Physiological foundations of spectral imaging-based monitoring of apple fruit ripening

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Abstract

An important aspect of precision horticulture methodology is comprised by online monitoring of apple crop load, gauging fruit expansion, and estimating the extent and the rate of ripening. Remote monitoring based on multi- and hyperspectral imaging is a powerful tool for solving this problem. Still, its potential is often limited by insufficient understanding of the relationships between the observed changes in fruit optical properties and the evolution of the fruit biochemistry and other properties in the course of ripening. Non-invasive estimations of apple ripening are based predominantly on the chlorophyll degradation kinetics derived from the spectral reflectance data calibrated against biochemical and rheological assays. Nevertheless, the fruit skin chlorophyll displays irregularities in response to fluctuation in environmental (sunlight, temperature) and other stimuli obscuring the changes caused by ripening itself. Another major pigment group, carotenoids display the opposite pattern of changes: these pigments are often retained or even accumulated during apple ripening. Consequently, the ratio of the contents of carotenoids and chlorophylls displays more robust trends in apple fruit ripening reflecting the variation in on-tree and off-tree ripening rate as affected by environmental stimuli as compared to assessment of degradation of the chlorophylls alone. We propose to use the previously developed in our group plant senescence reflectance index (PSRI), which is closely related with carotenoid-to-chlorophyll contents ratio in fruit and hence with their ripening. The PSRI values plotted vs. chlorophylls (also derived from fruit reflectance) provide for a robust recording of apple ripening both on tree and in storage yielding data sets comparable across orchards and growing seasons. We demonstrate the applicability of this approach to reveal the effect of apple fruit picking date on their ripening rate in storage. We also test the 'PSRI vs. chlorophylls' approach for visualization of apple ripening on hyperspectral images and discuss its limitations and possible workarounds.

Keywords: hyperspectrometer, proximal sensing, chlorophyll, carotenoids, postharvest monitoring, reflectance

INTRODUCTION

The priority goal of any fruit grower is maintaining the highest quality of fruit produce at all stages of the production chain, from farm to table. This goal calls for the development of affordable and efficient, preferably non-invasive techniques for monitoring the quality of the fruit produce during pre- and postharvest period. Apart from sorting out damaged fruit, it is important to monitor the fruit ripeness stage. This is essential to i) choose a correct harvest window and ii) pick the fruit at a similar ripening stages to simplify their postharvest handling and extend their shelf life (Barden and Bramlage, 1994; Zude and Herold, 2002; Geyer et al., 2007).

Implementation of the modern approaches of precision farming, including

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horticulture, dictate the need for knowledge-based algorithms for deriving actionable insights from (hyper)spectral data. Development of such algorithms requires a deep understanding of the biology of fruit maturation and ripening. A considerable body of evidence accumulated to the date suggests that the changes in the optical properties of ripening fruit reflect the implementation of genetic program of maturation modulated by a plethora of environmental stimuli and the employed horticulture practices (Gross and Gross, 1987; Vendrell and Palomer, 1998; Solovchenko et al., 2019). There were numerous attempts to employ the changes in total chlorophylls (Chl) and/or carotenoids (Car) as ripeness markers but with a limited success (Gross and Gross, 1987; Knee, 1988). Robust approaches have been developed employing monitoring of the Car-to-Chl ratio together with Chl content as an internal reference of the ripeness condition of apple fruit (Merzlyak et al., 1999; Zude, 2003; Solovchenko et al., 2005).

Since the reflection of light used for non-invasive monitoring of fruit is governed by the pigments contained in fruit, apart from the fruit tissue structure, the patterns of pigment transformation accompanying fruit ripening are of special importance in this context (Knee, 1980; Merzlyak et al., 1999, 2003b). Several spectral indexes taking into account the biology of fruit ripening was developed to the date like Plant Senescence Reflectance Index, PSRI. The latter has been successfully applied for the monitoring of apple fruit ripening (Merzlyak et al., 1999; Solovchenko et al., 2005) but we are not aware of the application of such 'physiology-based' indexes in hyperspectral monitoring of fruit which remain mostly empirical.

A wide array of methods has been proposed for both remote and proximal non-invasive monitoring of plants (He et al., 2018; Schadewijk et al., 2018; Thomas et al., 2018). Currently the most widespread approaches are based on recording of light reflected by the plant object (Gamon et al., 2019; McCormick and Biegert, 2019). One of the most significant limitation of these methods was linked with its limited spatial resolution. With the advent of affordable imaging spectrometers, a breakthrough was made in this field: devices of these types capture spatially resolved information about the object. Nevertheless, the relationship between the changes in the parameters constituting what is perceived as 'quality' of fruit and the (hyper)spectral data remains in many cases uncertain, if not to say enigmatic. This is exacerbated by a significant heterogeneity of the monitored parameter exhibited by plants even within a single fruit (Solovchenko et al., 2006; Li and Cheng, 2008). Another set of problems arise during transition i) from the lab to the 'real-life' measurements in the field and ii) from conventional point-based reflectance measurements to hyperspectral imaging-based approaches. These problems are related with the complex geometry of the fruit and canopy of apple tree, its uneven illumination, and the complexity of plant pigment optics in situ (Merzlyak et al., 2003b; Sabzi et al., 2019).

The present work is a proof-of-concept aiming at coupling the previously developed robust indices e.g., PSRI and hyperspectral reflectance imaging for monitoring of apple fruit ripening. Toward this end, we i) re-capitulated the main features and limitations of the PSRI and ii) tested the applicability of PSRI as a model for processing of hyperspectral reflectance images of ripening apple fruit.

MATERIALS AND METHODS

Plant material

Visually undamaged and anthocyanin-free apple (*Malus domestica* 'Antonovka') grown in the Fruit Crop Department of Botanical Garden of Lomonosov Moscow State University (Moscow, Russia) were studied throughout several seasons (1992-2019). The fruits from the Botanical Garden were studied immediately and those from Michurinsk – within 1 day. Since the results of the multi-season observation (carried out with the conventional spectrophotometer) were discussed in detail in our previous publications (Merzlyak et al., 2003b; Solovchenko et al., 2005, 2006), in this work we show only representative data obtained with this method during the years 2017-2018.

In these experiments, six fruits were randomly picked each 4-7 days and after initial

measurements (80-110 days after full bloom, DAFB) were incubated at 25°C under dim light and ambient atmosphere up to one month (off-tree ripening). During off-tree ripening, the whole-fruit spectral reflectance was measured each 4-7 days.

For the hyperspectral measurements, fruit of the same harvest stored in a cold room (+4°C) in conventional atmosphere were used. Three samples consisting of 20 fruit each were removed from the storage and measured at 7-8-days intervals.

Conventional reflectance measurements

Whole-fruit reflectance was recorded using a 150-20 (Hitachi, Japan) or Cary 300 Bio (Agilent, USA) spectrophotometer equipped with tungsten and deuterium lamps as light sources and an integrating sphere. The spectral resolution was set at 2 nm. The spectra were recorded against barium sulfate or Spectralon as a white standard. In the course of ripening the spectra were taken from the same zones of fruit surface.

Hyperspectral reflectance image recording

The hyperspectral reflectance data-containing images were captured with a frame-based imaging hyperspectrometer IQ (SPECIM, Finland). For each pixel of the hyperspectral image (512×512 pixels), a reflectance spectrum (spectral range 400-1000 nm; spectral resolution 1 nm) was recorded against a white standard made of Spectralon under illumination with two 150 W cold daylight fluorescent lamps.

Spectral data analysis

The areas with background and specks were masked taking into account the corresponding reflectance signatures. For each pixel of the hyperspectral images, two indices were calculated. The first index, CI_{678}, is among the most sensitive spectral indices indicative of Chl content of the sample (Merzlyak et al., 2003b). It was calculated as follows:

$$CI_{678} = \frac{R_{800}}{R_{678}} \quad (1)$$

where R_{800} is the reflectance in a band in the near infrared (NIR) region unaffected by pigment absorption of light and R_{678} is the reflectance in the band of the red Chl absorption maximum.

The second spectral index was PSRI, an index tightly related with Car/Chl ratio in the samples and, ultimately, indicative of the rate and stage of senescence of plants (Merzlyak et al., 1999, 2003a, b):

$$PSRI = \frac{R_{678} - R_{480}}{R_{800}} \quad (2)$$

where R_{800} is the reflectance in a band in the near infrared (NIR) region unaffected by pigment and water absorption of light, R_{480} is the reflectance in a band affected by both Car and Chl, and R_{678} is the reflectance in the band of the red Chl absorption maximum.

For each index and each hyperspectral image, histograms were calculated (Tables 1 and 2). The histograms were binned (integrated) over specific ranges, normalized to the total pixel number in the corresponding image and the time-courses of changes of the corresponding integral values were analyzed.

As a result of the analysis, two combined hyperspectral indexes were suggested. The first one is a CI_{678}-based index, hyperspectral fruit CI_{678} ($hfCI_{678}$):

$$hfCI_{678} = \int_2^4 CI_{678} \left(\int_{1.5}^2 CI_{678} \right)^{-1} \quad (3)$$

The second one was the PSRI-based index, hyperspectral fruit PSRI (hfPSRI):

$$\text{hfPSRI} = \int_{0.3}^{0.5} PSRI \left(\int_{0.1}^{0.2} \text{PSRI} \right)^{-1} \quad (4)$$

Statistical treatment

Averages ± standard deviation are shown in the figures and tables. Number of samplings is indicated in the corresponding figure legend. The significance of the average differences was tested using Student's *t*-test in Origin software (OriginLabs, USA).

RESULTS AND DISCUSSION

Reflection of light by apple fruits during their on-tree maturation and ripening in postharvest period is governed by changes in their pigment content and composition (Gross and Gross, 1987; Solovchenko et al., 2019). At the initial stages of ripening, apples possess fully functional photosynthetic apparatus (Blanke and Lenz, 1989). Their pigment apparatus is comprised by Chl, Car, flavonoids and (in red-colored cultivars) anthocyanins. Maturation and ripening is accompanied with a gradual degradation of Chl and photosynthetic (primary) Car and accumulation of secondary Car uncoupled from photosynthesis (Gross and Gross, 1987; Knee, 1988; Solovchenko et al., 2010). The biology of the postharvest pigment transformation in apple fruit is discussed in detail elsewhere (Gross and Gross, 1987; Knee, 1988; Giovannoni, 2004; Solovchenko et al., 2005, 2019).

Generally, Car content declines monotonously and at a lower rate than Chl during on-tree ripening. After picking, Car content increases considerably mainly due to formation of secondary xanthophylls (Knee, 1988; Solovchenko et al., 2006, 2010). An illustrious example of the apple pigment transformation pattern is constituted by 'Antonovka' apples (Figure 1). Collectively, the findings obtained in our previous works form a ground to believe that the PSRI index adequately captures the timing of apple fruit ripening (Figure 2). At the same time, it seems to be general enough to serve as a foundation of models applicable across cultivars and different growing practices (upon appropriate parametrization). In view of this, we tested the applicability of PSRI for the processing of hyperspectral images of ripening apple fruits.

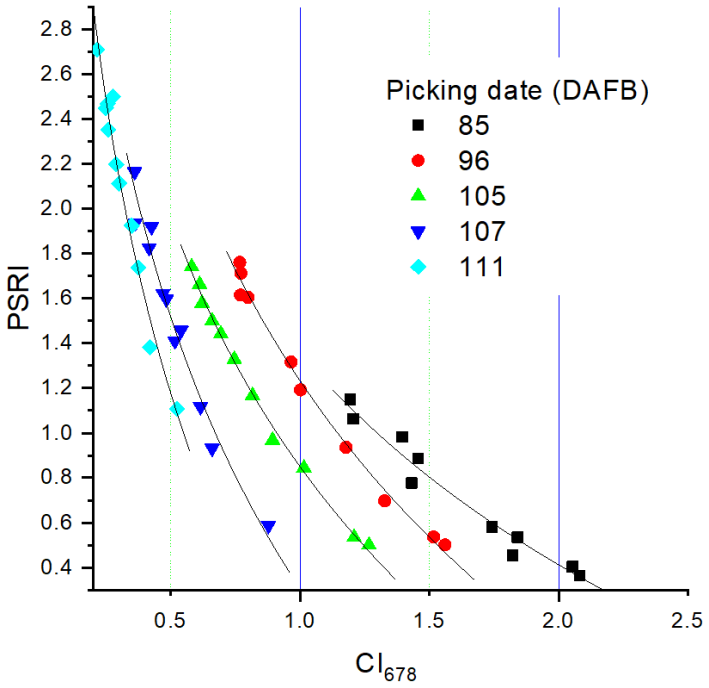


Figure 1. Typical trends of changes in PSRI (a proxy or ripeness) in stored apple ('Antonovka') fruit vs. CI_{678} (a proxy of Chl content) as a function of their picking date (indicated on the figure). Modified from (Solovchenko et al., 2005).

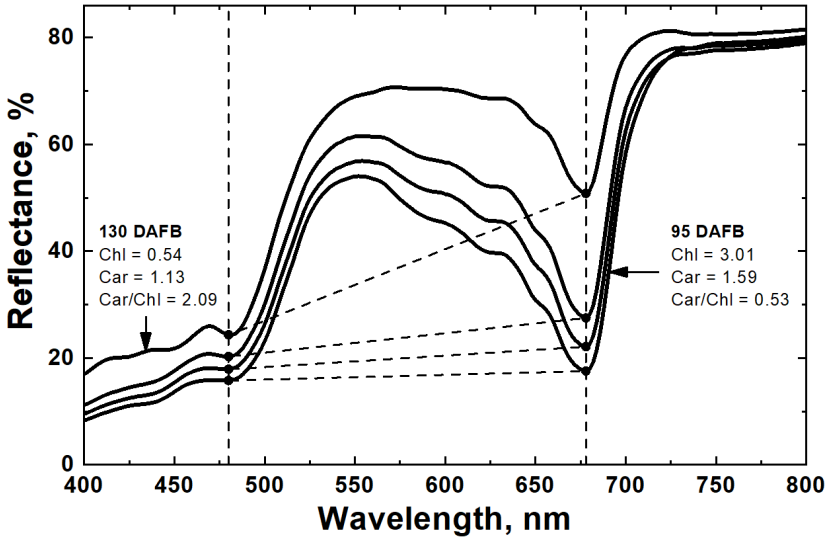


Figure 2. Typical changes in apple ('Antonovka') fruit reflectance during their ripening. The time after full bloom (DAFB) and pigment contents are indicated on the figure. The key spectral bands for construction of spectral indexes for Chl (CI_{678}, 678 nm) and Car/Chl ratio (PSRI, 578 and 480 nm) are shown by vertical dashed lines. Modified from (Merzlyak et al., 2003b).

To test the applicability of PSRI to the analysis of the hyperspectral reflectance image of stored fruit we used the apples removed from cold storage room as a model (see Materials and Methods). After pre-processing of the images (masking the background and specks), the spectral indexes reflecting Chl content (Table 1) and ripeness (Table 2) were calculated for each pixel remained in the image and the results were presented in form of color-coded images and histograms.

Table 1. Changes in fruit surface area exhibiting different values of CI_{678} index (color-coded) indicative of Chl content (top image row) and the corresponding distribution of the pixel values (bottom image row) in the hyperspectral images of the in stored apple ('Antonovka') fruit (picked at 105 DAFB), see also Materials and Methods.

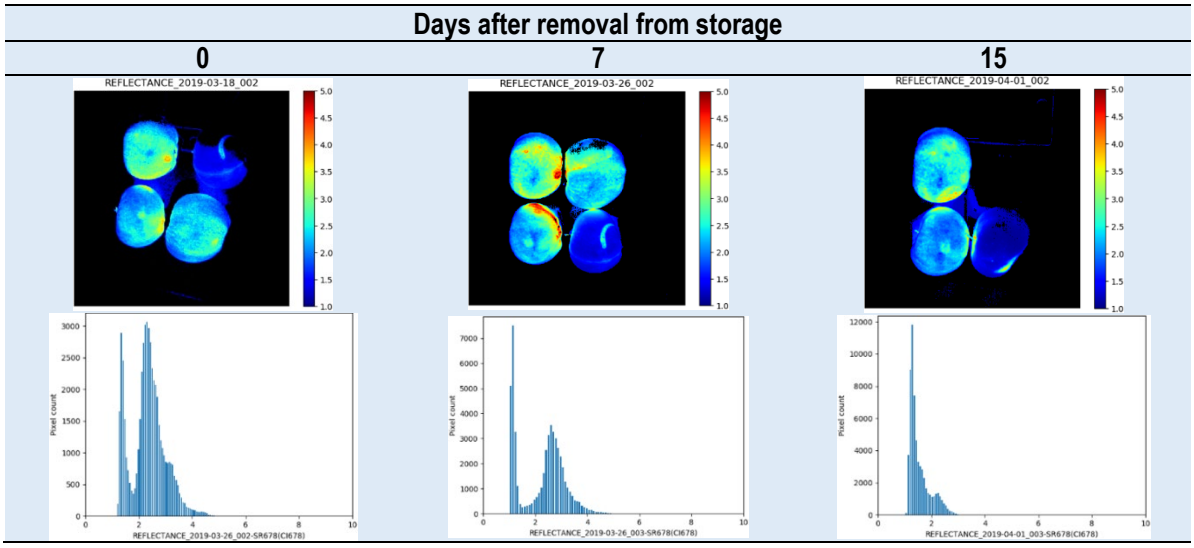
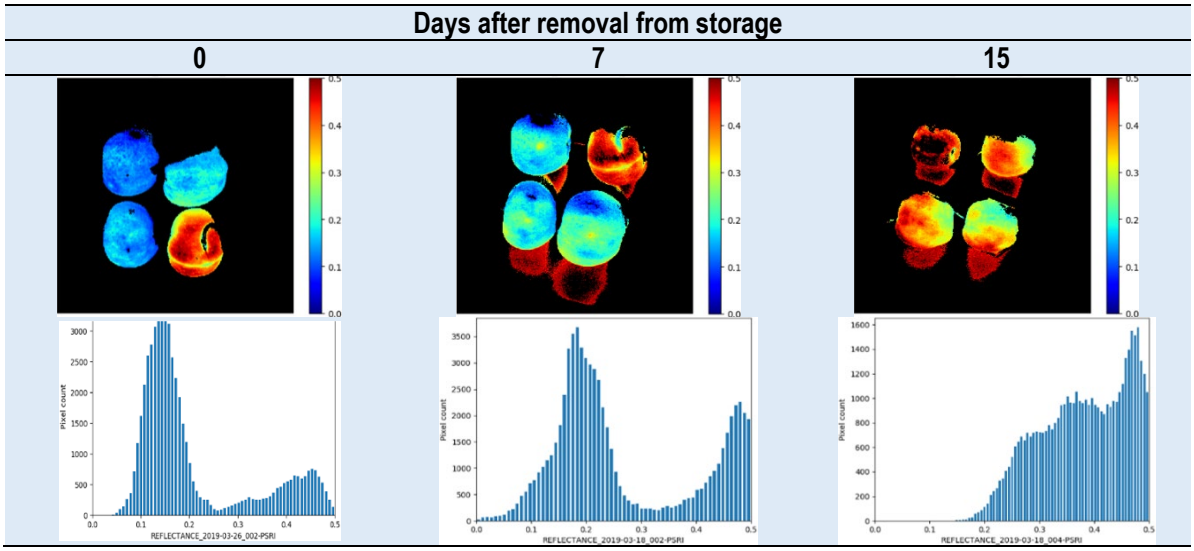


Table 2. Changes in fruit surface area exhibiting different values of PSRI index (color-coded) indicative of ripeness (Car/Chl ratio; top image row) and the corresponding distribution of the pixel values (bottom image row) in the hyperspectral images of the in stored apple ('Antonovka') fruit (picked at 105 DAFB), see also Materials and Methods.



As one can see from the representative histograms, the studied apple fruits were characterized by a considerable heterogeneity of surface pigment content and the corresponding actual ripeness. This is in line with our previous findings showing different rates of ripening even within the same fruit determined e.g., by the position of the fruit within the canopy (Solovchenko et al., 2006).

After pre-processing, we binned the pixels in each image according to their corresponding values of the CI_{678} and PSRI indexes and normalized each class to the total pixel number of the filtered image (Table 3). Analysis of the binned images highlighted the main trends of pigment transformation characteristic of fruit ripening outlined above and document in previous publications (Solovchenko et al., 2005; McCormick and Biegert, 2019), namely increase in Car/Chl ratio on the background of a decline in Chl. The high PSRI values are consistent with an increase in ripening after harvest. Thus, the detachment of fruits shifts their hormonal balance due to cessation of auxins and gibberellins, the hormones – antagonists of ethylene (Vendrell and Palomer, 1998) so ethylene quickly takes over leading to a fast onset of climacteric (Gross and Gross, 1987; Vendrell and Palomer, 1998; Giovannoni, 2004).

Table 3 shows that these trends were reflected by a gradual decline in the amounts of pixels associated with the values of the indexes typical of the unripe fruit. Accordingly, the relative amounts of pixels exhibiting the index values corresponding to ripe fruit increased. To further highlight the ripening-associated changes, we combined the classes of pixels showing the opposite trends in the compound indexes hfPSRI and $hfCI_{678}$ (see Materials and Methods and Figure 3).

The resulting index hfPSRI possessed a reasonably wide dynamic range allowed to reliably distinguish the fruit at different stages of ripeness (Figure 3) in spite of the internal heterogeneity of the fruit. The results of the analysis of the hyperspectral images of the stored fruit are in line with the current understanding of the key trends of post-harvest pigment transformation in apple fruit indicative of its ripeness. The non-invasive analysis carried out both with conventional reflectance or hyperspectral reflectance imaging showed that fruit ripening kinetics in storage is determined by a maturity state attained by the picking date but not the harvest date per se. More robust approaches for proximal sensing of apple fruit ripeness will require the destructive assessments e.g., Streif index measurements.

Table 3. Changes in the distribution of the hyperspectral image pixel values of the of the in stored apple fruit 'Antonovka' (2018, *n*=20), see also Tables 1 and 2.

Value interval		Normalized pixel count		
		DAFB		
		180	188	195
Ripeness (PSRI)				
0.0-0.1	(Unripe)	0.03	0.09	0.01
0.1-0.2		0.25	0.18	0.10
0.2-0.3	(Underripe)	0.29	0.14	0.21
0.3-0.4		0.16	0.23	0.32
0.4-0.5	(Ripe)	0.28	0.37	0.36
Chl content (CI_{678})				
1.1-1.5	(Yellow)	0.23	0.15	0.31
1.5-2	(Yellow green)	0.23	0.26	0.26
2.0-3.0	(Green yellow)	0.42	0.35	0.31
3.0-4.0	(Green)	0.05	0.06	0.02

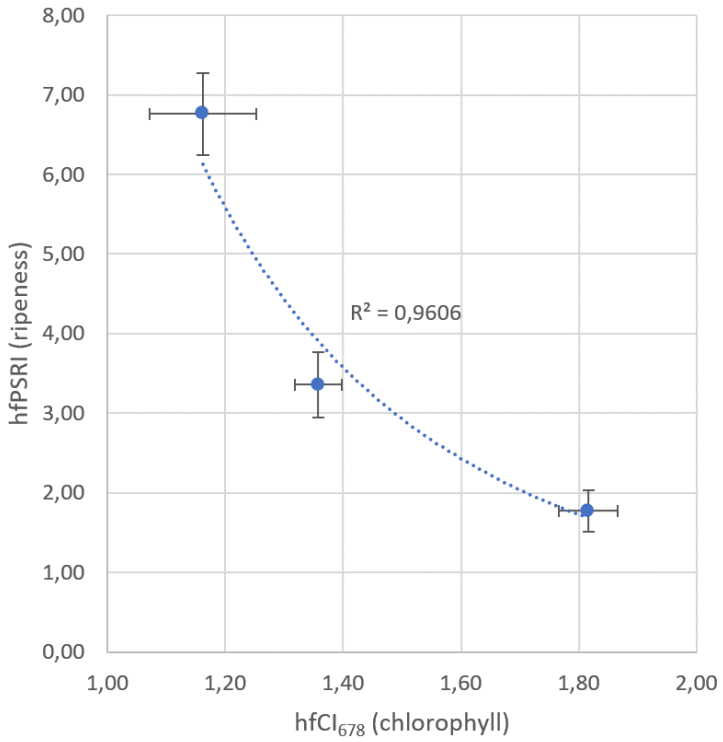


Figure 3. Trend of changes in hPSRI as a function of decline in Chl (hCI_{678} index) in apple ('Antonovka') fruit removed from controlled storage (*n*=20).

CONCLUSIONS

PSRI index is suitable for processing of hyperspectral reflectance images of ripening apple fruit. The analysis of hyperspectral reflectance images processed with the use of PSRI-based indexes gives adequate information about apple ripening despite the natural heterogeneity of the fruit with regard to actual ripeness. In the monitoring of apple ripening via hyperspectral reflectance imaging, Chl content can serve as an internal reference indicative of the actual physiological condition of the fruit. To increase robustness of fruit ripeness assessment and predictions, the results of proximal sensing should be calibrated against "wet" analytical assessments e.g., Streif index.

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