the procedure of Vanoli et al. (2018), a total of eight parameters were extracted, three from the mechanical graph and five from the acoustic profile, from the combined mechanical/acoustic profile (Table 2).

The intercellular spaces were estimated by computing the relative internal space volume $RISV=100\times[1-(d_f-d_j)]$ where d_f is the density of the fruit (i.e., ratio of fruit mass to fruit volume under water), and d_j is the density of the fruit juice extracted as described for SSC and TA determinations (Baumann and Henze, 1983).

2.2.3 SSC and TA

Soluble solids content (SSC) and titratable acidity (TA) were determined on frozen fruit, thawed at room temperature and squeezed for recovering the juice. SSC was measured putting some drops of juice on an automatic refractometer (RFM81, Bellingham-Stanley Ltd., England) and the data were expressed as %. TA was measured by titrating 5 g of juice plus 50 mL of distilled water with 0.1 N NaOH to pH 8 using a 682 titroprocessor equipped with a 665 dosimat (Metrohm, Herisau, Switzerland) and the data were expressed g malic acid kg⁻¹ juice.

2.3 Optical measurements

2.3.1 Time resolved reflectance set-up

An existing instrumentation for TRS developed at the Department of Physics of Politecnico di Milano was used (Fig. 1). Briefly, this instrument consists of: a supercontinuum fiber laser (SC450-6W, Fianium, UK) as light source that provides white-light picosecond pulses, adjustable in power by a variable neutral-density attenuator; a filter wheel loaded with 14 band-pass interference filters for spectral selection in the range 540-1064 nm; a custom-made solid-state SiPM detector, developed at the Department of Physics of Politecnico di Milano (Martinenghi et al., 2016). The photon distribution of time-of-flight is measured by a time-correlated single-photon counting board (SPC-130, Becker&Hickl, Germany). Light is delivered to the sample by means of a multimode

279 graded-index 100- μm glass optical fiber and is collected from the sample at a distance of 1.5 cm by
 280 exploiting a multimode graded-index 1-mm plastic optical fiber. The set-up is controlled by means
 281 of a computer and all the operations are fully automated. Typical temporal system resolution is 100
 282 ps (full width at half maximum of the instrument response function). As for the measurement
 283 protocol, TRS measurements were performed at seven wavelengths: 580 nm, 670 nm, 730 nm, 780
 284 nm, 830 nm, 980 nm, and 1064 nm. These wavelengths were selected according to the absorption
 285 spectra of chlorophyll and water as the main absorbers within the wavelength range considered: no
 286 other absorber shows a detectable contribution in this spectral range (□670 to 1064 nm).
 287 Furthermore, the three wavelengths 730 nm, 780 nm, and 830 nm allow a reliable determination of
 288 the reduced scattering coefficient of the samples as no significant absorption expected at these
 289 wavelengths. After an automatic adjustment of the injected signal power, the acquisition time was
 290 1 s for each wavelength, which guarantees an adequate signal-to-noise ratio.

291

292

293 Fig 1: Schematic of the TRS setup. GRIN: graded-index; POF: plastic optical fiber; CFD: constant
 294 fraction discriminator signal; SYNC: synchronization signal; TCSPC: time-correlated single-photon
 295 counting.

296

297 2.3.2 Spatially resolved laser reflectance set-up

298 The SRS setup used in the current research has been described in more detail by Van Beers et al.
299 (2015) and illustrated in Fig. 2. This setup uses a monochrome laser illumination resulting from the
300 combination of a supercontinuum laser (SC450-4, Fianium Ltd., Southampton, UK) with a
301 monochromator (Oriel Cornerstone 260 ¼ m, Newport, Irvine, USA). The laser has a total optical
302 power of 4 W and a spectral broadening over the range from 450 nm to 2400 nm. The monochrome
303 light is guided into an optical fiber (multimode, NA 0.22, 200 µm core diameter) after which it is
304 focused on the sample (COL-UV/VIS, Avantes BV, Apeldoorn, The Netherlands). The focused
305 light generates a diffuse reflectance glow spot, representing photons leaving the sample after one or
306 more scattering events, thus reflecting the optical properties of the measured sample. To measure
307 the diffuse reflectance at multiple distances from the point illumination, the diffuse reflectance glow
308 spot is imaged with a 12-bit panchromatic CCD camera (TXG-14NIR, Baumer, Frauenfeld,
309 Switzerland). This camera, combined with a 16 mm monofocal manual iris lens (C1614A, Pentax,
310 Tokyo, Japan) in combination with an extender (10 mm) (2-EX, Pentax, Tokyo, Japan), has been
311 mounted above the sample. Using this setup configuration, the diffuse reflectance glow spot was
312 measured in the 550 nm to 1000 nm range with a step size of 5 nm, resulting in 91 images per apple.
313 The intact apples were placed on a sample holder, which assured a constant distance from the apple
314 to the CCD camera (12.4 cm) using a manually controlled vertical translational stage (Van Beers et
315 al., 2015). First, the obtained raw images were dark corrected using a dark image, after which a
316 white reference measurement allowed the conversion to a relative reflectance image. This white
317 reference measurement was performed by directing the beam into an integrating sphere (5 cm Ø)
318 (AvaSphere-50-REFL, Avantes BV, Apeldoorn, The Netherlands) and measuring the diffuse
319 reflectance coming out of the sphere port (1 cm Ø). After these corrections, a diffuse reflectance
320 profile with the relative reflectance as a function of the distance from the point illumination was
321 obtained through a radial averaging procedure (Van Beers et al., 2015). Under the assumption of
322 tissue isotropy, the radial averaging procedure assures signal robustness against small irregularities
323 in the image (e.g. lenticels, dark spots, cracks, ...). Moreover, the signal-to-noise ratio is increased

324 by the averaging procedure, especially at further distances from the point illumination. LabView 8.5
 325 (National Instruments Corporation, Texas, USA) was used for the automatic control of all the used
 326 components and for programming the measurement procedure.

327

328

329 Fig. 2 - Schematic overview of the SRS setup used for Vis/NIR hyperspectral spatially resolved
 330 laser reflectance measurements in the 550-1000 nm range; BS = Polka-dot beam splitter; CCD =
 331 charge-coupled device; D = Silica detector; DAQ = data acquisition card; F = Filter; FW = Filter
 332 Wheel; L = Lens; M = Mirror. Reproduced with permission from Van Beers et al. (2015).
 333

334 2.4 Data processing

335 2.4.1 Optical properties estimation from time resolved reflectance signals

336 For the analysis of TRS data (Fig. 3), in order to retrieve the BOP of samples, i.e. the absorption
 337 coefficient μ_a and the reduced scattering coefficient μ'_s , a model for photon diffusion in turbid media
 338 was exploited (Martelli et al., 2009). In particular, a diffuse spherical model was used for describing
 339 photon migration inside the apple: to this purpose, the radius of each apple was computed by
 340 measuring with a digital caliper the minimum and the maximum equatorial diameters, averaging the
 341 two measures and dividing per 2 to obtain the radius. As a matter of fact, a diffuse spherical medium

342 is pretty close to the real geometry of an apple. In this way, the accuracy of the retrieved optical
343 properties of apples was increased with respect to the use of a semi-infinite diffuse medium as is
344 generally done. Indeed, the use of spherical geometry allows the photons lost throughout the apple
345 boundaries to be taken into account, these would be interpreted, by adopting the semi-infinite
346 geometry, as an overestimation of the absorption coefficient with effects also on reduced scattering
347 coefficient. It was noticed that the adoption of the spherical model in inversion procedures is very
348 time consuming, and this is the main reason why it has been rarely used in the past. As for the
349 reduced scattering coefficient, after inspecting the values retrieved at the seven wavelengths
350 measured, we noticed that the values were more or less constant, without a well-defined decreasing
351 trend with wavelength, as foreseen by the Mie's law. This fact, together with the limited wavelength
352 dependence of μ'_s for apples found in past studies (Seifert et al., 2014; Vanoli et al., 2014), suggested
353 the reduced scattering coefficient should be calculated by averaging the μ'_s values retrieved at 730
354 nm, 780 nm, and 830 nm and considered constant for all the wavelengths. As stated above, the three
355 chosen wavelengths allow a reliable estimation of the reduced scattering coefficient due to the
356 corresponding low absorption values. Furthermore, by fixing the reduced scattering coefficient, we
357 also get a more reliable estimation for the absorption coefficient, mainly at the wavelengths where
358 it assumes large values.

359

Fig. 3 - Schematic illustration of the TRS data analysis.

2.4.2 Optical properties estimation from spatially resolved reflectance signals

The approach presented by Watté et al. (2015) was used to estimate the **BOP** from the acquired diffuse reflectance profiles, as illustrated in Fig. 4. This approach involves measurement of a set of liquid phantoms with known optical properties to calibrate a so-called metamodel. To do this, a phantom calibration grid with different levels of scattering and absorption was measured using the SRS setup. As the **BOP** of these phantoms are known, a metamodel can be built linking a diffuse reflectance profile to a set of **BOP**. The liquid phantoms were prepared by mixing distilled water with both a scattering and an absorbing component. As a scattering agent, Intralipid® 20_% (Fresenius Kabi, Sweden) was used, while a water-soluble, light-stable azo dye called Naphthol Blue Black (195243, Sigma Aldrich, Missouri, USA) was added as an absorber. The concentration of these components was chosen in order to create liquid phantoms with the desired absorbing and scattering properties. In the considered wavelength range, from 550 nm to 1000 nm, naphthol blue black (NBB) has a maximal absorbing wavelength at 618 nm. A total of 49 calibration phantoms

376 were prepared, including 7 absorption (ranging from 0 cm^{-1} to 58 cm^{-1} at 620 nm) and 7 scattering
377 levels (ranging from 3.1 cm^{-1} to 106.2 cm^{-1} at 600 nm) (Watté et al., 2015). Compared to the
378 phantoms described in Watté et al. (2015), one extra absorption level (level 7: $\mu_a = 58\text{ cm}^{-1}$ at 620
379 nm) was included. The exact BOP values at all wavelengths were determined through measurements
380 with a double integrating sphere (DIS) setup. For a detailed description of this setup and its
381 application on liquid phantoms the reader is referred to Aernouts et al. (2013) and Watté et al.
382 (2015). The measurements on these liquid optical phantoms were used to build a so-called
383 metamodel, which links the diffuse reflectance profiles to the corresponding BOP. Stochastic
384 Kriging, also known as regression Kriging, was used to build this metamodel (Watté et al., 2013).
385 Actually, a metamodel was built for every distance from the point of illumination to predict the
386 diffuse reflectance value from the BOP values. This model predicts the diffuse reflectance values
387 from the absorption coefficient μ_a and the reduced scattering coefficient μ'_s , independent of the
388 wavelength. By including three independent measurements per phantom, the stochastic or
389 regression Kriging was trained to take measurement variance into account and to avoid overfitting
390 (Watté et al., 2013). This forward light propagation model was developed using the ooDACE
391 toolbox in Matlab (Couckuyt et al., 2012). As the forward model describes the SRS profiles as a
392 function of the BOP, it has to be inverted to estimate BOP values from SRS measurements. To do
393 so, an optimization procedure was implemented that searches the set of BOP for which the simulated
394 SRS profiles match with the measured SRS profiles for the different liquid phantoms. This is
395 performed by minimizing a cost function at each wavelength, defined by Watté et al. (2013). The
396 Nelder-Mead algorithm was used to find the optimum for this inverse problem. This optimization
397 algorithm was used with several starting points evenly distributed over the search space in order to
398 find the global minimum even when the optimization problem would not be strictly convex (Watté
399 et al., 2013).

Fig. 4 - Schematic illustration of the metamodeling approach for estimating the BOP from the SRS measurements

2.5 Statistical analysis

Optical properties acquired by TRS and SRS at specific wavelengths and quality data were submitted to analysis of variance and means were compared by a Tukey's test at $P \leq 0.05$ % (Statgraphics v.7, Manugistic Inc., Rockville, MD, USA). Pearson correlation analysis was performed in order to explore the relationships among TRS and SRS optical properties and among BOP and quality parameters.

3. Results

3.1 TRS and SRS absorption and scattering spectra

Absorption and scattering spectra estimated from the TRS and SRS measurements on 'Braeburn' apples stored for □ 1 month after harvest without any shelf life treatment (sample d0, see Table 1) are illustrated in Fig. 5 and 6, respectively. A large variation in the optical properties among apples belonging to the same batch can be noticed for both the TRS and SRS measurements.

418

419 Fig. 5 – Absorption coefficient μ_a spectra estimated by TRS (a) and SRS (b) for ‘Braeburn’ apples
 420 (n=20) stored for 1 month after harvest without any shelf life treatment (sample d0, see Table 1).
 421 Each line corresponds to an apple. The inset figure shows the absorption coefficient spectra for SRS
 422 in the 700- 1000 nm range.
 423

424 TRS absorption spectra (Fig. 5a) show two maxima, the first at 670 nm, indicating the presence of
 425 chlorophyll, and the second at 980 nm corresponding to water, as previously observed on apples
 426 (Vanoli et al., 2011, 2014; Seifert et al., 2014; Rizzolo and Vanoli, 2016). High variability can also
 427 be observed at 580 nm.

428 Considering the SRS absorption spectra (Fig. 5b), two absorption peaks can be noticed: the first at
 429 550 nm, corresponding to anthocyanins and the second at 670 nm; a third but smaller peak can be
 430 observed at 980 nm. Similar patterns in the absorption spectra of various apple cultivars have been
 431 reported by Qin and Lu (2008), Nguyen Do Trong et al. (2014) and Cen et al. (2016).

432 Comparing the TRS and SRS absorption spectra, we notice that for TRS spectra the main peak was
 433 at 980 nm, while for SRS the highest values were measured at 550 nm. Considering all data, on
 434 average, μ_{a670} values were $0.093 \pm 0.046 \text{ cm}^{-1}$ (mean $\pm$ standard deviation) for TRS and $1.351 \pm$
 435 0.604 cm^{-1} for SRS and those for μ_{a580} were $0.036 \pm 0.010 \text{ cm}^{-1}$ for TRS and $2.748 \pm 0.965 \text{ cm}^{-1}$
 436 for SRS. On average, the μ_a values at 670 nm obtained with SRS were about 15 times larger than
 437 those for TRS, while those at 580 nm were about 70 times higher. However, the absorption
 438 coefficient values measured at 980 nm were more comparable, but significantly higher ($p < 0.0001$)
 439 for TRS than for SRS, being $\mu_{a980\text{TRS}} = 0.399 \pm 0.065 \text{ cm}^{-1}$ and $\mu_{a980\text{SRS}} = 0.287 \pm 0.080 \text{ cm}^{-1}$.

440 The values of the absorption coefficients measured in the 700-900 nm range are small and the
 441 spectra quite flat for both TRS and SRS, indicating a limited absorption in this region by the
 442 chemical constituents of the apples. These μ_a values were always higher for SRS ($0.058 \pm 0.053 \text{ cm}^{-1}$,
 443 average $\pm$ standard deviation) than for TRS ($0.023 \pm 0.005 \text{ cm}^{-1}$).
 444 As for the reduced scattering coefficients, when μ'_s were measured by TRS (Fig. 6a), no change can
 445 be observed with wavelength progression, because we fixed it during the data analysis (see Sec.
 446 2.4.1 for a detailed explanation). For the μ'_s values estimated from SRS measurements (Fig. 6b),
 447 low values can be observed at 550 nm, a minimum at 670 nm, and a quite flat trend in the 720-1000
 448 nm range in agreement with Nguyen Do Trong et al., (2014) and Cen et al. (2016). The valleys
 449 appearing in the scattering spectra around 670 nm could be explained as the inability of the inverse
 450 metamodeling method to completely separate the absorption and the scattering phenomena for apple
 451 tissue in the wavelength regions where the absorption is relatively high compared to scattering
 452 (Nguyen Do Trong et al., 2014).

454
 455 Fig. 6 – Reduced scattering coefficient μ'_s spectra estimated by TRS (a) and SRS (b) for ‘Braeburn’
 456 apples (n=20) stored for 1 month after harvest without any shelf life treatment (sample d0, see
 457 Table 1). Each line corresponds to an apple.

459 Comparing the scattering values at 800 nm, where no pigments are present, the values of μ'_s
 460 measured by TRS and SRS were comparable, being $\mu'_{s\text{TRS}} = 16.96 \pm 2.29 \text{ cm}^{-1}$ and $\mu'_{s\text{SRS}} = 16.04 \pm$
 461 2.48 cm^{-1} , but significantly lower for SRS ($p < 0.001$).

462 The relationships among the TRS and SRS optical properties were studied by selecting the
 463 absorption coefficients measured at the same wavelengths, that is 580, 670, 730, 780, 830 and 980
 464 nm, and the reduced scattering coefficients measured at 800 nm (Table 3). Significant correlations
 465 among μ_a measured by TRS and SRS were found only between $\mu_{a670\text{SRS}}$ and $\mu_{a670\text{TRS}}$ ($r=0.642$)
 466 and between $\mu_{a670\text{SRS}}$ and $\mu_{a580\text{TRS}}$ ($r=0.622$). In contrast, a higher significant and positive
 467 correlation ($r=0.800$) exists between the reduced scattering coefficients measured by the two
 468 spectroscopic techniques.

469

Table 3- Pearson correlation matrix between TRS and SRS optical properties considering the common wavelengths. Significant correlations are in bold (n=180; $r \geq 0.5$, $P\text{-value} \leq 0.05$).

	μ_{a580} TRS	μ_{a670} TRS	μ_{a730} TRS	μ_{a780} TRS	μ_{a830} TRS	μ_{a980} TRS	$\mu'_{s\text{TRS}}$	μ_{a580} SRS	μ_{a670} SRS	μ_{a730} SRS	μ_{a780} SRS	μ_{a830} SRS	μ_{a980} SRS	$\mu'_{s\text{SRS}}$
$\mu_{a580\text{TRS}}$	1													
$\mu_{a670\text{TRS}}$	0.964	1												
$\mu_{a730\text{TRS}}$	0.485	0.332	1											
$\mu_{a780\text{TRS}}$	0.270	0.116	0.938	1										
$\mu_{a830\text{TRS}}$	0.217	0.078	0.916	0.958	1									
$\mu_{a980\text{TRS}}$	0.085	0.106	-0.011	-0.055	-0.067	1								
$\mu'_{s\text{TRS}}$	-0.061	-0.208	0.183	0.184	0.165	-0.192	1							
$\mu_{a580\text{SRS}}$	-0.168	-0.284	0.020	0.077	0.042	-0.117	0.385	1						
$\mu_{a670\text{SRS}}$	0.622	0.642	0.126	0.029	-0.004	0.143	-0.387	-0.030	1					
$\mu_{a730\text{SRS}}$	0.185	0.156	0.089	0.036	0.016	0.122	-0.096	0.152	0.524	1				
$\mu_{a780\text{SRS}}$	0.047	0.020	0.056	0.029	0.011	0.123	-0.068	0.102	0.338	0.926	1			
$\mu_{a830\text{SRS}}$	0.015	0.003	0.018	-0.004	-0.018	0.124	-0.137	0.073	0.365	0.843	0.862	1		
$\mu_{a980\text{SRS}}$	0.093	0.109	0.046	0.020	-0.004	0.159	-0.333	0.002	0.416	0.816	0.791	0.715	1	
$\mu'_{s\text{SRS}}$	-0.067	-0.197	0.118	0.104	0.112	-0.169	0.800	0.428	-0.338	-0.063	-0.050	-0.173	-0.403	1

470

471

472 3.2 Evolution of TRS and SRS optical properties during shelf life

473 The evolution of the absorption coefficients measured at 580 nm, 670 nm and 980 nm by TRS and
 474 SRS during the shelf life period and the evolution of the reduced scattering coefficients measured
 475 at 800 nm for untreated apples (no 1-MCP) are illustrated in Fig. 7 and 8, respectively, while data
 476 of optical properties for 1-MCP treated apples (sample d0-MCP, see Table 1) in comparison with
 477 untreated ones at d0 (sample d0, see Table 1) are reported in Table 4. We chose the absorption at

580 nm and at 670 nm, as these wavebands are related to absorption by pigments (anthocyanins and chlorophyll, respectively) and the absorption at 980 nm as it is related to water.

No statistically significant difference was found among 1-MCP treated fruit and untreated ones at d0 (sample d0, see Table 1) for both absorption and scattering coefficients (Table 4). No tissue browning was found in 1-MCP treated apples.

Table 4 - Absorption coefficients μ_a at 580 nm, 670 nm and 980 nm and scattering coefficient μ'_s measured by TRS and SRS on 'Braeburn' apples treated or not with 1-MCP at harvest and stored for □ 1 month (see Table 1) (mean $\pm$ standard error)

	<u>sample d0-MCP</u>	<u>sample d0</u>
<u>$\mu_{a580\text{TRS}}(\text{cm}^{-1})$</u>	<u>0.035 ± 0.001</u>	<u>0.038 ± 0.003</u>
<u>$\mu_{a670\text{TRS}}(\text{cm}^{-1})$</u>	<u>0.079 ± 0.004</u>	<u>0.103 ± 0.012</u>
<u>$\mu_{a980\text{TRS}}(\text{cm}^{-1})$</u>	<u>0.409 ± 0.012</u>	<u>0.419 ± 0.009</u>
<u>$\mu'_{s\text{TRS}}(\text{cm}^{-1})$</u>	<u>15.06 ± 0.211</u>	<u>14.84 ± 0.350</u>
<u>$\mu_{a580\text{SRS}}(\text{cm}^{-1})$</u>	<u>3.084 ± 0.194</u>	<u>2.705 ± 0.181</u>
<u>$\mu_{a670\text{SRS}}(\text{cm}^{-1})$</u>	<u>1.802 ± 0.105</u>	<u>1.816 ± 0.090</u>
<u>$\mu_{a980\text{SRS}}(\text{cm}^{-1})$</u>	<u>0.372 ± 0.017</u>	<u>0.328 ± 0.015</u>
<u>$\mu'_{s\text{SRS}}(\text{cm}^{-1})$</u>	<u>14.36 ± 0.320</u>	<u>14.36 ± 0.441</u>

Considering the trend in untreated apples, $\mu_{a670\text{TRS}}$ was constant during the first 2 d of shelf life, showed a maximum after 4 d, decreased up to 8 d, remained constant up to 14 d and decreased at 21 d reaching the lowest values (Fig. 7a). The $\mu_{a670\text{SRS}}$ showed a quite similar evolution, being quite constant during the first 4 d, decreasing after 6 d keeping these values up to 10 d and then decreasing until the end of the shelf life period (Fig. 7b).

The $\mu_{a580\text{TRS}}$ and the $\mu_{a980\text{TRS}}$ did not significantly change during shelf life (Fig. 7a). The $\mu_{a580\text{SRS}}$ remained constant up to 14 d, then increased showing the highest values at the end of the shelf life period, while the $\mu_{a980\text{SRS}}$ decreased up to 6 days of shelf life keeping these values up to 21 d (Fig. 7b). The $\mu_{a980\text{TRS}}$ was significantly higher ($p < 0.0001$) than $\mu_{a980\text{SRS}}$ at each day of shelf life, except for apples treated at harvest with 1-MCP (Table 4).

500

501 Fig. 7 – Changes in the absorption coefficients μ_a for 'Braeburn' apples measured 1 month after
 502 harvest at 580 nm, 670 nm and 980 nm by TRS (a) and SRS (b) following 8 different shelf life
 503 temperature treatments of 0, 2, 4, 6, 8, 10, 14 and 21 d at 20 °C, with fruit then kept at 1.3 °C for
 504 the remaining time. Bars indicate the standard error of the mean.
 505

506 Considering the coefficient of variation, TRS measurements showed the highest variability at 670
 507 nm and the lowest at 980 nm; SRS measurements had the highest variability at 580 nm and 670 nm
 508 and the lowest at 980 nm. Comparing the variability in TRS and SRS measurements, higher
 509 variability was found for SRS measurements at 580 nm and at 980 nm than for TRS, while the
 510 opposite was found for the measurement at 670 nm.

511 The reduced scattering coefficients measured by TRS and SRS had a similar behavior during shelf
 512 life showing an increasing trend up to the end of the shelf life period (Fig. 8). Moreover, the two
 513 spectroscopic techniques showed about the same variability for μ'_s estimation. The μ'_s measured by
 514 TRS and SRS did not significantly differ from each other considering the day of shelf life, except
 515 at 6, 8 and 10 d when the TRS μ'_s values were higher ($p < 0.05$).
 516

517

518 Fig. 8 – Changes in the reduced scattering coefficient μ'_s for 'Braeburn' apples measured 1 month
 519 after harvest at 800 nm by TRS and SRS following 8 different shelf life temperature treatments of
 520 0, 2, 4, 6, 8, 10, 14 and 21 d at 20 °C, with fruit then kept at 1.3 °C for remaining time. Bars indicate
 521 the standard error of the mean.
 522

523 3.4 Evolution of quality parameters during shelf life

524 The evolution of 'Braeburn' apple quality in terms of skin color, mechanical-acoustic parameters
 525 and SSC and TA contents during the shelf life period is reported in Table 5.

526 Considering skin color, L^* remained constant up to 8 d of shelf life, then slightly increased reaching
 527 the highest values at 14 d; a^* was constant up to 14 d and dramatically increased in the following
 528 week; b^* did not change up to 10 d, increased up to 14 d keeping these values up to 21 d; C^*
 529 increased from day 4 towards the end of the shelf life period; H° did not significantly change during
 530 the shelf life period. All these changes in color parameters indicate, as expected, that with shelf life
 531 skin color became lighter, redder and more intense. Shelf life time influenced also the spectral
 532 indices. I_{AD} , which is linked to chlorophyll content, remained constant up to 4 d and then decreased
 533 up to 21 d. A decreasing trend of I_{AD} with shelf life in 'Braeburn' apples was also reported by
 534 Zanella et al. (2013). I_{CAROT} , related to carotenoids content, slightly increased up to 8 d showing a
 535 dramatic increase in the last week of the shelf life period, whereas ARI, related to anthocyanin
 536 pigments, did not change up to 8 d, decreased up to 14 d, when it reached the lowest values and
 537 increased the following week showing the same values observed at 0 d. The increase of I_{CAROT} could

be due not only to an increase in carotenoid content in the skin, but also to a decrease in the interference of chlorophyll on the spectral measurements, as suggested by Merzlyak et al. (2003).

Table 5 – Changes in the qualitative parameters for ‘Braeburn’ apples measured ~1 month after harvest following 7 different shelf life temperature treatments of 0, 2, 4, 6, 8, 10, 14 and 21 d at 20°C, with fruit then kept at 1.3°C for remaining time (see Table 1)

	d0-MCP	d0	d2	d4	d6	d8	d10	d14	d21
L*	45.2 b	46.6 b	47.2 b	46.1 b	47.4 b	46.6 b	49.1 ab	53.0 a	49.7 ab
a*	28.1 b	26.4 b	26.2 b	26.5 b	30.2 b	32.6 ab	28.6 b	29.8 b	39.2 a
b*	19.8 c	21.4 bc	22.5 bc	21.0 c	22.6 bc	22.5 bc	25.7 ab	29.2 a	27.5 a
H°	35.7 a	40.2 a	41.4 a	39.0 a	37.5 a	35.1 a	42.4 a	44.6 a	35.5 a
C*	35.0 e	34.9 e	36.1 de	34.4 e	38.4 cd	39.9 bc	39.3 c	42.5 b	48.3 a
I _{AD}	0.485 a	0.482 a	0.458 ab	0.509 a	0.395 bc	0.365 c	0.383 c	0.288 d	0.128 e
I _{CAROT}	4.396 c	4.465 c	4.931 bc	4.616c	5.521 bc	6.219 b	5.809 bc	5.734 bc	8.440 a
ARI	0.094 a	0.087 a	0.088 a	0.088 a	0.088 a	0.094 a	0.075 ab	0.060 b	0.094 a
F _{max} (N)	85.9 a	71.2 b	57.2 c	57.0 c	49.9 d	49.1 d	50.6 d	48.9 d	47.0 d
St (N mm ⁻¹)	23.8 a	21.6 b	18.3 c	18.1 cd	16.4 de	15.3 efg	15.5 ef	14.1 fg	13.6 g
W (N×mm)	492.6 a	413.8 b	331.0 c	332.2 c	296.4 d	286.5 de	294.4 d	281.8 de	270.7 e
acMAX (mV)	25.1 a	19.1 b	12.9 c	11.6 cd	10.1 cde	8.4 def	7.3 def	8.6 ef	5.9 f
MEAN (mV)	7.6 a	6.0 b	3.8 c	3.5 cd	3.1 cde	2.8 def	2.5 ef	2.6 ef	2.1 f
LD (mV×s)	2571 a	2498 b	2436 c	2433 c	2428 c	2421 c	2418 c	2422 c	2417 c
PK (n°)	55.6 a	41.0 b	17.4 c	16.4 c	12.3 cd	8.9 de	6.2 e	6.3 de	4.3 e
acFmax (mV)	12.0 a	9.7 b	6.3 c	5.8 c	4.9 cd	3.9 de	3.6 de	3.8 de	2.7 e
RISV (%)	15.0 f	15.3 ef	16.0 cde	15.7 def	16.2 bcd	16.2 bcd	16.9 ab	16.7 bc	17.6 a
SSC (%)	11.3 a	10.8 bc	11.2 ab	11.2 ab	10.6 c	10.8 bc	11.0 abc	10.8 abc	11.2 ab
TA (g malic acid kg ⁻¹)	5.0 a	4.8 a	4.3 b	4.8 a	4.3 bc	4.1 bc	4.3 b	4.1 bc	3.9 c

Within each row, means followed by different letters are statistically different at Tukey's test (p < 0.05).

Mechanical and acoustic parameters were higher in 1-MCP treated apples than in untreated ones at day 0 (Table 5). The maximum force ($F_{\max}$) decreased up to day 6, then remained constant until the end of the shelf life period, while stiffness (St) and work (W) decreased over the entire period at 20 °C. The acoustic parameters decreased during the shelf life period, except for linear distance (LD), which decreased from day 0 to day 2 and then remained constant. RISV showed an increasing trend with shelf life. TA slightly decreased, while SSC did not change in a significant way. Spectral indices were well correlated with skin color parameters: I_{AD} was negatively correlated to C^* ($r=-0.89$) and a^* ($r=-0.59$); I_{CAROT} was positively correlated to a^* ($r=0.89$) and C^* ($r=0.82$) and negatively with H° ($r=-0.67$) and L^* ($r=-0.50$), while ARI was positively related to a^* ($r=0.75$) and negatively to L^* ($r=-0.93$), b^* ($r=-0.79$) and H° ($r=-0.89$). I_{AD} was also positively related to mechanical parameters with r ranging from 0.50 to 0.61 and to acidity ($r=0.56$) and negatively to

554 RISV ($r=-0.52$), while I_{CAROT} was negatively related to acidity ($r=-0.60$). Among the color
555 attributes, C^* was related (negatively) to mechanical and to some acoustic parameters with r ranging
556 from -0.52 to -0.63 and to RISV ($r=-0.56$), while C^* and a^* were negatively related to TA with
557 $r=-0.63$ and -0.55 , respectively. Mechanical and acoustic parameters were well related each other
558 with r ranging from 0.77 to 0.99 and also to RISV with r ranging from -0.51 to -0.66 and to TA
559 with r ranging from 0.52 to 0.65 .

560

561 3.4 Relationships among optical properties estimated by TRS and SRS and quality parameters

562 Table 6 reports the relationships among the TRS and SRS optical properties and the quality
563 parameters of 'Braeburn' apples. The correlation among μ_a730 , μ_a780 and μ_a830 measured by TRS
564 and SRS and quality parameters are not reported as not significant. The μ_a580_{TRS} was negatively
565 related to a^* , C^* and positively related to I_{AD} , while μ_a670_{TRS} was negatively related to a^* , C^* and
566 I_{CAROT} and positively related to I_{AD} . Higher correlations were found between μ_a580_{SRS} and μ_a670_{TRS}
567 and color parameters and spectral indices: μ_a580_{SRS} was negatively related to L^* , b^* and h° and
568 positively related to a^* , I_{CAROT} and ARI, while μ_a670_{SRS} was negatively related to a^* , C^* and I_{CAROT}
569 and positively related to I_{AD} . The μ_a670_{SRS} was also positively related to St , W and TA. No
570 significant correlations were found between the quality parameters and absorption coefficients
571 measured by TRS in the 730-980 nm range, as well as between the quality parameters and the
572 absorption coefficients measured by SRS in the 730-830 nm range while positive correlations were
573 found between μ_a980_{SRS} and mechanical properties and PK .

574 As for scattering, $\mu'_{\text{s TRS}}$ and $\mu'_{\text{s SRS}}$ showed positive correlations with C^* and I_{CAROT} and $\mu'_{\text{s TRS}}$ had
575 also a negative correlation with I_{AD} (Table 6). The $\mu'_{\text{s TRS}}$ was also negatively related to all
576 mechanical and acoustic parameters, except for LD , while $\mu'_{\text{s SRS}}$ was negatively related to St , PK
577 and $acFmax$ with lower correlation coefficient values. Both $\mu'_{\text{s TRS}}$ and $\mu'_{\text{s SRS}}$ were positively related
578 to RISV and negatively to TA (Table 6).

579

580

581

582

583

Table 6 - Pearson correlation matrix between TRS and SRS optical properties and quality characteristics. Significant correlations are in bold (n=180; $r \geq 0.5$, P-value ≤ 0.05).

	μ_a580	μ_a670	μ_a980	$\mu'_{s\text{ TRS}}$	μ_a580	μ_a670	μ_a980	$\mu'_{s\text{ SRS}}$
	TRS	TRS	TRS		SRS	SRS	SRS	
L^*	0.166	0.201	0.150	0.016	-0.694	-0.129	0.003	0.080
a^*	-0.501	-0.572	-0.220	0.438	0.643	-0.578	-0.241	0.385
b^*	0.098	0.087	0.070	0.222	-0.548	-0.257	-0.147	0.313
h°	0.365	0.405	0.180	-0.156	-0.683	0.217	0.074	-0.068
C^*	-0.521	-0.604	-0.173	0.615	0.315	-0.830	-0.364	0.632
I_{AD}	0.507	0.554	0.087	-0.501	-0.112	0.881	0.213	-0.484
I_{CAROT}	-0.476	-0.567	-0.197	0.556	0.632	-0.624	-0.315	0.529
ARI	-0.336	-0.393	-0.155	0.161	0.759	-0.124	-0.083	0.100
Fmax	0.114	0.105	0.118	-0.550	0.017	0.496	0.510	-0.482
St	0.123	0.142	0.139	-0.601	0.021	0.575	0.501	-0.544
W	0.119	0.112	0.126	-0.561	0.029	0.523	0.512	-0.488
acMAX	0.046	0.052	0.114	-0.555	0.033	0.455	0.461	-0.468
MEAN	0.024	0.033	0.136	-0.591	0.027	0.471	0.493	-0.498
LD	-0.019	-0.028	0.105	-0.482	0.086	0.367	0.456	-0.386
PK	0.034	0.041	0.121	-0.600	0.032	0.471	0.531	-0.507
acFmax	0.035	0.058	0.120	-0.622	-0.025	0.467	0.489	-0.572
RISV	-0.115	-0.163	-0.135	0.570	0.108	-0.420	-0.327	0.580
SSC	-0.078	-0.184	-0.129	0.171	0.340	0.019	0.044	0.168
TA	0.328	0.377	0.133	-0.600	-0.328	0.509	0.304	-0.574

584

585

586

4. Discussion

587

588

589

590

591

592

593

594

595

596

During the shelf life period the absorbance spectra in the 550-1000 nm wavelength of 'Braeburn' apples revealed several peaks, which can be attributed to carotenoids (around 500 nm), anthocyanins (around 550 nm), chlorophyll (around 670 nm) and water (around 980 nm). The content and the proportions of chlorophylls, carotenoids and anthocyanins determine the fruit color: chlorophylls are responsible for the green color, carotenoid constitutes the background yellow color, while anthocyanins are responsible for the red color. Pigments also contribute to flesh coloration, even if chlorophylls and carotenoids are present in lower amounts than in the peel and anthocyanins are not commonly found in the flesh of commercial apple cultivars, but only in the flesh of some apple genotypes, such as crab apples and red-fleshed apples (Ampomah-Dwamena et al., 2012; Delgado-Pelayo et al., 2014; Honda and Moriya, 2018; Rupasinghe et al., 2010). In the peel, the carotenoid

content is much lower than that of chlorophyll, while the opposite was found for the flesh (Ampomah-Dwamena et al., 2012; Delgado-Pelayo et al., 2014). Despite the low presence of chlorophyll in the flesh, chlorophylls have been quantified in the flesh of white fleshed fruit such as ‘Royal Gala’, ‘Pink Lady’ and ‘Fuji’ (Ampomah-Dwamena et al., 2012; Delgado-Pelayo et al., 2014). In addition, the carotenoid composition differs in the peel and flesh as lutein is the main carotenoid of the skin, while in the flesh there is a prevalence of violaxanthin and neoxanthin (Ampomah-Dwamena et al., 2012; Delgado-Pelayo et al., 2014). It is well known that in the skin chlorophyll content declines, while carotenoids and anthocyanins increase during apple ripening (Solovchenko et al., 2005; Tian et al., 2018). In the flesh, carotenoids generally decrease during fruit maturation and increase during ripening. Lutein was completely lost at ripeness, while xanthophylls appear only in ripe fruit (Ampomah-Dwamena et al., 2012). Chlorophyll is present at high contents at the beginning of fruit development and decreases with fruit maturation and ripening (Ampomah-Dwamena et al., 2012).

Comparing the absorption spectra obtained from the TRS and SRS measurements, some differences appear. In TRS spectra, there were 2 peaks, the highest at 980 nm and the second at 670 nm, while in SRS spectra the main peak was measured at 550 nm followed by that measured at 670 nm. The values of μ_a580_{SRS} and of μ_a670_{SRS} were much higher than those measured at the same wavelengths by TRS suggesting that TRS and SRS actually explore the apple tissues (skin and flesh) in a different way. Van Beers et al. (2017) also found that absorption peaks around 678 nm in the apple flesh were at least 10 times smaller than those found in the skin confirming that μ_a670_{TRS} is more linked to the flesh, while μ_a670_{SRS} is strongly affected by the skin. Considering the correlations among the SRS and TRS absorption coefficients, a positive but low correlation was found between μ_a670_{TRS} and μ_a670_{SRS} , while higher relationships were found between μ_a670_{SRS} and skin color (C^* , I_{AD}). The correlations between I_{AD} and μ_a670_{TRS} were lower than those calculated for μ_a670_{SRS} , in agreement with Zanella et al. (2013) who found a logarithmic correlation between μ_a670_{TRS} and I_{AD} and between μ_a670_{TRS} and NDVI (a spectral index related to the chlorophyll content of the skin) and a

623 linear and very high relationship between I_{AD} and NDVI suggesting that μ_a670_{TRS} has a higher
624 contribution from the flesh, while I_{AD} and NDVI are dominated by the skin. In addition, the fact that
625 the absorption peak of chlorophyll is much higher than that of water in the SRS measurements
626 suggests that the photons captured by the SRS technique have not travelled so deep into the flesh
627 where the absorption by water is much higher than the absorption by chlorophyll, as found by
628 Nguyen Do Trong et al. (2014).

629 Considering the absorption coefficients measured at 580 nm by TRS and SRS, no significant
630 correlations were found between μ_a580_{TRS} and μ_a580_{SRS} . On the other hand, μ_a580_{SRS} showed a high
631 and positive relationship to ARI, indicating that it is linked to the development of the red color in
632 the skin, as observed by Van Beers et al. (2017) for ‘Braeburn’ and ‘Kanzi’ apples. The μ_a580_{TRS}
633 was negatively related to a^* and b^* and positively with I_{AD} with relative low r values. In this case
634 it is difficult to find some relationships with the presence of pigments in the flesh, as this wavelength
635 is more related to anthocyanins than to carotenoids, while the flesh of ‘Braeburn’ apples is pale
636 yellow. Van Beers et al. (2017) noticed substantial absorption around 500 nm in the cortex of
637 ‘Braeburn’ apples which might be attributed to the presence of carotenoids.

638 As for the water absorption peak at 980 nm, the values of μ_a980_{TRS} were higher than those of
639 μ_a980_{SRS} . This is probably due to the fact that water content was lower in the skin (mostly probed
640 by SRS) than in the flesh (mostly probed by TRS). Thus, TRS could be used for the detection of
641 water and other relevant indicators bound to water such as dry matter, even if further research are
642 needed to better study the relationships between TRS measures and the water content in the fruit
643 flesh also with the purpose of developing predicting model.

644 During the shelf life period, both μ_a670_{TRS} and μ_a670_{SRS} showed a similar decreasing trend. The
645 decreasing trends of μ_a670_{TRS} and of μ_a670_{SRS} with shelf life are in agreement with previous findings
646 on ‘Braeburn’ and on other apple cultivars (Zanella et al., 2013; Nguyen Do Trong et al., 2014;
647 Seifert et al., 2014; Rizzolo and Vanoli, 2016; Van Beers et al., 2017). The maximum value found
648 for μ_a670_{TRS} in sample 4d could be due to the fact that optical measurements were carried out on

different fruit at each shelf life temperature treatment variant and hence, with fruit having different biological age. The decrease of $\mu_{a670\text{TRS}}$ during ripening means that there is a decrease in the chlorophyll content of the fruit flesh as described by Ampomah-Dwamena et al. (2012) in different apple genotypes during fruit growth and ripening and by Vanoli et al. (2011) for ‘Pink Lady®’ apples during cold storage.

The $\mu_{a580\text{TRS}}$ did not significantly change during shelf life, while $\mu_{a580\text{SRS}}$ followed more or less the same trend of the color parameter a^* which is linked to the red color: this is a further evidence that SRS absorption coefficients are strongly influenced by the content of skin pigments. However, a stronger correlation with the internal parameters may be obtained if we could eliminate the information on the skin from the SRS data. Similarly to what was found for $\mu_{a580\text{SRS}}$, in a study on optical properties of developing apples, Van Beers et al. (2017) found that the absorption coefficient values at 550 nm for the skin samples increased significantly towards the moment of harvest (and afterwards) in ‘Braeburn’ and ‘Kanzi’ apple cultivars.

During the shelf life period, the apples became soft, as mechanical and acoustic parameters decreased and RISV increased. This trend is accompanied by changes in the reduced scattering coefficients which showed an increasing trend for both $\mu'_{s\text{TRS}}$ and $\mu'_{s\text{SRS}}$. An upward trend of $\mu'_{s\text{TRS}}$ with shelf life has been modelled by Rizzolo et al. (2019) and was previously observed by Vanoli et al. (2013) in ‘Braeburn’ and in ‘Cripps Pink’ apples. Nguyen Do Trong et al. (2014) found a slight increase of $\mu'_{s\text{SRS}}$ in ‘Braeburn’ apples during 14 d of storage at 18 °C, while Van Beers et al. (2017) observed a significantly increasing trend of the reduced scattering coefficient in the skin of ‘Braeburn’ apples, but a downwards trend in the cortex during fruit maturation, as also observed by Seifert et al. (2014) in developing ‘Elstar’ apples. Probably, during fruit maturation, the expansion of newly differentiated cells resulted in less cell walls and air pores on a volume basis with the consequent reduction of the scattering events, as suggested by Seifert et al. (2014). Cen et al. (2013) observed that the values of the reduced scattering coefficient generally decreased in ‘Golden

674 Delicious' and 'Granny Smith' apples over a 30_d period of storage at 22_°C, concurrently with
675 decreases in the cells' area and equivalent diameter and in the acoustic/impact firmness.

676 In our work, we found that μ'_{sTRS} , and μ'_{sSRS} to a lesser extent, were negatively related to mechanical
677 and acoustic parameters, in agreement with Rizzolo et al. (2010, 2014) and Vanoli et al. (2010,
678 2015, 2018) for μ'_{sTRS} . During the shelf life period, 'Braeburn' apples softened as firmness
679 significantly decreased. Softening involves degradation and modifications of the cell wall and
680 middle lamella structures and Vanoli et al. (2009) observed that pectin solubilization was
681 accompanied with increasing values of μ'_{sTRS} and decreasing firmness.

682 During the shelf life period, 'Braeburn' apples probably became mealy, as acoustic parameters also
683 significantly decreased together with a dramatic increase of RISV: high values of mechanical and
684 acoustic parameters accompanied with low RISV are characteristic of firm, crispy and juicy apples,
685 while low values of mechanical and acoustic parameters with high RISV are typical of mealy apples,
686 as found for 'Kanzi' and 'Braeburn' apples, respectively, by Vanoli et al. (2018). Thus, the increase
687 of μ'_{sTRS} observed in the present work could be due to the change of flesh texture from firm, crispy
688 and juicy at harvest to mealy at the end of shelf life, as observed by Rizzolo et al. (2014, 2019). In
689 addition, Rizzolo et al. (2010, 2014) and Vanoli et al. (2010, 2015, 2018) found that μ'_{sTRS} was
690 inversely related to sensory firmness, crispness and juiciness and positively related to mealiness and
691 RISV. In the present work, we also found a similar positive correlation of μ'_{sTRS} and μ'_{sSRS} with
692 RISV, in agreement with Wang et al. (2020) who found a clear ($R^2=0.89$) and positive linear
693 relationship between μ'_{sSRS} and porosity and pore surface density measured up to 3 mm in depth
694 from the fruit surface, suggesting that variation in scattering is more linked to the variation in
695 porosity/pore surface density, whereas cell size and cell density played a limited role in the
696 scattering differences. This scenario was also confirmed by the fact that among eight microstructure
697 parameters used in a PLS model to predict scattering, porosity contributed most in predicting the
698 variation in scattering properties (Wang et al., 2020). This confirms that both SRS and TRS capture
699 valuable information on the changes in flesh porosity.

700

701 5. Conclusions

702 In this study, the performance of TRS and SRS were compared by measuring ‘Braeburn’ apples
703 after ripening in shelf life. TRS and SRS explore apple fruit tissue in different ways. It was found
704 that the absorption coefficients measured by TRS were more related to the pigment and water
705 content of the fruit flesh, while the absorption coefficients measured by SRS were strongly affected
706 by the pigments present in the skin. The reduced scattering coefficients measured by TRS and SRS
707 were correlated in a similar way to fruit porosity and texture, even if TRS probed the cortex to a
708 depth of $\square$ 1.0 cm while SRS explored a shallower volume of fruit cortex underneath the skin. Both
709 the techniques are able to follow the ripening processes occurring in ‘Braeburn’ apples during the
710 shelf life period, as the changes in absorption phenomena were related to changes in pigments
711 present in the flesh and in the skin, while scattering events mirrored changes in the flesh texture.

712

713 Acknowledgements

714 The present work was carried out in the framework of the project 'Monitoring key environmental
715 parameters in the alpine environment involving science, technology and application' (MONALISA),
716 funded by the Autonomous Province of Bolzano (Italy).

717

718

719 References

720 Adebayo, S. E., Hashim, N., Abdan, K., Hanafi, M., 2016. Application and potential of
721 backscattering imaging techniques in agricultural and food processing – A review. J. Food Eng.
722 169, 155–164. <http://dx.doi.org/10.1016/j.jfoodeng.2015.08.006>
723 Aernouts, B., Zamora-Rojas, E., Van Beers, R., Watté, R., Wang, L., Tsuta, M., Lammertyn, J.,
724 Saeys, W., 2013. Supercontinuum laser based optical characterization of Intralipid® phantoms in

the 500-2250 nm range. Optics Express, 21(26), 32450–32467.
<https://doi.org/10.1364/OE.21.032450>.

Ampomah-Dwamena, C., Dejnopratt, S., Lewis, D., Sutherland, P., Volz, R.K., Allan, A.C., (2012).
Metabolic and gene expression analysis of apple (*Malus × domestica*) carotenogenesis. J. Exp.
Bot., 63(2): 695–709. <https://doi.org/10.1093/jxb/err313>

Askoura, M., Vaudelle, F., L’Huillier, J.-P., 2016. Experimental study of light propagation in apple
tissues using a multispectral imaging system. Photonics. doi:10.3390/photonics3030050

Baranyai, L., Zude, M., 2009. Analysis of laser light propagation in kiwifruit using backscattering
imaging and Monte Carlo simulation. Comput. Electron. Agric. 69, 33–39.
doi:10.1016/j.compag.2009.06.011

Baumann, H., Henze, J., 1983. Intercellular space volume of fruit. Acta Hort. 138, 107–112.
<https://doi.org/10.17660/ActaHortic.1983.138.11>.

Cen, H., Lu, R., Mendoza, F., Beaudry, R.M., 2013. Relationship of the optical absorption and
scattering properties with mechanical and structural properties of apple tissue. Postharvest Biol.
Technol. 85, 30–38. <http://dx.doi.org/10.1016/j.postharvbio.2013.04.014>.

Cen, H., Lu, R., Nguyen-Do-Trong, N., Saeys, W., 2016. Spatially Resolved Spectroscopic
technique for measuring optical properties of food. In: Renfu Lu (Ed) “Light scattering technology
for food property, quality and safety assessment.”, CRC Press, Taylor & Francis Group, Boca
Raton, FL 33487-2742, ISBN: 978-1-4822-6334-3, pp.159–185.

Cubeddu, R., D’Andrea, C., Pifferi, A., Taroni, P., Torricelli, A., Valentini, G., Ruiz-Altisent, M.,
Valero, C., Ortiz, C., Dover, C., Johnson, D., 2001. Time-resolved reflectance spectroscopy
applied to the non-destructive monitoring of the internal optical properties in apples. Appl.
Spectrosc. 55, 1368–1374.

748 Couckuyt, I., Forrester, A., Gorissen, D., De Turck, F., Dhaene, T., 2012. Blind Kriging:
 749 Implementation and performance analysis. *Advances in Engineering Software*, 49 (1), 1–13.
 750 <https://doi.org/10.1016/j.advengsoft.2012.03.002>

751 Delgado-Pelayo, R., Gallardo-Guerrero, L., Hornero-Mendez, D., 2014. Chlorophyll and
 752 carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Res. Int.* 65
 753 (PB): 272-281. <http://dx.doi.org/10.1016/j.foodres.2014.03.025>.

754 Eccher Zerbini, P., Grassi, M., Cubeddu, R., Pifferi, A., Torricelli, A., 2002. Nondestructive
 755 detection of brown heart in pears by time-resolved reflectance spectroscopy. *Postharvest Biol.*
 756 *Technol.* 25, 87–97. [https://doi.org/10.1016/S0925-5214\(01\)00150-8](https://doi.org/10.1016/S0925-5214(01)00150-8).

757 Eccher Zerbini, P., Vanoli, M., Rizzolo, A., Grassi, M., Pimentel, R. M. A., Spinelli, L., Torricelli,
 758 A., 2015. Optical properties, ethylene production and softening in mango fruit. *Postharvest Biol.*
 759 *Technol.* 101, 58–65. <https://doi.org/10.1016/j.postharvbio.2014.11.008>.

760 Farrell, T. J., Patterson, M. S., Wilson, B., 1992. A diffusion theory model of spatially resolved,
 761 steady-state diffuse reflectance for the noninvasive determination of tissue optical properties in
 762 vivo. *Med. Phys.* 19, 879–888.

763 Gitelson, A. A., Merzlyak, M. N., & Chivkunova, O. B., 2001. Optical properties and non-
 764 destructive estimation of anthocyanin content in plant leaves. *Photochemistry and Photobiology*,
 765 74, 38–45. [https://doi.org/10.1562/0031-8655\(2001\)0740038OPANEO2.0.CO2](https://doi.org/10.1562/0031-8655(2001)0740038OPANEO2.0.CO2).

766 Herrero-Langreo, A., Fernández-Ahumada, E., Roger, J.-M., Palagós, B., Lleó, L., 2012.
 767 Combination of optical and non-destructive mechanical techniques for the measurement of
 768 maturity in peaches. *J. Food Eng.* 108, 150–157. doi:10.1016/j.jfoodeng.2011.07.004.

769 Honda C., Moriya, S., 2018. Anthocyanin [biosynthesis](#) in [apple fruit](#). *Hort. J.* 87 (3): 305–314. 2018.
 770 doi: 10.2503/hortj.OKD-R01

771 Huang, Y., Lu, R., Xu, Y., Chen, K., 2018a. Prediction of tomato firmness using spatially-resolved
 772 spectroscopy. *Postharvest Biol. Technol.* 140, 18–26.
 773 <https://doi.org/10.1016/j.postharvbio.2018.02.008>

774 Huang, Y., Lu, R., Chen, K., 2018b. Assessment of tomato soluble solids content and pH by
 775 spatially-resolved and conventional Vis/NIR spectroscopy. *J. Food Eng.* 236, 19–28.
 776 <https://doi.org/10.1016/j.jfoodeng.2018.05.008>

777 Lorente, D., Zude, M., Idler, C., Gómez-Sanchis, J., Blasco, J., 2015. Laser-light backscattering
 778 imaging for early decay detection in citrus fruit using both a statistical and a physical model. *J.*
 779 *Food Eng.* 154, 76–85. <http://dx.doi.org/10.1016/j.jfoodeng.2015.01.004>

780 Lu, R., Ariana, D. P., Cen, H., 2011. Optical absorption and scattering properties of normal and
 781 defective pickling cucumbers for 700–1000 nm. *Sens. Instrum. Food Qual. Saf.* 5, 51–56.
 782 <https://doi.org/10.1007/s11694-011-9108-6>

783 Lu, R., Van Beers, R., Saeys, W., Li, C., Cen, H., 2020. Measurement of optical properties of fruits
 784 and vegetables: A review. *Postharvest Biol. Technol.* 159, 111003.
 785 <https://doi.org/10.1016/j.postharvbio.2019.111003>

786 Martelli, F., Del Bianco, S., Ismaelli, A., Zaccanti, G., 2009. Light propagation through biological
 787 tissue and other diffusive media: theory, solutions and software. SPIE Press, Washington, USA.

788 Martinenghi, E., Di Sieno, L., Conti, D., Sanzaro, M., Pifferi, A., Dalla Mora, A. 2016. Time-
 789 resolved single-photon detection module based on silicon photomultiplier: A novel building block
 790 for time-correlated measurement systems, *Rev. Sci. Instrum.* 87, 073101.
 791 <https://doi.org/10.1063/1.4954968>.

792 Mendoza, F., Lu, R., Cen, H., 2014. Grading of apples based on firmness and soluble solids content
 793 using Vis/SWNIR spectroscopy and spectral scattering techniques. *J. Food Eng.* 125, 59–68.
 794 <http://dx.doi.org/10.1016/j.jfoodeng.2013.10.022>.

795 Merzlyak, M.N., Solovchenko, A.E., Gitelson, A.A., 2003. Reflectance spectral features and non-
796 destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit.
797 Postharvest Biol. Technol. 27, 197–211. [https://doi.org/10.1016/S0925-5214\(02\)00066-2](https://doi.org/10.1016/S0925-5214(02)00066-2).

798 Mollazade, K., Omid, M., Tab, F. A., Mohtasebi, S. S., 2012. Principles and [applications](#) of [light](#)
799 [backscattering imaging](#) in [quality evaluation](#) of [agro-food products](#): a [review](#). Food Bioprocess
800 Technol. 5, 1465–1485. DOI 10.1007/s11947-012-0821-x

801 Mollazade, K., Arefi, A., 2017. Optical analysis using monochromatic imaging-based spatially-
802 resolved technique capable of detecting mealiness in apple fruit. Sci. Hortic. 225, 589–598.
803 <https://doi.org/10.1016/j.scienta.2017.08.005>

804 Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. A [user-friendly guide](#) to [multivariate calibration](#)
805 and [classification](#). 1st Edition, NIR Publications, Chichester. ISBN 0-9528666-2-5.

806 Nguyen Do Trong, N., Erkinbaev, C., Tsuta, M., De Baerdemaeker, J., Nicolai, B., Saeys, W., 2014.
807 Spatially resolved diffuse reflectance in the visible and near infrared wavelength range for non-
808 destructive quality assessment of “Braeburn” apples. Postharvest Biol. Technol. 91, 39–48.
809 <http://dx.doi.org/10.1016/j.postharvbio.2013.12.004>.

810 Qin, J., Lu, R., 2008. Measurement of the optical properties of fruits and vegetables using spatially
811 resolved hyperspectral diffuse reflectance imaging technique. Postharvest Biol. Technol. 49 (3),
812 355–365. <http://dx.doi.org/10.1016/j.postharvbio.2008.03.010>.

813 Qin, J., Lu, R., Peng, Y., 2009. Prediction of [apple internal quality using spectral absorption](#) and
814 [scattering properties](#). Trans. ASABE. 52 (2), 499–507. doi: 10.13031/2013.26807.

815 Qing, Z., Ji, B., Zude, M., 2007. Predicting soluble solid content and firmness in apple fruit by
816 means of laser light backscattering image analysis. J. Food Eng. 82, 58–67.
817 <https://doi.org/10.1016/j.jfoodeng.2007.01.016>

818 Rady, A., Guyer, D., Lu, R., 2015. Evaluation of [sugar content](#) of [potatoes](#) using [hyperspectral](#)
819 [imaging](#). Food Bioprocess Technol. 8, 995–1010. DOI 10.1007/s11947-014-1461-0

820 Rizzolo, A., Grassi, M., Vanoli, M., 2015. Influence of storage (time, temperature, atmosphere) on
821 ripening, ethylene production and texture of 1-MCP treated ‘Abbé Fétel’ pears. Postharvest Biol.
822 Technol. 109 (2015) 20–29. <http://dx.doi.org/10.1016/j.postharvbio.2015.06.003>

823 Rizzolo, A., Vanoli, M., 2016. Time-resolved technique for measuring optical properties and
824 quality of food. In Renfu Lu (Ed) “Light scattering technology for food property, quality and
825 safety assessment.”, CRC Press, Taylor & Francis Group, Boca Raton, FL 33487-2742, ISBN:
826 978-1-4822-6334-3, pp 187–224.

827 Rizzolo, A., Vanoli, M., Bianchi, G., Zanella, A., Grassi, M., Torricelli, A., Spinelli, L., 2014.
828 Relationship between texture sensory profiles and optical properties measured by time-resolved
829 reflectance spectroscopy during post-storage shelf life of ‘Braeburn’ apples. J. Hort. Res. 22(1),
830 113–121. <https://dx.doi.org/10.2478/johr-2014-0014>.

831 Rizzolo, A., Vanoli, M., Buccheri, M., Grassi, M., Lovati, F., Torricelli, A., Spinelli, L., Sadar, N.,
832 Zanella, A., 2019. Relationship between bulk scattering, sensory texture and water spectral pattern
833 in Braeburn apples. Book of abstracts 6th International Symposium on Modeling in horticultural
834 supply chain, June, 9-12, Molfetta, Italy, Abstract P050.

835 Rizzolo, A., Vanoli, M., Spinelli, L., Torricelli, A., 2010. Sensory characteristics, quality and
836 optical properties measured by time-resolved reflectance spectroscopy in stored apples.
837 Postharvest Biol. Technol. 58, 1–12. <https://dx.doi.org/10.1016/j.postharvbio.2010.05.003>.

838 Rowe, P. I., Künnemeyer, R., McGlone, A., Talele, S., Martinsen, P., Seelye, R., 2014. Relationship
839 between tissue firmness and optical properties of “Royal Gala” apples from 400 to 1050nm.
840 Postharvest Biol. Technol. 94, 89–96. <https://dx.doi.org/10.1016/j.postharvbio.2014.03.007>

841 Rupasinghe, H. P. V., Huber G. M., Embree C., Forsline P. L., 2010. Red-fleshed apple as a source
842 for functional beverages. Can. J. Plant Sci. 90: 95–100.

843 Saeys, W., Velazco-Roa, M. a, Thennadil, S. N., Ramon, H., Nicolaï, B. M., 2008. Optical
844 properties of apple skin and flesh in the wavelength range from 350 to 2200 nm. *Applied Optics*,
845 47(7), 908–919. <https://doi.org/10.1364/AO.47.000908>.

846 Seifert, B., Zude, M., Spinelli, L., Torricelli, A., 2014. Optical properties of developing pip and
847 stone fruit reveal underlying structural changes. *Physiol. Plant.* 153, 327-336.
848 <http://dx.doi.org/10.1111/ppl.12232>.

849 Solovchenko, A.E, Chivkunova, O.B, Merzlyak, M.N., Gudkovsky, V.A., 2005. Relationships
850 between chlorophyll and carotenoid pigments during on and off-tree ripening of apple fruit as
851 revealed non-destructively with reflectance spectroscopy. *Postharvest Biol. Technol.*, 38: 9–17.
852 doi:10.1016/j.postharvbio.2005.05.004

853 Spinelli, L., Rizzolo, A., Vanoli, M., Grassi, M., Eccher Zerbini, P., Meirelles de Azevedo Pimentel,
854 R., Torricelli, A. 2012. Optical properties of flesh and skin in Brazilian mangoes in the 540-900
855 nm spectral range: implication for non-destructive maturity assessment by time-resolved
856 reflectance spectroscopy. *Proceedings of the 3rd CIGR International Conference of Agricultural*
857 *Engineering (CIGR-AgEng2012)*, July 8-12, Valencia, Spain, ISBN 84-615-9928-4

858 Tian, X., Li, J., Wang, Q., Fan, S., Huang, W., 2018. A bi-layer model for nondestructive prediction
859 of soluble solids content in apple based on reflectance spectra and peel pigments. *Food Chem.*,
860 239: 1055–1063. <http://dx.doi.org/10.1016/j.foodchem.2017.07.045>

861 Tjiskens, L.M.M., Eccher Zerbini, P., Schouten, R.E. 2007. Biological variation in ripening
862 nectarines, *Veg. Crops Res. Bull.* 66, 205–212. <https://doi.org/10.2478/v10032-007-0023-7>.

863 Torricelli, A., Spinelli, L., Contini, D., Vanoli, M., Rizzolo, A., Eccher Zerbini, P., 2008. Time-
864 resolved reflectance spectroscopy for non-destructive assessment of food quality. *Sens. Instrum.*
865 *Food Qual. Safety*, 2, 82-89. <https://doi.org/10.1007/s11694-008-9036-2>.

866 Torricelli, A.; Spinelli, L.; Vanoli, M.; Leitner, M.; Nemeth, A.; Trong, N. N. D.; Nicolaï, B., Saeys,
867 W., 2013. Optical coherence tomography (OCT), space-resolved reflectance spectroscopy (SRS)

and time-resolved reflectance spectroscopy (TRS): principles and applications to food microstructures. In Morris V.J.; Groves K. (Eds) "Food microstructures: microscopy, measurement and modeling." Woodhead Publishing Ltd, Cambridge, UK, pp 132-162.

Tuchin, V., 2007. Tissue Optics – Light scattering methods and instruments for medical diagnosis. 2nd ed. SPIE Press, USA, ISBN 978-0-8194-6433-0.

Van Beers, R., Aernouts, B., León Gutiérrez, L., Erkinbaev, C., Rutten, K., Schenk, A., Nicolaï, B.M., Saeys, W., 2015. Optimal illumination-detection distance and detector size for predicting Braeburn apple maturity from vis/NIR laser reflectance measurements. Food Bioprocess Technol. 8(10), 2123–2136. <https://doi.org/10.1007/s11947-015-1562-4>.

Van Beers, R., Aernouts, B., Watté, R., Schenk, A., Nicolaï, B., Saeys, W., 2017. Effect of maturation on the bulk optical properties of apple skin and cortex in the 500e1850 nm wavelength range. J. Food Eng. 214, 79-89. <http://dx.doi.org/10.1016/j.jfoodeng.2017.06.013>.

Vanoli, M., Bucchini, M., 2012. Overview of the methods for assessing harvest maturity. Stewart Postharvest Rev. 1, 4. DOI: 10.2212/spr.2012.1.4.

Vanoli, M., Eccher Zerbini, P., Grassi, M., Rizzolo, A., Fibiani, M., Zanella, A., Pifferi, A., Spinelli, L., Torricelli, A., Cubeddu, R., 2005. The quality and storability of apples cv. 'Jonagored' selected at-harvest by time-resolved reflectance spectroscopy. Acta Hortic. 682, 1481–1488. <https://doi.org/10.17660/ActaHortic.2005.682.199>

Vanoli, M., Eccher Zerbini, P., Rizzolo, A., Spinelli, L., Torricelli, A., 2010. Time-resolved reflectance spectroscopy for the non-destructive detection of inner attributes and defects of fruit. Acta Hortic. 877, 1379-1386. <https://doi.org/10.17660/ActaHortic.2010.877.189>

Vanoli, M., Eccher Zerbini, P., Spinelli, L., Torricelli, Rizzolo, A., 2009. Polyuronide content and correlation to optical properties measured by time-resolved reflectance spectroscopy in 'Jonagored' apples stored in normal and controlled atmosphere. Food Chem. 115, 1450–1457. <https://doi.org/10.1016/j.foodchem.2009.01.081>.

893 Vanoli, M., Grassi, M., Buccheri, M., Lovati, F., Sadar, N., Zanella, A., Torricelli, A., Rizzolo, A.,
 894 Spinelli, L., 2018. Time-resolved reflectance spectroscopy reveals different texture characteristics
 895 in 'Braeburn', 'Gala' and 'Kanzi®' apples. *Acta Hortic.* 1194, 1273-1282.
 896 <https://doi.org/10.17660/ActaHortic.2018.1194.180>.

897 Vanoli, M., Rizzolo, A., Grassi, M., Farina, A., Pifferi, A., Spinelli, L., Torricelli, A., 2011. Time-
 898 resolved reflectance spectroscopy nondestructively reveals structural changes in 'Pink Lady®'
 899 apples during storage. *Procedia Food Sci.* 1, 81 – 89.
 900 <https://doi.org/10.1016/j.profoo.2011.09.014>.

901 Vanoli, M., Rizzolo, A., Grassi, M., Spinelli, L., Verlinden, B.E., Torricelli, A., 2014. Studies on
 902 classification models to discriminate 'Braeburn' apples affected by internal browning using the
 903 optical properties measured by time-resolved reflectance spectroscopy. *Postharvest Biol. Technol.*
 904 91, 112–121. <http://dx.doi.org/10.1016/j.postharvbio.2014.01.002>.

905 Vanoli, M., Rizzolo, A., Grassi, M., Torricelli, A., Zanella, A., Spinelli, L., 2015. Characterizing
 906 apple texture during storage through mechanical, sensory and optical properties. *Acta Hortic.*
 907 1079, 383-390. <https://doi.org/10.17660/ActaHortic.2015.1079.48>.

908 Wang, Z., Van Beers, R., Aernouts, B., Watté, R., Verboven, P., Nicolaï, B., Saeys, W., 2020.
 909 Microstructure affects light scattering in apples. *Postharvest Biol. Technol.*, 159, 110996.
 910 <https://doi.org/10.1016/j.postharvbio.2019.110996>

911 Watté, R., Aernouts, B., Van Beers, R., Saeys, W., 2015. Robust metamodel-based inverse
 912 estimation of bulk optical properties of turbid media from spatially resolved diffuse reflectance
 913 measurements. *Optics Express*, 23(21), 27880- 27698. <https://doi.org/10.1364/OE.23.027880>.

914 Watté, R., Nguyen Do Trong, N., Aernouts, B., Erkinbaev, C., De Baerdemaeker, J., Nicolaï, B.,
 915 Saeys, W., 2013. Metamodeling approach for efficient estimation of optical properties of turbid
 916 media from spatially resolved diffuse reflectance measurements. *Optics Express*, 21(26), 32630.
 917 <https://doi.org/10.1364/OE.21.032630>.

918 Ying Y., Xie L., Fu X., 2016. Spectral scattering for assessing the quality of fruits and vegetables.
919 In Renfu Lu (Ed) "Light scattering technology for food property, quality and safety assessment.",
920 CRC Press, Taylor & Francis Group, Boca Raton, FL 33487-2742, ISBN: 978-1-4822-6334-3, pp
921 225-250.

922 Zanella, A., Vanoli, M., Rizzolo, A., Grassi, M., Eccher Zerbini, P., Cubeddu, R., Spinelli, L.,
923 Torricelli, A., 2013. Correlating optical maturity indices and firmness in stored 'Braeburn' and
924 'Cripps Pink' apples. *Acta Hortic.* 1012, 1173–1180.
925 <https://doi.org/10.17660/ActaHortic.2013.1012.158>.

926 Zhang, S., Wu, X., Zhang, S., Cheng, Q., Tan, Z., 2017. An effective method to inspect and classify
927 the bruising degree of apples based on the optical properties. *Postharvest Biol. Technol.* 127, 44–
928 52. <https://dx.doi.org/10.1016/j.postharvbio.2016.12.008>.

929 Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., Costa, G., 2008. A new
930 index based on VIS spectroscopy to characterize the progression of ripening in peach fruit.
931 *Postharvest Biol. Technol.* 49, 319–329. <https://doi.org/10.1016/j.postharvbio.2008.01.017>.

932 Zude, M., Pflanz, M., Spinelli, L., Dosche, C., Torricelli, A., 2011. Non-destructive analysis of
933 anthocyanins in cherries by means of Lambert–Beer and multivariate regression based on
934 spectroscopy and scatter correction using time-resolved analysis. *J. Food Eng.* 103, 68–75.
935 <https://doi.org/10.1016/j.jfoodeng.2010.09.021>.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AUTHOR STATEMENT

- Maristella Vanoli   Conceptualization, Methodology, Formal analysis, Writing- Original draft, Writing - Review & Editing,
- Robbe Van Beers   Conceptualization; Methodology; Software; Validation; Investigation; Formal analysis; Writing - Original draft
- Nadja Sadar   Conceptualization, Investigation, Review & Editing
- Anna Rizzolo   Writing - Review & Editing
- Marina Buccheri   Investigation
- Maurizio Grassi   Investigation
- Fabio Lovati   Investigation
- Bart Nicolaï   Conceptualization; Resources; Project administration
- Ben Aernouts   Methodology; Software; Writing – Review & Editing
- Rodrigo Watté   Methodology; Software
- Alessandro Torricelli Investigation, Writing - Review & Editing
- Lorenzo Spinelli   Conceptualization, Software, Formal analysis, Investigation, Writing - Review & Editing
- Wouter Saeys   Conceptualization; Resources; Writing – Review & Editing; Project administration
- Angelo Zanella   Conceptualization, Methodology, Resources, Review & Editing, Project administration, Funding acquisition