Robustness of pigment analysis in tree fruit

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Abstract. *The non-destructive application of spectrophotometry for analyzing fruit pigments has become a promising tool in precise fruit production. Particularly, the pigment contents are interesting to the growers as they provide information on the harvest maturity and fruit quality for marketing. The absorption of chlorophyll at its Q band provides quantitative information on the chlorophyll pool of fruit. As a challenge appears the in-situ measurement at varying developmental stage of the fruit due to its non-linear changes of absorption as well as scattering properties, which appear in the sum signal measured.*

Studies were carried out to analyze the absorption and effective scattering coefficients, μ_a and μ_s', respectively, by means of spatially resolved spectroscopy. Example is given for pear (Pyrus communis L. 'Conference') over a period of 60 - 150 days after full bloom.

Results are encouraging for calculating μ_a and μ_s' by means of Farrell diffusion theory model using Levenberg-Marquardt algorithm. The measuring uncertainty was <5% when reducing the 300 data points to 3 readings showing a potential for data reduction. With this approach, robust calibration can be carried out for non-destructively analyzing the chlorophyll content of pear over the period ranging from unripe to post-climacteric pears.

Keywords. *Backscattering imaging, Farrell, Pear, Plum, Precision horticulture, Spatially resolved spectroscopy*

Introduction

In horticulture, maturity analysis of climacteric fruit is even more important than in any other crop, since the developmental stage of fruit at harvest determines its eating quality and storability. Analyzing spectral-optical properties of fruit and, particularly, the absorption of fruit chlorophyll represents a potentially valuable approach for characterizing the fruit developmental stage. In the chlorophyll pool of fruits, the chlorophyll a, b, and pheophytin are the quantitatively most important molecules. The degradation of chlorophyll, when the chloroplasts of fruit are transformed to chromoplasts, can be measured at the chlorophyll absorption in the red wavelength range. This Q band appears around 680 nm in vivo (Seifert and Zude-Sasse, 2016), where no coinciding absorption of other pigments occurs.

For proximal analysis of fruit, the normalized difference vegetation index (NDVI) or red-edge are used to estimate the absorption of the chlorophyll pool at the Q passband. The indices, originally developed in remote sensing (Carter, 1994; Richardson et al., 2002), are frequently employed to reduce effects of disturbing scattering properties of the sample on the apparent signal (Merzlyak et al., 1999; Zude and Herold, 2002; Zude, 2003). In apple, the calibration of NDVI values on the chlorophyll content appears robust with coefficient of determination, $r^2 > 0.80$, even for varying cultivars (Zude, 2003). However, it was shown that this simple approach may fail to correct signals in other fruit. In plum, sampled 75 – 145 days after full bloom, dafb, the effective scattering coefficient measured at Q band, changed by 41.5% (8.5 – 5.0 cm^{-1}). In apple, sampled 65 – 135 dafb, the change of 14.7% (18.2 – 15.5 cm^{-1}) was measured (Seifert et al., 2015). Consequently, for in-situ analysis of fruit in varying developmental stage, we need the distinguished absorption coefficient, μa, rather than the apparent sum signal of absorption coefficient and effective scattering coefficient, μs'.

The separated data on μa and μs' can be obtained by means of analyzing the distribution of time of flight of photons in the fruit tissue. The necessary time-resolution is in the ps range (Seifert et al., 2015) and measurements in the field are difficult with existing equipment. In addition, the size of the fruit limits the application, since small fruits provide no reliable data. The spatially resolved measurement may serve as an alternative to this approach. The set-up of spatially resolved spectroscopy captures the light source for injecting photons at a certain point on the fruit surface and camera to acquire the spatial photon transport in the fruit tissue non-destructively. From this backscattering imaging, the radial profiles of photon attenuation can be derived. By means of the diffusion theory model of Farrell, the μa and μs' can be calculated (Farrell and Patterson, 1992). First attempts were made by the work group of Lu (Qin and Lu, 2007). In own work, the method has been used to calculate the soluble solids content and fruit flesh firmness in apple and kiwi, and fungal infection in citrus fruit (Qing et al., 2007; Baranyai and Zude, 2009; Lorente et al., 2015). The approach was reasonable even on smaller fruit as long as the profile was visible in the image taken and the μa was moderate. Enhanced pigment contents limited the application of the Farrell model of diffusion theory, since scattering appears no longer the dominant process (Lorente et al., 2015). Calculation are relatively fast, but data reduction would support the use of method in real-time systems.

The aims of the present study were (i) to use spatially resolved spectroscopy for analyzing μa and μs' at the Q band of chlorophyll absorption in pear and (ii) to test a data reduction considering the measuring uncertainty of μa and μs'.

Material & Methods

Fruits were sampled in commercial orchard from marked trees. At each measurement day, fruits were picked and all measurements were carried out. Fresh and dry mass were recorded gravimetrically subsequently to oven-drying at 65°C, respectively. Pigment contents were analyzed by high pressure liquid chromatography considering chlorophyll a, - b, and pheophytin. Sampling (n=30) was done 12 times over eight weeks beginning from 29th July – 10th September 2015.

All fruits (n=360) were non-destructively measured by spatially resolved spectroscopy. The imaging system was assembled in-house. The system comprised of a charge coupled device (CCD) camera (CV-A50IR, JAI Ltd, Japan) with a F2.5 zoom lens and 18-108 mm focal lengths (12VG1040 ASIR-SQ, Tamron Co. Ltd, Japan) and personal computer to control the camera, capturing the images, and its storage. Acquisition of the images was done in the dark to prevent direct illuminations from the external environment that may results in interference. 720 × 576 pixel images having a resolution of 0.133 mm/pixel were acquired. The images were captured, when injecting photons from laser diode module (2 mW, Newport, USA) emitting at 660 nm. The camera captured the fraction of backscattered light to the fruit surface and transferred the image to the computer. Mean radial profiles were calculated, starting from the outer end of saturation area at the incidence point of photon injection until total attenuation of the photons in the fruit tissue. Radial profiles were fitted by Farrell diffusion theory model using Levenberg Marquardt algorithm by optimizing the chi square error for calculating the absorption coefficient, μa, and effective scattering coefficient, μs'. 200 iterations were run starting from μa=0.001 and μs'=0.3.

$$R_F(r) = \frac{a'}{4\pi}\left[\frac{1}{\mu_t'}\left(\mu_{eff} + \frac{1}{r_1}\right)\frac{\exp(-\mu_{eff} r_1)}{r_1^2} + \left(\frac{1}{\mu_t'} + \frac{4A}{3\mu_t'}\right)\left(\mu_{eff} + \frac{1}{r_2}\right)\frac{\exp(-\mu_{eff} r_2)}{r_2^2}\right]$$

where r is the distance from the center of photon injection; a' is the transport albedo; μ_{eff} is the effective attenuation coefficient, $\mu_{eff} = [3\mu_a(\mu_a + \mu_s')]^{1/2}$; μ_t' is the total interactance coefficient; the variables r_1 and r_2 are given by the equations $r_1 = [(1/\mu_t')^2 + r^2]^{1/2}$ and $r_2 = [((1/\mu_t') + (4A/3\mu_t'))^2 + r^2]^{1/2}$, respectively; A is the internal refractive index.

All data analysis was done in Labview (National Instruments, U.S.) or MATLAB® (R2010B, MathWorks, U.S.).

RESULTS & DISCUSSION

Table 1. Absorption coefficient, μa, effective scattering coefficient, μs', of pears, when fitting the radial profiles be means of the model of Farrells' diffusion theory.

Date of year 2015	μa [r.u.]	μs' [r.u.]
29.7.	0.00152	0.24837
6.8.	0.00142	0.24293
13.8.	0.00100	0.21079
20.8.	0.00089	0.20700
24.8	0.00062	0.17788
27.8	0.00082	0.20021
31.8.	0.00061	0.18122
1.9.	0.00052	0.17083
2.9.	0.00068	0.18912
3.9.	0.00059	0.17861
7.9.	0.00052	0.17004
10.9.	0.00057	0.17523

The absorption coefficient considering the Q band of chlorophyll at 660 nm appeared low compared

to the effective scattering coefficient in pears at all ripeness stages of the fresh fruit from the tree. The diffusion theory should hold true in this conditions with μs'>>μa.

During the development of fruit the absorption decreased, indicating the degradation of chlorophyll in the climacteric fruit. The effective scattering coefficient decreased during fruit development by 29 % (Table 1). This finding appeared unexpected, since pip fruit such as pear and apple are built from the bottom of the flowers resulting in isotropic tissue that shows stable texture over the period of fruit development.

Comparing the present results on pear and former data found in apple (Seifert et al., 2015), the change of scattering coefficient is much higher in pear. Consequently, more accurate results can be expected for non-destructive chlorophyll analysis, if this 29% perturbation due to variation in the μs' can be diminished by using μa instead of the apparent sum signal of measured diffuse reflection.

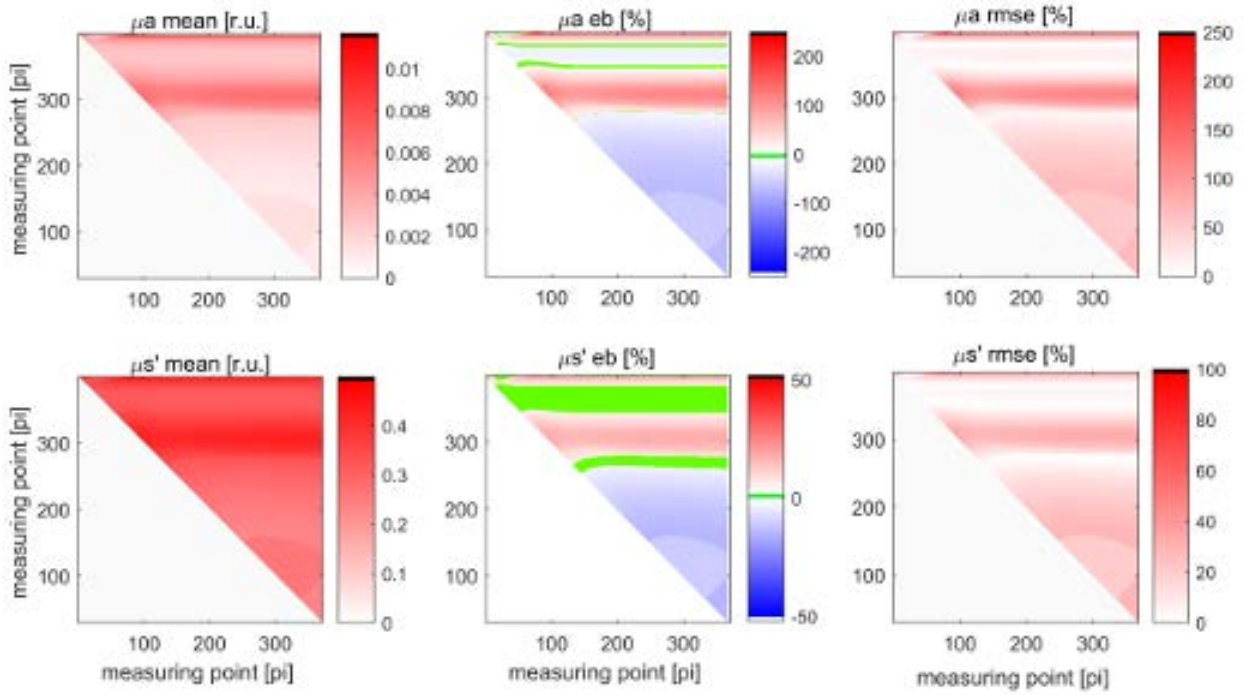

Figure 1. Calculated values of absorption coefficient, μa, and effective scattering coefficient, μs', at 660 nm are given when using varying position of data point 2 and data point three of the mean radial profile (left). The bias, eb, is provided for μa and μs' considering the difference of calculated values based on 300 and 3 data points (middle). The root mean square error is provided comparing calculated values using 300 and 3 data points accordingly (right).

This approach was further optimized by evaluating the influence of data reduction: The values calculated for μa and μs' were considered as reference values, when using the entire mean radial profile (300 pi) from the images. These values obtained with 300 data points were compared to the calculated μa and μs', when using a reduced number of data points. We assumed that the position of those reduced number of point would influence the result. Consequently, results from all combination of positions were calculated. In figure 1, the measuring uncertainty is presented, when using only 3 data points of the mean radiale profile for the fitting. Here, the first data point was always set at the threshold between saturation and the start of the mean radial profile. The second and third data points were taken in all combinations possible for the two points. The values of μa and μs' resulting from the position of the second and third data point are presented in figure 1, left. The bias, eb, and root mean square error, rmse, when compared to the data obtained with 300 data points are

provided. For the eb, the green areas and for rmse the white areas mark positions, which are suitable to calculate μa and μs' with errors below 5%.

In former studies on the spatially resolved spectroscopy, indices were calculated from the mean radial profile. Particularly, the full wide at half maximum, FWHM, was frequently used for simple analysis. With the results presented, it can be pointed out that the position of FWHM was indeed prominent for the data reduction as well.

Resulting, we are able to analyze the chlorophyll pool non-destructively – even in fruit with varying optical properties.

Conclusion

In pear, the effective scattering coefficient changed over the period of fruit development at the tree by 29 %. The resulting perturbation, when analyzing the chlorophyll of fruit, can be reduced by working on the separated μa. A data reduction from 300 to 3 can be obtained when using the optimized position of data points.

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