Effects of harvest time, fruit size and cultivar on the bulk optical properties of Satsuma mandarin

Chanjun Sun^a, Ben Aernouts^b, Wouter Saeys^{a,*}

^aKU Leuven, Department of Biosystems-MeBioS, Kasteelpark Arenberg 30, 3001 Leuven, Belgium

^bKU Leuven, Department of Biosystems-A2H, Campus Geel, Kleinhoefstraat 4, 2440 Geel, Belgium

Abstract

As Satsuma mandarin is a multi-layered fruit, the optical properties of the different tissue layers influence the performance of spectroscopy-based quality detection models. Therefore, the variation in the bulk optical properties (BOP) of the inner (juice vesicles) and outer (flavedo) tissue layer of Satsuma mandarin was investigated for different harvest times, fruit sizes and cultivars. Satsuma mandarin fruit from three different cultivars (Iwasaki, Okitsu and Goku Wase) were harvested at three different times around the recommended harvest date and the BOP of the inner and outer tissue layers were estimated from double integrating spheres measurements. Along the harvest time, the absorption peak related to carotenoids in the bulk absorption coefficient (μ_a) spectrum increased in both tissue layers, while the values attributed to chlorophylls decreased in the flavedo. Moreover, a slightly increasing trend over the harvest time was observed in the bulk scattering coefficient (μ_s) spectra of juice vesicles, especially at the early stage. The μ_a values of both tissue layers and the μ_s values of juice vesicles deviated among different fruit sizes, which can be related to the development stage. Smaller fruit presented a higher carotenoids concentration in juice vesicles, an advanced color change in flavedo throughout the maturation stages and higher μ_s values in juice vesicles at earlier maturation stages. The μ_a differences in pigment absorption among different cultivars were related to the maturation order. Earlier maturing cultivars possessed higher μ_a values related to carotenoids absorption in juice vesicles and advanced color changes in flavedo. The μ_s values of the flavedo differed among the cultivars and these differences were enhanced during maturation. These insights in the sources of variation in the bulk optical properties of Satsuma mandarin tissues can be used for simulation-based optimal design of an optical measurement configuration

and interpretation of the acquired signals in spectroscopy-based quality detection and monitoring.

Keywords: Flavedo; Juice vesicle; absorption coefficient; scattering coefficient; Spectroscopy; Citrus fruit

1. Introduction

Satsuma mandarin (*Citrus unshiu* Marc.) is one of the most popular citrus cultivars and is widely grown in the main citrus production regions, because it combines easy peeling with the absence of seeds and a high nutritional value (Tietel et al., 2010; Tao et al., 2012). Satsuma mandarin is mainly consumed fresh (Ozkaya et al., 2019). With the increasing demand for high quality fruit, industries are in urgent need of efficient and non-destructive techniques for fruit quality evaluation on the sorting lines. Thanks to the availability of instruments and the development of the chemometrics methods (Magwaza et al., 2012), Vis-NIR spectroscopy has been widely investigated for the assessment of quality parameters for citrus fruit (Blasco et al., 2009; Magwaza et al., 2014; Lorente et al., 2015a; Lorente et al., 2015b; Folch-Fortuny et al., 2016; Ncama et al., 2017; Simeone et al., 2017).

The most frequently used modes of Vis-NIR spectroscopy in the quality detection of citrus fruit are transmittance and reflectance mode (Jamshidi et al., 2014). In the transmittance mode, light has to go through the whole fruit. Therefore, the size of the fruit largely determines the optical path and has an important impact on the model performance. Moreover, the peel of citrus fruit has a much higher attenuation coefficient compared to the flesh (Fraser et al., 2003), leading to low light transmission into the flesh. In other words, the signal obtained in the transmittance mode would be quite low. On the contrary, the signal obtained in the reflectance mode can be much higher. This has been confirmed by simulations of the light propagation in citrus fruit, where the largest part of the energy was either reflected or absorbed, and only a very small proportion of the energy at around 800 nm and almost no energy outside this wavelength range were transmitted (Sun et al., 2020a). It was also found that in the reflectance mode only a tiny fraction of the signal comes from the flesh part. According to the results reported by Sun et al. (2020a), the fraction absorbed by flesh was still below 0.6 % for grapefruit, orange and lemon, even at a source-detector distance as long as 0.88

cm. This limited penetration depth of the light is considered as one of the biggest disadvantages of the reflectance mode (Krivoshiev et al., 2000; Magwaza et al., 2012).

Even though the information obtained in the reflectance mode is dominated by the outside tissue layer, good results have been reported for prediction of the inner quality from reflectance spectra of intact fruit. Gómez et al. (2006) used reflectance spectra in the 350-2500 nm wavelength range, smoothed and pretreated with multiplicative scatter correction (MSC), to predict the soluble solids content (SSC) and acidity (pH) of Satsuma mandarin, obtaining correlation coefficient values of 0.94 and 0.80, respectively. Antonucci et al. (2011) built SSC and titratable acidity (TA) prediction models for Satsuma mandarin based on Savitzky-Golay smoothed spectra between 400 nm and 1000 nm, obtaining correlation coefficients of 0.85 and 0.88, respectively. In the research of Cayuela and Weiland (2010), reflectance spectra of oranges in the 350-2500 nm range were mean normalized and MSC pretreated for the prediction of SSC and TA. The models performed well with correlation coefficient values of 0.92 and 0.86. Ncama et al. (2017) applied the reflectance mode with standard normal variate (SNV) and smoothing preprocessing to predict the SSC, TA and BrimA in orange and grapefruit, obtaining R^2 values of 0.93-0.96 for orange and 0.84-0.90 for grapefruit.

Most of the models were built based on samples from a single batch. Even though some may have included samples with different fruit sizes, harvest times and cultivars, the prediction performance was typically evaluated based on the samples that originated from the same batch as the samples used in model building. That means the samples used for prediction had similar characteristics as the ones used in the model building. This allowed to obtain good prediction performances in the study, but once the already built model would be applied on the samples from a different batch, the prediction performance would generally be poor, which has been verified by Guthrie et al. (2005). This illustrates that these models are generally not robust against the variability present in practice. To our knowledge, only Guthrie et al. (2005) and a few other researchers (Miyamoto and Kitano, 1995) have investigated the robustness of the quality prediction models for mandarin. According to Guthrie et al. (2005), to improve the prediction performance of the model, samples from the new

population should be involved in the training set and the original model should be retrained. While this may help to improve the prediction performance in that specific case, it does not solve the problem fundamentally as the unspecific correlations hidden in the model building may change from sample to sample. Insight in the sources of variation in the bulk optical properties would allow to design measurement configurations and calibration models that maximize the information on the quality traits of interest, while minimizing the interferences.

In addition, these quality prediction models were built with various wavelength regions, as well as preprocessing methods. Furthermore, different preprocessing methods are generally tested and the best one is selected. This result-based process, undeniably, can work well in some fields, but still there are some shortcomings. For instance, in MSC, one of the most frequently used preprocessing methods, both the scaling and offset variations are corrected based on the average spectra of the samples (Windig et al., 2008), which is regarded as the ideal spectrum free from scattering effects. In this way, the relative scattering interference can be reduced before the model is built. However, this model would not be suitable and robust for a sample of a completely new data set with other scattering and absorption properties and thus another reference spectrum. By measuring and analyzing the absorption and scattering coefficient separately, the characteristics attributed to chemical or physical properties could be studied independently. Thus, the wavelength regions of the quality properties of interest can be studied and confirmed more easily.

Therefore, the objectives of this research were to: (1) measure the BOP of flavedo and juice vesicles, as well as relevant color parameters (L^* , a^* and b^*) and inner quality parameters (SSC, TA and BrimA) for Satsuma mandarin; (2) investigate the BOP differences among different harvest times, fruit sizes and cultivars; (3) explore the link between the changes in the BOP and the changes in the inner and outer quality parameters.

2. Materials and methods

2.1 Satsuma mandarin

Three cultivars of Satsuma mandarin (*C. unshiu* Marc.): Iwasaki, Okitsu and Goku Wase were used in this

study. These mandarins were obtained from an orchard in Santa Magdalena de Polpís (40.36°N, 0.30°E), Castellón, Spain, in three different times during the harvest season: 16/09/2019, 07/10/2019, 05/11/2019. At each harvest time, a batch of 10 kg fruit was randomly collected for each cultivar. After arriving at the laboratory by road transport, these samples were stored in a climate chamber (WKL100, Weiss Technik, Liedekerke, Belgium) at 10 °C and 90 % relative humidity until the measurements. All the measurements were finished within 4 days. For each combination of harvest time and cultivar, 5 samples from each of three different sizes (big, medium and small) were selected from each batch, resulting in a total of 135 samples for further analysis. The detailed information about the size of the samples can be found in Table 1. All the mandarins were allowed to equilibrate to room temperature before measurements.

Table 1. Diameter (mm) of the samples

		Big	Medium	Small
1 st harvest time (HT 1)	Iwasaki	61.41 ± 0.46	55.50 ± 0.77	48.75 ± 0.55
	Okitsu	60.59 ± 1.55	55.34 ± 1.20	50.41 ± 0.82
	Wase	66.67 ± 1.37	55.65 ± 0.54	51.60 ± 0.74
2 nd harvest time (HT 2)	Iwasaki	62.76 ± 0.50	56.70 ± 0.68	52.23 ± 0.75
	Okitsu	62.92 ± 0.90	57.09 ± 0.56	49.53 ± 0.36
	Wase	65.42 ± 1.44	58.19 ± 0.64	52.29 ± 1.26
3 rd harvest time (HT 3)	Iwasaki	85.49 ± 0.58	75.31 ± 1.57	67.35 ± 1.13
	Okitsu	65.82 ± 1.24	60.56 ± 1.06	56.76 ± 1.08
	Wase	73.60 ± 0.83	66.95 ± 0.98	60.23 ± 0.70

2.2 Color measurement

A spectrophotometer (CM-2500d, Konica Minolta, Osaka, Japan) with a specular reflection included (SCI) mode equipped with color data software (SpectraMagic™ NX, Konica Minolta, Osaka, Japan) was used to measure the color with a D65 illuminant at an observation angle of 10°. The color was estimated in the CIE

111 $L^*a^*b^*$ color space by three parameters: L^* represents the lightness from black (0) to white (100); a^* indicates
112 the green-red tendency, with a negative value towards green (-) and a positive value towards red (+); b^*
113 indicates the blue-yellow tendency, with a negative value towards blue (-) and a positive value towards yellow
114 (+) (Granato and Masson 2010; Porras et al., 2015). The spectrophotometer was first calibrated with standard
115 black and white reference. Then, three measurements were taken at the positions trisecting the equator for
116 each sample and the average value was used as the color parameter for the sample.

117 2.3 Sample preparation

118 A peel block with an approximate size of 3 cm * 3 cm (length * width) was sampled around the equator of the
119 intact fruit. Then, a scalpel was used to scrape off excessive tissues (including albedo tissue and parts of
120 flavedo) to reduce the flavedo sample to a thickness of 0.04 cm. A digital caliper was used to verify the
121 thickness. Successively, the flavedo slice was sandwiched by sample holders ready for optical measurements.
122 The segments were individually separated after peeling. All the segments were randomly split into two groups,
123 which were respectively used to prepare the juice and the juice vesicles. The segments from the first group
124 were loaded in a stainless-steel press and the squeezed juice was stored in a container for internal quality
125 measurements. For the other group of segments, the juice vesicles were placed one by one into a glass cuvette
126 (Borofloat 33, Schott, Germany), which was pre-filled with the squeezed juice. By immersing the juice
127 vesicles into the juice one by one, the air space among adjacent juice vesicles was filled to avoid a refractive
128 index mismatch and to retain the juice vesicles in their original shape. The cuvette filled with the juice vesicles
129 and juice was then used for the optical measurements.

Fig. 1 Schematic diagram of the vertical section of Satsuma mandarin

2.4 Internal quality measurements

Soluble solids content (SSC) was determined by using a digital handheld refractometer (DR301-95, A.KRUSS Optronic GmbH, Hamburg, Germany). Results were expressed as TSS (%). The titratable acidity (TA) was determined with the potentiometric titration method. Results were expressed as TA (% g/100ml). The detailed procedures for measuring SSC and TA were according to Sun et al. (2020b). Brix minus acid (BrimA) is obtained using the following equation (Clark, 2016) based on the California Standard:

$$BrimA = (SSC - TA * 4) * 16.5$$

2.5 Optical measurements

The optical measurements were performed with the setup for double integrating spheres (DIS) and unscattered transmittance measurements which was also used by Aernouts et al. (2013) and Sun et al. (2020b). A supercontinuum laser (SC450-4, Fianium Ltd., Southampton, UK) coupled into a monochromator (Oriel Cornerstone 260 ¼ m, Newport, Irvine, USA) was used to scan the wavelength range from 450 nm to 1880 nm. A beam splitter is installed after the monochromator to split the light beam into three parts: 5 % of the light is sent to a Si detector (PDA100A, Thorlabs Inc., New Jersey, USA), collecting the signal in the 440 nm - 1050 nm range, and another 5 % is sent to an extended-InGaAs detector (PDA10DT-EC, Thorlabs Inc., New Jersey, USA), collecting the signal in the 1050 nm - 1880 nm range. These two detectors are used to monitor the stability of this tunable light source. The remaining 90 % is used to illuminate the sample either in the double integrating spheres (DIS) measurement path or the unscattered transmittance measurement path. The path conversion was achieved by using a flip mirror. After obtaining the spectra measured from the setups, the inverse adding-doubling (IAD) approach (Prahl et al., 1993) was used to estimate the BOP. More detailed information about the parameters used in the IAD routine was according to Sun et al. (2020b). The BOP of juice vesicles and flavedo were recorded from 450 nm to 1880 nm. However, due to the high absorption by water in juice vesicles, extreme low signals were obtained around 1450 nm, resulting in a reduced accuracy

of the estimated BOP in that wavelength range. Therefore, the BOP between 1390 nm and 1560 nm were omitted for juice vesicles.

2.6 Statistical analysis

One-way and two-way analysis of variance (ANOVA) with a significance level of 5 % were respectively used to determine whether the inner quality parameters and the BOP are statistically significant different for different harvest times, fruit sizes and cultivars. In the evaluation of the BOP difference, the average μ_a values around the absorption peaks (carotenoids: 460 nm - 520 nm; chlorophylls: 660 nm - 690 nm; water: 1420 nm - 1500 nm) and the average μ_s values in the wavelength range from 800 nm to 1300 nm were used in the ANOVA test. OriginPro (version 8.5, OriginLab Corporation, Northampton, USA) software was used to process the data.

3. Results

3.1 Comparison of color parameters

As shown in Fig. 2 (a), along the harvest time, the L^* first dramatically increased from HT 1 to HT 2 and then slightly reduced, especially for Iwasaki and Wase. The increase in the L^* index between HT 1 and HT 2 may be attributed mainly to the water accumulation (Iglesias et al., 2007). As water accumulates, the concentration of the chlorophylls (main pigments at these harvest times) would reduce, thereby reducing the darkness of the fruit (Itle and Kabelka, 2009), resulting in an increase in the L^* index. While getting closer to HT 3, the fruit growth is mostly arrested and the metabolism is shifted to trigger a series of biochemical and physiological changes (Iglesias et al., 2007), among which, the biosynthesis of carotenoids is also included (Llorente et al., 2016). Therefore, with the increase of carotenoids, the L^* index reduced again for Iwasaki and Wase. However, the values for Okitsu kept increasing throughout these three harvest times. Considering the late degreening of Okitsu which will be discussed below, the L^* index of Okitsu was speculated to reduce again at a later time.

177 The a^* index is a measure for the color tendency between green and red. As shown in Fig. 2 (b), almost all
 178 the a^* indexes of the samples harvested at HT 1 are below 0 (around -10), which indicates that the samples
 179 are very green at this stage (Conesa et al., 2019). With increasing harvest time, the a^* indexes gradually
 180 changed from negative values to positive values. The biggest deviation of the a^* index among different
 181 cultivars happened at HT 2, where all the a^* values for Okitsu were still below 0, while the values for Wase
 182 were already up to around 20 and the values for Iwasaki were fluctuating around 0. As described by Manera
 183 et al. (2012) and Conesa et al. (2019), any decrease in the chlorophyll content of the fruit is associated with
 184 the colorimetric a^* coordinate. The variation of the a^* index among different cultivars at HT 2 indicated that
 185 the degreening process is asynchronous for different cultivars. Moreover, according to the changes of the a^*
 186 index from HT 1 to HT 2, the degreening process was speculated to first happen in Wase, then in Iwasaki and
 187 finally in Okitsu.

188 The variation in the b^* index among different cultivars and its change along the harvest time were similar to
 189 those observed for the L^* index. The positive values of b^* indicate that the color of the fruit was expressed
 190 towards yellow. Although the b^* values were around 40 and 50 at HT 1, the yellow color was not yet be visible
 191 at this stage, because it was masked by the green color of chlorophylls (Conesa et al., 2019), as indicated by
 192 the negative value of a^* at HT 1. In citrus fruit, the content of carotenoids is mainly responsible for the yellow
 193 color (Rodrigo et al., 2013). As shown in Fig. 2 (c), the content of carotenoids continues to accumulate for
 194 Iwasaki and Okitsu between HT 2 and HT 3, while for Wase, a slight decrease in the b^* index can be observed.

195
 196 *Fig. 2 Distribution of (a) L^* , (b) a^* and (c) b^* of Satsuma mandarin with different cultivars and harvest*
 197 *times (HT)*

3.2 Comparison of SSC, TA and BrimA

The SSC is commonly used as an index for the sweetness of citrus fruit. It is mainly composed of carbohydrates (75-80 %), in which, sucrose, glucose and fructose play the major roles (Lado et al., 2018). As shown in Fig. 3 (a1) and (b1), the difference in the SSC index among different fruit sizes for a specific harvest time is not significant ($P>0.05$) and the index generally decreases slightly and then increases thereafter along the harvest time for Iwasaki and Okitsu. This is in contrast with the reports that soluble carbohydrates accumulate during fruit growth (Iglesias et al., 2007; Lado et al., 2018). However, it should be noted that the SSC changes reported in this research correspond to samples with different sizes harvested at different times. When the fruit size was taken into account, the SSC changes along harvest time were more in line with these reported observations. In addition, it is interesting to report that a uniform and stable trend of the SSC index among different fruit sizes gradually emerged at later harvest times (e.g. HT 2 and HT 3 for Iwasaki; HT 3 for Okitsu). For Wase, the situation was slightly different compared to the other two cultivars. As shown in Fig. 3 (c1), only the SSC index of the samples with a similar fruit size (HT 1: big size; HT 2: big size; HT 3: medium size) along harvest time follows the same pattern as in Iwasaki and Okitsu, and a significant difference ($P<0.05$) between the big size and the other sizes can be found at HT 2. The increasing trend of the SSC index with decreasing fruit size at HT 2 for Wase was similar to that observed in other cultivars at the full maturation stage. However, this trend faded away after that. Moreover, the SSC index for the medium and small size fruit at HT 3 seemed to be decreased compared to HT 2. One possible reason for this could be the deviation in the fruit size scale, as the real fruit size of Wase at HT 3 was larger than that with the same labelled size at HT 1 and HT 2. When we could correct for the size difference, a similar SSC level observed at HT 2 could be expected at HT 3.

Organic acid content, another important quality parameter for satsuma mandarin (Lado et al., 2018), is generally expressed in terms of the TA index. As shown in Fig. 3 (a2), (b2) and (c2), the TA index follows a similar pattern along the harvest time for all these three cultivars. At HT 1, the index is relatively high for all

the samples, with values above 0.75 %. Subsequently, a dramatic decrease of the index can be seen from HT 1 to HT 2, resulting in TA index values between 0.25 % and 0.75 %. Thereafter, the TA index remained more or less constant below 0.75 %. As for the different fruit sizes, some patterns can be observed at HT1. As shown in Fig. 3 (b2), a potential negative correlation between TA content and fruit size can be found at HT 1 for Okitsu. As the content of titratable acid has been reported to increase with decreasing light intensity (Izumi et al., 1992), the negative correlation between fruit size and TA content observed at HT 1 for Okitsu may be related with the difference in the lighting condition among different fruit sizes. However, for Iwasaki and Wase, such a correlation was also observed for the big and medium size fruit, but the TA content of small size fruit seems to decrease earlier than for other sizes. From HT 2 on, no obvious difference or trend in the TA values among different fruit sizes can be seen, which is consistent with the findings reported by Biais et al. (2014) that the metabolic responses to the environment tend to decrease after fruit has attained the final size at ripening. Additionally, Lee et al. (2015) also reported that no significant differences could be observed in the TA values of ripe mandarin for fruit grown with or without shading.

BrimA, a sensory-based alternative to SSC/TA ratio which better aligns with consumers' preference for good taste, is also used to describe the maturity level of citrus fruit (Ncama et al., 2018). As shown in Fig. 3 (a3), (b3) and (c3), regardless of the variation at each harvest time, the index in general increased along the harvest time. At an early stage (from HT 1 to HT 2), the increasing trend was obvious, while later on (from HT 2 to HT 3), the index tended to stabilize at around 160. According to the Australian internal maturity standard for 2020, the minimum BrimA for mandarin is 110 (Lacey, 2019). So, the fruit harvested at HT 1 would not yet be qualified as mature. Although the SSC content at HT 1 already met the requirement proposed by the Plant Protection and Inspection Services with values above 9.0 % (Tietel et al., 2010), the slow decrease in TA resulted in the unqualified BrimA index (Richardson et al., 1997). When focusing on the difference among different fruit sizes at an individual harvest time, it could be found that the variation was generally in agreement with the one in the SSC index and opposite to the one in the TA index, which is consistent with

246 maturation of citrus fruit involving sugar accumulation and acid decrease (Liao et al., 2019; Sadka et al., 2019).
 247 However, since the increase in SSC with harvest time started later than the decrease in TA, the changes in
 248 BrimA with harvest time did not follow the exact same or opposite pattern as the other two indexes.

252 *Fig. 3 Distribution of (1) soluble solids content (SSC %), (2) titratable acidity (TA %) and (3) BrimA of*
 253 *(a) Iwasaki, (b) Okitsu and (c) Goku Wase with different fruit sizes and harvest times (HT)*

254 3.3 Bulk optical properties

255 3.3.1 Relation with harvest time

256 In Fig. 4, the average BOP values of all the fruit sizes and cultivars are illustrated for the different harvest
 257 times. As shown in Fig. 4 (a1), the absorption peaks for juice vesicles are roughly the same among different

258 harvest times. Absorption peaks between 450 nm and 560 nm are attributed to the absorption by carotenoids
259 (Hager, 1970). The subsequent peaks appeared at 980 nm, 1200 nm, 1780 nm. Together with the omitted peak
260 around 1450 nm, these are attributed to the absorption by water (Workman and Weyer, 2012). Although the
261 positions of the pigment absorption peaks in juice vesicles are fairly consistent throughout the different harvest
262 times, the intensity of the absorption peaks increases with harvest time, especially from HT 1 to HT2. In
263 addition to these peaks observed in juice vesicles, an extra peak corresponding to the absorption by chlorophyll
264 can be noticed at around 680 nm for the flavedo samples in Fig. 4 (b1). Compared to the relatively simple and
265 uniform changes in the absorption by pigments in the juice vesicles along harvest time, the changes in the
266 flavedo are more complex. The absorption peak related to chlorophyll (around 680 nm) presented a negative
267 correlation with harvest time, while the absorption peaks attributed to carotenoids presented a positive
268 correlation. Moreover, compared to HT 1 and HT 2, a slight wavelength redshift (towards longer wavelengths)
269 in the position of the carotenoids' absorption peak can be observed at HT3.

270 The bulk scattering coefficient spectra of juice vesicles and flavedo are respectively displayed in Fig. 4 (a2)
271 and (b2). All the spectra decrease with increasing wavelength throughout the spectral range from 450 nm to
272 1880 nm. However, differences can still be noticed between these two tissue layers. In the juice vesicles, μ_s
273 mainly varies between 2.5 mm^{-1} and 0.5 mm^{-1} for all the harvest times. The difference in μ_s values of juice
274 vesicles between HT 1 and HT 2 is significant ($P < 0.05$), moreover, a slight baseline increasing trend can be
275 observed from HT 1 to HT 2. In addition, the μ_s trend over the wavelength range becomes flatter at HT 3. In
276 the flavedo, μ_s mainly ranges from 26 mm^{-1} to 22 mm^{-1} for all the samples except for the clearly increased μ_s
277 values below 550 nm that are likely the result of the low signal to noise ratio of the total reflectance
278 measurements due to the strong absorption by the pigments. In contrast to the slight increase of the μ_s values
279 from HT 1 to HT 2 and the flatter trend over the wavelength at HT3 observed in juice vesicles, the μ_s values
280 of flavedo are quite stable over the harvest times.

Fig. 4 Evolution of (1) the bulk absorption coefficient spectra and (2) the bulk scattering coefficient spectra of (a) juice vesicles and (b) flavedo with harvest time (HT). The lines indicate the mean values, while the shaded areas indicate the mean $\pm$ standard deviation.

3.3.2 Relation with fruit size

Fruit size is an important feature in the grading and determines its desired markets (Lacey, 2019). While the average fruit size increased with harvest time, we observed considerable variation in fruit size at each harvest time. Therefore, the relation between the measured fruit sizes and BOP spectra is also explored. The average value of all the cultivars with the same labelled fruit size in each harvest time was used for the BOP comparison among different fruit sizes.

As shown in Fig. 5 (a1), (a2) and (a3), although the position of the pigment absorption peaks in juice vesicles remains consistent among different fruit sizes, the intensity varies. At HT 1 and HT 3, a clear difference ($P < 0.05$) can be observed between big and small fruit, with higher absorption values for small fruit than for

big fruit. At HT 2, the difference can be noticed between all the fruit sizes with a negative correlation between fruit size and absorption values for the pigments in juice vesicles. In the case of the flavedo, the differences in the μ_a spectra among different fruit sizes mainly locate in the absorption peaks attributed to chlorophyll and carotenoids, as well as the water absorption at around 1450 nm. As shown in Fig. 5 (b1), the μ_a spectra of the big and medium fruit are almost entirely overlapping, while the small fruit tends to have a slightly lower intensity of the chlorophyll absorption and a higher water absorption. At HT 2, the difference in the flavedo μ_a spectra among different fruit sizes becomes more obvious. Both the absorption peaks attributed to carotenoids and water negatively correlated with fruit size, while a positive correlation was found between the chlorophyll absorption and fruit size. At HT 3, the difference among different fruit sizes was considerably reduced. Nonetheless, the peak around 515 nm and the peak around 470 nm respectively presented a slight decreasing and increasing trend with decreasing fruit size.

Fig. 5 Bulk absorption coefficient spectra of (a) juice vesicles and (b) flavedo at the (1) 1st harvest time, (2) 2nd harvest time and (3) 3rd harvest time for different fruit sizes. The lines indicate the mean values, while the shaded areas indicate mean +/- standard deviation.

For the earlier harvest times (HT 1 and HT 2), a decreasing trend in the μ_s values of the juice vesicles can be

observed for increasing fruit sizes. In the case of flavedo, minor differences in the μ_s values were found among different fruit sizes. The μ_s values of flavedo in small fruit were slightly higher than the values in other fruit sizes only at HT 2. For the other harvest times, the values of μ_s clearly overlapped among different fruit sizes.

Fig. 6 Bulk scattering coefficient spectra of (a) juice vesicles and (b) flavedo at the (1) 1st harvest time, (2) 2nd harvest time and (3) 3rd harvest time for different fruit sizes. The lines indicate the mean values, while the shaded areas indicate mean $\pm$ standard deviation.

3.3.3 Relation with cultivar

As illustrated in section 3.3.2, the BOP of the fruit tissues are more or less influenced by fruit size. Therefore, to study the variation of BOP among different Satsuma cultivars, the effect of fruit size was excluded by only considering fruit with a similar size range. Considering the size fluctuation among labelled sizes and harvest times (Table 1), only a group of samples for each harvest time (HT 1: 55.34-55.65 mm; HT 2: 56.70-58.19 mm; HT 3: 65.82-67.35 mm) was used for this comparison.

As shown in Fig. 7 (a1) and (a2), the positions of the pigment absorption peaks for juice vesicles are coincident among different cultivars for HT 1 and HT 2. Moreover, it is worth noting that the difference in the pigment absorption intensity among different cultivars increased from HT 1 towards HT 2, where the highest and the

lowest values were respectively observed in Wase and Okitsu, and the values for Iwasaki were somewhere in between. At HT 3, a slight wavelength shift of the pigment absorption peaks can be noticed among different cultivars, which could be attributed to the variation in the carotenoids' composition. Compared to the situation in the juice vesicles, the difference among different cultivars in the flavedo was more obvious. As shown in Fig. 7 (b1), the positions of the pigment absorption peaks in the flavedo are consistent among different cultivars, with a slightly lower intensity for Wase compared to the other cultivars. Moreover, the intensity of the peak attributed to water absorption in Okitsu tends to be lower than the ones in the other cultivars. At HT 2, the peak attributed to the absorption by chlorophylls around 680 nm in Wase is remarkably ($P<0.05$) lower than the ones in Iwasaki and Okitsu, while the peak attributed to the absorption by carotenoids below 550 nm is higher ($P<0.05$). Meanwhile, a difference ($P<0.05$) in the water absorption among different cultivars can be observed at HT 2, where Wase has the highest value followed by Iwasaki and then Okitsu. At HT 3, the absorption caused by chlorophylls almost disappeared for all the cultivar, while the position of the peak around 515 nm and the intensity of the peak around 470 nm varied among different cultivars. The difference in the water absorption between Iwasaki and Okitsu at HT 3 follows a similar pattern as at HT 2. However, the relative intensity of water absorption in Wase compared to that in the other cultivars is totally different at these two harvest times. At HT 3, the absorption by water in Wase seems to be greatly reduced, which suggests that the flavedo has dried from HT 2 to HT 3.

Fig. 7 Bulk absorption coefficient spectra of (a) juice vesicles and (b) flavedo for fruit from different

cultivars with similar fruit size for the (1) 1st harvest time, (2) 2nd harvest time and (3) 3rd harvest time. The lines indicate the mean values, while the shaded areas indicate mean $\pm$ standard deviation.

The differences in the μ_s spectra among different cultivars change slightly with harvest time both for the juice vesicles and the flavedo. As shown in Fig. 8 (a1) and (a3), at HT 1 and HT 3, the differences in the μ_s values of juice vesicles among different cultivars are negligible ($P > 0.05$), while at HT 2 small deviations can be noticed. The situation for flavedo at HT 1 was similar to that for the juice vesicles as all the values overlapped with each other. The values for Wase begin to deviate from the other two cultivars at HT 2 (Fig. 8 (b2)), while at HT 3 significant differences ($P < 0.05$) can be noticed between all the three cultivars. As shown in Fig. 8 (b3), the decreasing trend of the μ_s values over the wavelength range is similar for Iwasaki and Okitsu, except for a baseline shift. However, for Wase not only the μ_s values are lower but also the decreasing trend is steeper compared to the other cultivars.

Fig. 8 Bulk scattering coefficient spectra of (a) juice vesicles and (b) flavedo for fruit from different cultivars with similar fruit size for the (1) 1st harvest time, (2) 2nd harvest time and (3) 3rd harvest time. The lines indicate the mean values, while the shaded areas indicate mean $\pm$ standard deviation.

4. Discussion

4.1 Influence of harvest time on BOP

The evolution in the absorption coefficient μ_a spectra of the juice vesicles and flavedo of Satsuma mandarin with harvest time clearly indicates an accumulation of carotenoid pigments, while a degradation of chlorophylls in the flavedo was also suggested. This is in line with the observations reported by other researchers based on chemical methods (Kato et al., 2004). β -cryptoxanthin and a β,β -xanthophyll are different types of carotenoids that have been reported to accumulate massively in juice vesicles when the flavedo color changes from green to orange (Kato et al., 2004), which corresponds to HT 2 as indicated by the change of the a^* index in Fig. 2 (b). The massive accumulation of these carotenoids is in accordance with the prominent increase of the pigment absorption peaks from HT 1 to HT 2 in the juice vesicles in Fig. 4 (a1). In the flavedo, the concentrations of β -carotene and α -carotene decrease and then remain constant at an extremely low level after the green stage. Meanwhile, other carotenoids such as β -cryptoxanthin and c -violaxanthin become abundant in the flavedo (Kato et al., 2004). The changes in the composition of the carotenoids can give an explanation for the slight wavelength redshift of the carotenoids' absorption peaks observed in the flavedo at HT3.

The variations in the scattering coefficient μ_s spectra are mainly determined by the structural changes of the tissues (Xia et al., 2007), which may result in changes in the textural properties. Extensive research has been

dedicated to the textural properties of fruit (Muramatsu et al., 1996; Goulao et al., 2007), among which the majority concentrated on the softening process during ripening and postharvest storage. Van Beers et al. (2017) observed both a decline in the firmness and the scattering coefficient spectra of apple flesh during ripening. However, in the current research, an increase in the μ_s spectra of juice vesicles of Satsuma mandarin was observed from HT 1 to HT 2. The changes in the texture are commonly attributed to the changes in cell size, cell wall composition, cell wall strength and water loss (Toivonen et al., 2008). As the samples at these two harvest times have a similar size range, the influence from fruit size and thus cell size, which will be discussed in the following section, can be neglected here. So, it is unlikely that the increasing trend in μ_s values from HT 1 and HT 2 can be attributed to a tissue softening process similar to what was observed in apples. It has been reported that the softening of cell walls in fruit pulp starts at a fully developed stage when fruit growth is ceased (Brownleader et al., 1999). Moreover, the high activity of sucrose synthase (SuSy) in rapidly growing tissues has been linked to the rapid utilization of sucrose for cell wall synthesis (Amor et al., 1995). Furthermore, the TA decrease happens within the rapid growing period (Etxeberria et al., 2004). Therefore, according to the TA decrease illustrated in Fig. 3, the μ_s increase between HT 1 and HT 2 could be related to the synthesis of cell walls rather than the softening process.

4.2 Influence of fruit size on BOP

The BOP differences among different fruit sizes were compared for each harvest time. Considering these different size fruits were obtained from the same orchard at the same harvest time under the same management, the main factor influencing the fruit size was most likely the lighting condition. Fruit inside the canopy and on shaded branches receive less light and are therefore typically smaller than the fruit on the outside and bright side branches (Mooney, 2001). Meanwhile, light is also one of the major environmental factors influencing coloration of citrus fruit (Lado et al., 2019). Light shading can accelerate the color change in the peel (Lado et al., 2019), while the color change during citrus maturation involves the biosynthesis of carotenoids and the degradation of chlorophylls (Llorente et al., 2016). This is in accordance with the advanced color change

406 observed in small fruit in Fig. 5 (b1), (b2) and (b3). However, researchers also reported that the effect of light
407 shading on the coloration of mandarin is restricted to the peel and will not affect the final color of the pulp
408 (Lado et al., 2019). This is in contrast with the negative correlation between the intensity of pigment absorption
409 and fruit size observed in the juice vesicles in Fig. 5 (a1), (a2) and (a3). One possible explanation for the
410 negative correlation may relate to the change in moisture content. As water accumulates during the increase
411 in fruit size (Iglesias et al., 2007), the accumulation rate of water may exceed the accumulation of pigments.
412 This would reduce the pigment concentrations, resulting in smaller absorption peaks in big fruit. Similar
413 situations can also be observed in the SSC and TA values, where big size fruit tend to have lower SSC content
414 at later harvest times and lower TA content at earlier harvest times in Fig. 3. Such trends may also be related
415 to the water content. As for the difference in the timing of these two occurrences, it can be explained by the
416 asynchronous changes of the components related to these two indices (Iglesias et al., 2007).

417 The development of citrus fruit follows a sigmoid growth curve with 3 overlapping stages, including cell
418 division (stage 1), cell enlargement (stage 2) and fruit maturation (stage 3) (Iglesias et al., 2007). HT 1 and
419 HT 2 in this research are the early maturation stages, close to stage 2, where the growth of the juice vesicles
420 is responsible for most of the fruit size increase (Bain, 1958). With the enlargement of cells in juice vesicles,
421 the intercellular space increases, resulting in relatively less cell wall interfaces. As McGlone et al. (1997)
422 pointed out that the cell wall interfaces are the dominant scatterers in fruit, it can be speculated that larger size
423 fruit, having larger cells, would have a lower scattering coefficient. The phenomena observed in Fig. 6 (a1)
424 and (a2) support this hypothesis. As the differences in μ_s values became negligible at HT 3, this can be regarded
425 as the maturation stage. During this maturation stage, fruit continues to grow, but at a sharply reduced rate
426 compared to stage 2. Moreover, the juice vesicles at this stage are mainly occupied by loosely connected large
427 cells at the center (Jeffery et al., 2012). As this structure does not change much, it might explain the relative
428 stability of the μ_s values among different fruit sizes at HT 3. In the case of the flavedo, although a positive
429 correlation can be generally found between the size of the oil glands in the flavedo and the fruit size throughout

fruit development (Voo et al., 2012), large deviations in the number of oil glands have also been reported at maturity. Therefore, the combination of the negative effects of the increasing size and the positive effects of the decreasing number of the oil glands in the flavedo may provide explanations for the coherence of the μ_s values of flavedo among different fruit sizes.

4.3 Influence of cultivar on BOP

The emerging intensity difference in the pigment absorption of juice vesicles among different cultivars may be attributed to the variation in the start and duration of the maturation period, and thus the ripening period among different cultivars (Okuda et al., 2005). Iwasaki has been reported to ripen about two weeks earlier than Okitsu (Merino et al., 2015). Moreover, the degradation of chlorophylls in the flavedo of Wase was observed to finish earlier than in the other two cultivars. This suggests that Wase ripened first, followed by Iwasaki and Okitsu. This hypothesis is supported by the evolution in the juice pigments, where the earlier maturing cultivar has a higher concentration of pigments in the juice vesicles at HT 2. In addition, the ripening order of the cultivars observed from the pigment absorption of juice vesicles is in agreement with the degreening order observed from the color index of the flavedo in Fig. 2. Although, peel and flesh can be considered as separated organs, the external and internal ripening process in citrus fruit is generally regarded as coincident (Iglesias et al., 2007). Moreover, fruit ripening is a complex, genetically programmed process (Pathare et al., 2013), during which some substances participating in the formation of one property may also be involved in the metabolism of another property (e.g. carotenoid biosynthesis), which further provides the possibility for an indirect connection between the properties of both tissue layers. After the green stage, a variety of carotenoids gradually accumulate in the juice vesicles. Since each type of carotenoid follows a specific increasing rate (Kato et al., 2004), the time to mature differs from cultivar to cultivar, resulting in variation in the carotenoid composition of the juice vesicles among different cultivars. As each type of carotenoid has a characteristic absorption spectrum (Kato et al., 2004), the difference in the carotenoids' composition results in variation between the μ_a spectra of different cultivars, which is in line with the

wavelength shift of the pigment absorption peaks observed in Fig. 7 (a3). Similarly, a change in the composition of carotenoids also happened in the flavedo after the green stage (Kato et al., 2004). Therefore, the differences in maturation among different cultivars may explain the obvious differences in the absorption signatures in Fig. 7 (b3).

In the early maturation stage, approaching stage 2, the increase in fruit size mainly results from enlargement of the juice vesicles (Bain, 1958). As samples of similar size were selected for the different cultivars, the size/structure of the juice vesicles can be assumed to be similar too. This is in agreement with the comparable μ_s values observed in Fig. 8 (a1). Meanwhile, as the cells enlarge, the cells surrounding the center of the vesicles with electron-dense cytoplasm begin to collapse, filling the central cavity with fibrillar material, lipid and cell wall (Burns et al., 1992). In line with the differences in the maturation timing between these cultivars, this process may also differ among different cultivars. This might explain the deviation in Fig. 8 (a2). As the central cells continue to enlarge, the central cavity becomes less apparent (Burns et al., 1992), resulting in a reduction of the interference from the central cavity on the μ_s values. This could explain why the μ_s values of the three cultivars overlapped again at HT 3. The oil glands in the flavedo have been reported to reach maturity in an immature green fruit and then enlarge with fruit growth (Knight et al., 2001). Actually, the ultrastructural development of the oil glands in flavedo is similar to the development of the oil droplet area (central cavity) in juice vesicles (Burns et al., 1992). However, the size of the oil glands is much larger compared to the surrounding cells, which is different from the relative size of the central cavity to the encircled cells in juice vesicles. Therefore, the unneglectable existence of the oil glands combining the difference in the maturation timing among cultivars may give explanations for the continuous deviation in the μ_s values of flavedo among these three cultivars. As for the relative μ_s difference among these cultivars at HT 3, as mentioned previously, water loss can be one of the factors influencing the textural properties. Therefore, the relative intensity of the water absorption peak in the flavedo shown in Fig. 7 (b3) may explain the change of the μ_s spectra among these cultivars.

5. Conclusions

The BOP of juice vesicles and flavedo of Satsuma mandarin were measured and the effects of harvest time, fruit size and cultivar were explored. An increasing trend in the absorption by carotenoids was observed in both the juice vesicles and the flavedo during maturation. Moreover, a negative correlation was found in the absorption attributed to chlorophylls in the flavedo with harvest time. The μ_s values of juice vesicles increased slightly from HT 1 to HT 2, and then became flatter over the wavelength range at HT 3, while the values in the flavedo were quite stable over the different harvest times. Lower absorption peaks related to the pigments in juice vesicles were observed in larger fruit, which were linked to a dilution effect in fruit expansion. Moreover, an accelerated color change was observed in the flavedo of small fruit. Small fruit had higher μ_s values in the juice vesicles compared to big fruit and the difference gradually diminished with fruit maturation, whereas the μ_s values in flavedo were basically the same for different fruit sizes. The μ_a comparison among different cultivars was related to the maturation order. Higher μ_a values related to carotenoids in juice vesicles and advanced color changes in flavedo were found in earlier maturing cultivars. Except for a small deviation at HT 2, almost no difference was observed in the μ_s values of the juice vesicles between the different cultivars. Similarly, the μ_s difference in the flavedo also appeared at HT 2, but became more obvious thereafter. These insights in the effects of harvest time, fruit size and cultivar on the interaction of light with Satsuma mandarin tissues will be valuable in designing optical sensors for non-destructive evaluation of Satsuma mandarin quality.

Acknowledgements

This work was supported by the China Scholarship Council (No. 201606320240).

References

- Aernouts, B., Zamora-Rojas, E., Van Beers, R., Watté, R., Wang, L., Tsuta, M., Lammertyn, J., Saeys, W.,
2013. Supercontinuum laser based optical characterization of turbid media in the 500-2250 nm range.
Opt. Express. 21, 32450-32467. doi:10.1364/OE.21.032450

Amor, Y., Haigler, C.H., Johnson, S., Wainscott, M., Delmer, D.P., 1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose. *Proc. Natl. Acad. Sci. USA* 92(20), 9353-9357. doi: 10.1073/pnas.92.20.9353

Antonucci, F., Pallottino, F., Paglia, G., Palma, A., D'Aquino, S., Menesatti, P., 2011. Non-destructive estimation of mandarin maturity status through portable Vis-NIR spectrophotometer. *Food Bioproc. Tech.* 4 (5), 809-813. doi:10.1007/s11947-010-0414-5

Bain, J.M, 1958. Morphological, anatomical, and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis* (L) Osbeck. *Aust. J. Bot.* 6(1), 1-23. doi:10.1071/BT9580001

Blasco, J., Aleixos, N., Cubero, S., Gómez-Sanchís, J., Moltó E., 2009. Automatic sorting of satsuma (*Citrus unshiu*) segments using computer vision and morphological features. *Comput. Electron. Agric.* 66, 1-8. doi: 10.1016/j.compag.2008.11.006

Biais, B., Bénard, C., Beauvoit, B., Colombié, S., Prodhomme, D., Ménard, G., Bernillon, S., Gehl, B., Gautier, H., Ballias, P., Mazat, J.P., Sweetlove, L., Génard, M., Gibon, Y., 2014. Remarkable reproducibility of enzyme activity profiles in tomato fruits grown under contrasting environments provides a roadmap for studies of fruit metabolism. *Plant Physiol.* 164, 1204-1221. doi:10.1104/pp.113.231241

Brownleader, M.D., Jackson, P., Mobasheri, A., Pantelides, A. T., Sumar, S., Trevan, M., Dey, P.M., 1999. Molecular aspects of cell wall modifications during fruit ripening. *Crit. Rev. Food Sci. Nutr.* 39(2), 149-164. doi: 10.1080/10408399908500494

Burns, J.K., Achor, D.S., Echeverria, E., 1992. Ultrastructural studies on the ontogeny of grapefruit juice vesicles (*Citrus paradise* Macf. cv Star Ruby). *Int. J. Plant Sci.* 153(1), 14-25. doi:10.1086/297002

Cayuela, J., Weiland, C., 2010. Intact orange quality prediction with two portable NIR spectrometers. *Postharvest Biol. Technol.* 58(2), 113-120. doi:10.1016/j.postharvbio.2010.06.001

Clark, C.J., 2016. Fast determination by Fourier-transform infrared spectroscopy of sugar–acid composition of citrus juices for determination of industry maturity standards. *N. Z. J. Crop Hortic. Sci.* 44 (1), 69-82.

doi:10.1080/01140671.2015.1131725

- Conesa, A., Manera, F.C., Brotons, J.M., Fernandez-Zapata, J.C., Simón, I., Simón-Grao, S., Alfosea-Simón, M., Martínez Nicolás, J.J., Valverde, J.M., García-Sánchez, F., 2019. Changes in the content of chlorophylls and carotenoids in the rind of Fino 49 lemons during maturation and their relationship with parameters from the CIELAB color space. *Sci. Hortic.* 243, 252-260. doi:10.1016/j.scienta.2018.08.030
- Ettxeberria, E., Gonzalez, P., Pozueta-Romero, J., 2004. Sucrose transport into citrus juice cells: evidence for an endocytic transport system. *J. Amer. Soc. Hort. Sci.* 130(2), 269-274. doi: 10.21273/JASHS.130.2.269
- Folch-Fortuny, A., Prats-Montalbán, J.M., Cubero, S., Blasco, J., Ferrer, A., 2016. VIS/NIR hyperspectral imaging and N-way PLS-DA models for detection of decay lesions in citrus fruits. *Chemometr. Intell. Lab. Syst.* 156, 241-248. doi: 10.1016/j.chemolab.2016.05.005
- Fraser, D.G., Jordan, R.B., Künnemeyer, R., McGlone, V.A., 2003. Light distribution inside mandarin fruit during internal quality assessment by NIR spectroscopy. *Postharvest Biol. Technol.* 27(2), 185-196. doi:10.1016/S0925-5214(02)00058-3
- Gómez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis/NIR-spectroscopy techniques. *J. Food Eng.* 77 (2), 313-319. doi:10.1016/j.jfoodeng.2005.06.036
- Goulao, L.F., Santos, J., Sousa, I., Oliveira, C.M., 2007. Patterns of enzymatic activity of cell wall-modifying enzymes during growth and ripening of apples. *Postharvest Biol. Technol.* 43(3), 307-318. doi: 10.1016/j.postharvbio.2006.10.002
- Granato, D., Masson, M.L., 2010. Instrumental color and sensory acceptance of soy-based emulsions: a response surface approach. *Ciência e Tecnologia de Alimentos.* 30(4), 1090-1096. doi:10.1590/S0101-20612010000400039
- Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. *Aust. J. Agric. Res.* 56(4), 417-426. doi:10.1071/AR04299

550 Hager, A., 1970. Formation of Maxima in the Absorption Spectrum of Carotenoids in the Region Around 370
551 Nm; Consequences for the Interpretation of Certain Action Spectra. *Planta*. 91(1), 38-53.
552 doi:10.1007/BF00390164.

553 Iglesias, D.J., Cercós, M., Colmenero-Flores, J.M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-Rivero, O.,
554 Lliso, I., Morillon, R., Tadeo, F.R., Talon, M., 2007. Physiology of citrus fruiting. *Braz. J. Plant Physiol.*
555 19(4), 333-362. doi:10.1590/S1677-04202007000400006

556 Itle, R.A., Kabelka, E.A., 2009. Correlation between L*a*b* color space values and carotenoid content in
557 pumpkins and squash (*Cucurbita* spp.). *HortScience* 44(3), 633-637. doi:10.21273/HORTSCI.44.3.633

558 Izumi, H., Ito, T., Yoshida, Y., 1992. Effect of light intensity during the growing period on ascorbic acid content
559 and its histochemical distribution in the leaves and peel, and fruit quality of Satsuma mandarin. *J. Japan.*
560 *Soc. Hort. Sci.* 61(1), 7-15. doi:10.2503/jjshs.61.7

561 Jamshidi, B., Minaei, S., Mohajerani, E., Ghassemian, H., 2014. Prediction of soluble solids in oranges using
562 Visible/Near-infrared spectroscopy: effect of peel. *Int. J. Food Prop.* 17, 1460-1468.
563 doi:10.1080/10942912.2012.717332

564 Jeffery, J., Holzenburg, A., King, S., 2012. Physical barriers to carotenoid bioaccessibility. Ultrastructure
565 survey of chromoplast and cell wall morphology in nine carotenoid-containing fruits and vegetables. *J.*
566 *Sci. Food Agric.* 92, 2594-2602. doi:10.1002/jsfa.5767

567 Kato, M., Ikoma, Y., Matsumoto, H., Sugiura, M., Hyodo, H., Yano, M., 2004. Accumulation of carotenoids
568 and expression of carotenoid biosynthetic genes during maturation in citrus fruit. *Plant Physiol.* 134(2),
569 824-837. doi:10.1104/pp.103.031104

570 Knight, T.G., Klieber, A., Sedgley, M., 2001. The relationship between oil gland and fruit development in
571 Washington Navel orange (*Citrus sinensis* L. Osbeck). *Ann. Bot.* 88, 1039-1047.
572 doi:10.1006/anbo.2001.1546

573 Krivoshiev, G.P., Chalucova, R.P., Moukarev, M.I., 2000. A possibility for elimination of the interference from

the peel in nondestructive determination of the internal quality of fruit and vegetables by vis-NIR spectroscopy. *LWT-Food Sci. Technol.* 33(5), 344-353. doi:10.1006/fstl.2000.0669

Lacey, K., 2019. Fruit size management of citrus. <https://agric.wa.gov.au/n/1828> (accessed 25 August 2020)

Lado, J., Alós, E., Manzi, M., J.R. Cronje, P., Gómez-Cadenas, A., J. Rodrigo, M., Zacarías, L., 2019. Light regulation of carotenoid biosynthesis in the peel of mandarin and sweet orange fruits. *Front Plant Sci.* 10, 1288. doi:10.3389/fpls.2019.01288

Lado, J., Gambetta, G., Zacarias, L., 2018. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. *Sci. Hortic.* 233, 238-248. doi:10.1016/j.scienta.2018.01.055

Lee, T.C., Zhong, P.J., Chang, P.T., 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of 'Ponkan' (*Citrus reticulata* Blanco) mandarin fruits. *Sci. Hortic.* 188, 57-65. doi:10.1016/j.scienta.2015.03.016

Liao, L., Dong, T., Qiu, X., Rong, Y., Wang, Z., Zhu, J., 2019. Nitrogen nutrition is a key modulator of the sugar and organic acid content in citrus fruit. *PLoS One* 14(10), e0223356. doi:10.1371/journal.pone.0223356

Llorente, B., D'Andrea, L., Ruiz-Sola, M.A., Botterweg, E., Pulido, P., Andilla, J., Loza-Alvarez, P., Rodriguez-Concepcion, M., 2016. Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism. *Plant J.* 85, 107-119. doi: 10.1111/tpj.13094

Lorente, D., Escandell-Montero, P., Cubero, S., Gómez-Sanchis, J., Blasco, J., 2015. Visible-NIR reflectance spectroscopy and manifold learning methods applied to the detection of fungal infections on citrus fruit. *J. Food Eng.* 163, 17-24. doi: 10.1016/j.jfoodeng.2015.04.010

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

Magwaza, L.S., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Nicolai, B.M., Terry, L.A.,

598 Opara, U.L., 2014. The use of Vis/NIRS and chemometric analysis to predict fruit defects and postharvest
599 behaviour of 'Nules Clementine' mandarin fruit. Food Chem. 163, 267-274.
600 doi:10.1016/j.foodchem.2014.04.085

601 Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolai, B., 2012. NIR spectroscopy
602 applications for internal and external quality analysis of citrus fruit-A review. Food Bioproc. Tech. 5(2),
603 425-444. doi:10.1007/s11947-011-0697-1

604 Manera, J., Brotons, J.M., Conesa, A., Porras, I., 2012. Relationship between air temperature and degreening
605 of lemon (*Citrus lemon* L. Burm. f.) peel color during maturation. Australian J. Crop Sci. 6(6), 1051-
606 1058. http://www.cropj.com/brontos_6_6_2012_1051_1058.pdf

607 McGlone, V.A., Abe, H., Kawano, S., 1997. Kiwifruit firmness by near infrared light scattering. J. Near
608 Infrared Spectrosc. 5, 83-89. doi:10.1255/jnirs.102

609 Merino, C., Hervalejo, A., Salguero, A., Arenas-Arenas, F.J., 2015. Yield and fruit quality of two satsuma
610 cultivars, 'OKITSU' and 'IWASAKI' in Andalusia, Spain. Acta Hort. 1065, 261-265.
611 doi:10.17660/ActaHortic.2015.1065.30

612 Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by
613 near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3(4), 227-237. doi:10.1255/jnirs.73

614 Mooney, P., 2001. Section 3. Orchard Management, in: Mooney, P. (Eds.), Growing citrus in New Zealand.
615 New Zealand Citrus Growers Inc., Wellington, pp. 60-64.

616 Muramatsu, N., Takahara, T., Kojima, K., Ogata, T., 1996. Relationship between texture and cell wall
617 polysaccharides of fruit flesh in various species of citrus. HortScience 31(1), 114-116.
618 doi:10.21273/HORTSCI.31.1.114

619 Ncama, K., Tesfay, S.Z., Opara, U.L., Fawole, O.A., Magwaza, L.S., 2018. Non-destructive prediction of
620 'Valencia' orange (*Citrus sinensis*) and 'Star Ruby' grapefruit (*Citrus × paradisi* Macfad) internal quality
621 parameters using Vis/NIRS. Acta Hort. 1194. doi:10.17660/ActaHortic.2018.1194.160

622 Ncama, K., Tesfay, S.Z., Opara, U.L., Tesfay, S.Z., Fawole, O.A., Magwaza, L.S., 2017. Application of
623 Vis/NIR spectroscopy for predicting sweetness and flavour parameters of ‘Valencia’ orange (*Citrus*
624 *sinensis*) and ‘Star Ruby’ grapefruit (*Citrus x paradisi* Macfad). *J. Food Eng.* 193, 86-94.
625 doi:10.1016/j.jfoodeng.2016.08.015

626 Okuda, H., Noda, K., Hirabayashi, T., Yoshimi Yonemoto, J., 2005. The relationship between bud dormancy
627 and the fruit maturing period in satsuma mandarin. *J. Japan. Soc. Hort. Sci.* 74(4), 342-344.
628 doi:10.2503/jjshs.74.342

629 Ozkaya, O., Yabaci Karaoglan, S., Incesu, M., Yesiloglu, T., 2019. The general and volatile properties and the
630 quality of two newly selected Satsuma clones (11/1 Izmir and 30/ Izmir) grown under Mediterranean
631 ecological conditions. *Food Sci. Technol.* 39(2), 451-457. doi:10.1590/fst.33017

632 Pathare, P.B., Linus Opara, U., Al-Julanda Al-Said, F., 2013. Colour measurement and analysis in fresh and
633 processed foods: A review. *Food Bioprocess Technol.* 6, 36-60. doi:10.1007/s11947-012-0867-9

634 Porras, I., Brotons, J.M., Conesa, A., Castañer, R., Pérez-Tornero, O., Manera, F.J., 2015. Quality and fruit
635 colour change in Verna lemon. *J. Appl. Bot. Food Qual.* 88, 215-221. doi:10.5073/JABFQ.2015.088.031

636 Prahl, S.A., van Gemert, M.J.C., Welch, A.J., 1993. Determining the optical properties of turbid media by
637 using the adding-doubling method. *Appl. Opt.* 32, 559. doi:10.1364/ao.32.000559

638 Richardson, A.C., Marsh, K.B., MacRae, E.A., 1997. Temperature effects on Satsuma mandarin fruit
639 development. *J. Hortic. Sci.* 72(6), 919-929. doi:10.1080/14620316.1997.11515584

640 Rodrigo, M.J., Alquézar, B., Alós, E., Medina, V., Carmona, L., Bruno, M., Al-Babili, S., Zacarías, L., 2013.
641 A novel carotenoid cleavage activity involved in the biosynthesis of Citrus fruit-specific apocarotenoid
642 pigments. *J. Exp. Bot.* 64, 4461-4478. doi:10.1093/jxb/ert260

643 Sadka, A., Shlizerman, L., Kamara, I., Blumwald, E., 2019. Primary metabolism in citrus fruit as affected by
644 its unique structure. *Front Plant Sci.* 10, 1167. doi:10.3389/fpls.2019.01167

645 Simeone, M.L.F., Parrella, R.A.C., Schaffert, R.E., Damasceno, C.M.B., Leal, M.C.B., Pasquini, C., 2017.

646 Near infrared spectroscopy determination of sucrose, glucose and fructose in sweet sorghum juice.
647 Microchem. J. 134, 125-130. doi:10.1016/j.microc.2017.05.020

648 Sun, C., Aernouts, B., Van Beers, R., Saeys W., 2020a. Simulation of light propagation in citrus fruit using
649 monte carlo multi-layered (MCML) method. J. Food Eng. 291, 110225.
650 doi:10.1016/j.jfoodeng.2020.110225

651 Sun, C., Van Beers, R., Aernouts, B., Saeys W., 2020b. Bulk optical properties of citrus tissues and the
652 relationship with quality properties. Postharvest Biol. Technol. 163, 111127.
653 doi:10.1016/j.postharvbio.2020.111127

654 Tao, N.G., Ao, T.T., Liu, Y.J., Huang, S.R., 2012. Effect of sucrose-based polymers on quality of Satsuma
655 mandarin fruit (Citrus unshiu Marc. cv. Miyagawa Wase). Int. J. Food Sci. Technol. 47, 997-1003.
656 doi:10.1111/j.1365-2621.2011.02933.x

657 Tietel, Z., Weiss, B., Lewinsohn, E., Fallik, E., Porat, R., 2010. Improving taste and peel color of early-season
658 Satsuma mandarins by combining high-temperature conditioning and degreening treatments. Postharvest
659 Biol. Technol. 57(1):1-5. doi:10.1016/j.postharvbio.2010.01.015

660 Toivonen, P.M.A., Brummell, D.A., 2008. Biochemical bases of appearance and texture changes in fresh-cut
661 fruit and vegetables. Postharvest Biol. Technol. 48(1), 1-14. doi:10.1016/j.postharvbio.2007.09.004

662 Van Beers, R., Aernouts, B., Watté, R., Schenk, A., Nicolaï, B., Saeys, W., 2017. Effect of maturation on the
663 bulk optical properties of apples skin and cortex in the 500 to 1850 nm wavelength range. J. Food Eng.
664 214, 79-89. doi:10.1016/j.jfoodeng.2017.06.013

665 Voo, S.S., Grimes, H.D., Lange, M.B., 2012. Assessing the biosynthetic capabilities of secretory glands in
666 citrus peel. Plant Physiol. 159, 81-94. doi:10.1104/pp.112.194233

667 Windig, W., Shaver, J.M., Bro, R., 2008. Loopy MSC: A simple way to improve multiplicative scatter
668 correction. Appl. Spectrosc. 62(10), 1153-9. doi:10.1366/000370208786049097

669 Workman, J., Weyer, L., 2012. Practical Guide and Spectral Atlas for Interpretive Near-Infrared Spectroscopy

670 (2nd ed.). CRC Press, Florida.

671 Xia, J.J., Berg, E.P., Lee, J.W., Yao, G., 2007. Characterizing beef muscles with optical scattering and
672 absorption coefficients in VIS-NIR region. Meat Sci. 75(1), 78-83. doi:10.1016/j.meatsci.2006.07.002