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Comparison of different chlorophylls determination methods for leafy vegetables

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Abstract. Modern agricultural farming requires precise, quick and nondestructive methods for determination of basic plant physiological parameters. One of the widely used and informative parameters is chlorophyll content in plant leaves. Determination of chlorophyll content by nondestructive methods is well elaborated for main field crops, but these methods are not widely used for chlorophyll content determination in leafy vegetables. The aim of the study was to compare two nondestructive methods with a classic biochemical chlorophylls determination method. Pigment content was expressed regarding to the leaf weight and leaf area. For nondestructive chlorophyll determination were used: a low cost handheld chlorophyll meter atLEAF+ and Miniature Leaf Spectrometer CI-710 (CID- Bio-Science). Chlorophylls content was determined using one of the 21 indices incorporated in Cl-710. For comparison of methods four different plant species (lettuce, leaf mustard, radish and cabbage) were used. Plants were grown at four illumination conditions – natural light, illumination supplemented with red, blue and mixed red/blue LED light. Results showed that at the majority of the investigated wavelengths, readings of the chlorophyll meter atLEAF+ and indices used for calculation are more sensitive to chlorophyll a content calculated per unit area. The maximum sensitivity of reflectance to variation with pigment content is found at 605 nm and 696 nm and in the near infrared region (740–930 nm). Higher correlation between non-destructive methods and biochemical analyses was observed in radish and leaf mustard leaves. The highest correlation coefficient was obtained with Difference Vegetation Reflectance index (NDVI) and Simple Ratio Pigment Index (SRPI). Nondestructive chlorophyll determination with chlorophyll meter atLEAF+ and Miniature Leaf Spectrometer CI-710 can completely replace biochemical analyses.

Key words: chlorophyll, atLEAF+, CI-710, non- destructive determination.

INTRODUCTION

Nowadays modern agricultural farming requires precise, quick and nondestructive methods for determination of basic plant physiological parameters. One of the widely used and informative parameters is the chlorophyll content in plant leaves. Chorophyll content varies in plant leaves depending on plant genetics, content of mineral elements, different stress factors, etc. (Haboudane et al., 2002; Torres et al., 2015; Wang et al.,

2016). Therefore leaf pigment content can provide valuable insight into the physiological performance of leaves (Sims & Gamon, 2002). Determination of chlorophyll content by nondestructive methods is well elaborated for main field crops, but these methods are not widely used for chlorophyll content determination in leafy vegetables. In the Database for remote sensing indices[1] totally 112 different indices of vegetation chlorophyll content are listed. Single wavelengths, simple ratios and more complex equations as well as derivations are used for determination of chlorophyll content in plant leaves. Eucalyptus plants showed maximum sensitivity of reflectance to variation in pigment content in the green wavelength region at 550 nm and at 708 nm in the far-red wavelengths. The reflectance in the main pigment absorption regions in the blue (400–500 nm) and in the red (660–690 nm) wavelengths proved to be insensitive to variation in pigment content (Datt, 1998). Similar results were obtained also with corn leaves were reflectance factors at 550 nm and 715 nm were inversely related to chlorophyll concentrations, but at 450 nm and 670 nm changed only slightly (Daughtry et al., 2000). Chainese researchers recommended 553 nm of detection chlorophyll content in frost damaged wheat leaves (Wang et al., 2016). Gathering more than 10 years research done in chlorophylls non destructive determination, Blackburn (2007) concluded that the major chlorophyll reflectance feature in the region 530–630 nm and in a narrower band around 700 nm, is most sensitive to pigment concentrations.

Recommended wavelengths in the NIR region for determination of chlorophyll content are 727–734 nm (Wang et al., 2016), 695–725 nm (Gitelson et al., 2003) and 710 nm and 925 nm (Maire et al., 2008).

The aim of this study was to compare two nondestructive methods with the classic biochemical chlorophylls determination method.

MATERIALS AND METHODS

Experiments were carried out in autumn 2015 in the heated polycarbonate greenhouse of the Faculty of Agriculture, Latvia University of Agriculture.

Plant material

Two varieties of lettuce plants: lettuce (*Lactuca sativa*) cv 'Lollo Bionda' and cv 'Lollo Rossa', leaf mustard (*Brassica juncea*) cv 'Scala' radish (*Raphanus sativus*) cv 'French breakfast, and cabbage seedlings (*Brassica oleracea*) cv 'Rufus', were grown in 3–5 L vegetation pots filled with commercial peat substrate *KKS-U*, pH 5.9 ± 0.3, PG Mix 15-10-20 0.6 kg m^{-3}. Lettuce cv 'Lollo Bionda', leaf mustard and cabbage has red leaves.

Additional LumiGrow The LumiBar LED strips were switched on from 6–9 AM and 16–20 PM used to obtain a 14 h photoperiod. Blue, Red and Mixed Blue/Red LED lighting was used. Control variant was without additional lighting. Plants were sown at August 29, analyses were performed at November 4, 2015. Totally 20 variants were examined. Earch variant was grown in 4 replicates.

[1] http://www.indexdatabase.de/db/a-single.php?id=17

Biochemical determination of pigments content

Content of chlorophyll a and b and sum of chlorophylls of were determined spectrophotometrically. 10 leaf discs were weighed and ground in a mortar with approximately 10 mg $CaCO_3$. Ground leaf material was extracted with three small volumes of ethanol, filled to 10 mL, centrifuged for 3 minutes and the absorbance of the supernatant solution in a 1cm cell was read at 440, 649 and 665 nm with a UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan). Chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophylls sum (Chl a+b) were calculated using the equations of Lichtenthaler & Buschmann (2001). Pigment content was expressed relative to the leaf weight and leaf area. Biochemical analyses were done in two replicates.

Nondestructive determination of pigments

For nondestructive chlorophyll determination were used: a low cost handheld chlorophyll meter atLEAF+ and Miniature Leaf Spectrometer CI-710 (CID-Bio-Science), which rapidly estimates plant properties using published and accepted indices. Chlorophylls content was calculated using one of the 21 different indices incorporated in Cl-710: Structure intensive pigment index (SIPI), Chlorophyll normalised vegetation index (CNDVI), Carter indexes (CTR1, CTR2), Greening index (G), Gitelson Merzlak indexes (GM1,GM2), Lichtenthaler indexes (LIC1, LIC20, Normalized difference vegetation reflectance (NDVI), Normalized pigment chlorophyll reflectance (NPCI), Normalized pheophitinization index (NPQI), Simple ratio pigment index (SRPI), Triangular vegetation index (TVI),Transformed Carter index (TCARI), Zarco-Tejada & Miller Index (ZMI), Modified red edge simple ratio index (MRESRI), Red edge normalized difference index (RENDVI), Vogelman red edge indexes (VREI1, VREI2 VREI3), Photochemical reflectance index (PRI), Plant senescence reflectance index (PSRI), Water band index (WBI). Nondestructive measurements were done in 10 replicates.

Significance for differences between methods and variants was analysed using two-way Anova at $P \leq 0.05$. Correlation analyses were performed between biochemical data, reflectance at a single wavelength and calculated indices by using Excel Data Analyses tool pack.

RESULTS AND DISCUSSION

The spectral absorbance properties of pigments are manifest in the reflectance spectra of leaves (Blackburn, 2007). Reflectance ability of vegetable leaves depends on pigment content and anatomical properties of leaves (Fig. 1). At the visible light range (400–700 nm) absorption of chlorophylls and carotenoides is well detectable. At the wavelength range 400–500 nm and 660–690 nm reflectance of plant leaves is less than 10%. The blue region is not recommended for estimation of chlorophylls content because it overlaps with absorbance of carotenoids. In the red region absorbance tends to saturate at low chlorophyll content and therefore sensitivity of the method decreases (Wu et al., 2008). The highest reflectance of leaves in visible spectrum was detected at 550 ± 5 nm which corresponds with literature data (Daughtry et al., 2000; Haboudane et al., 2002; Blackburn, 2007). Reflectance in the near infrared region varied from 32% (lettuce) till 56% (cabbage seedlings leaves).

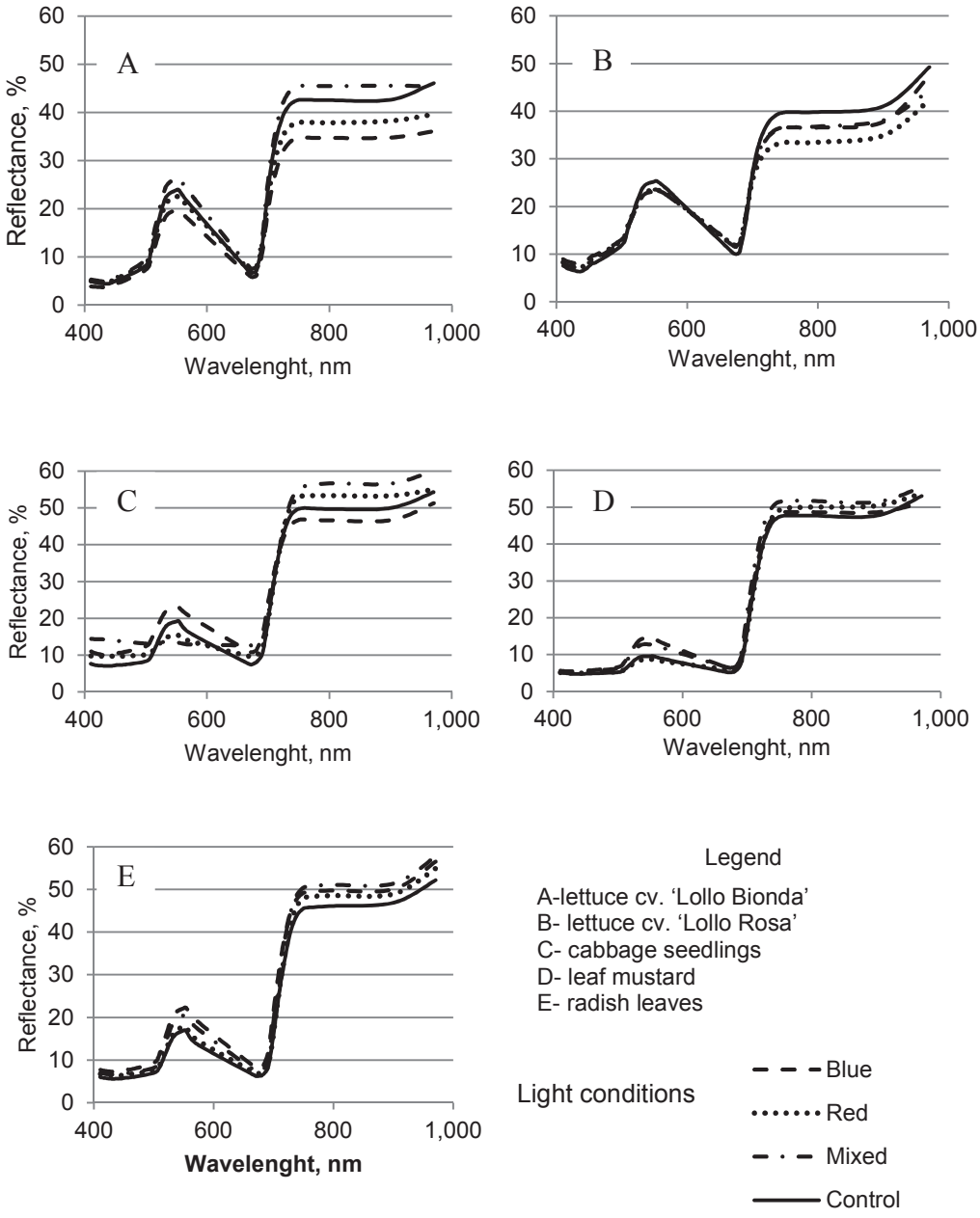

Figure 1. Reflectance spectra of leaves of; lettuce, leaf mustard, cabbage and redish grown with additional illumination with LED light of different colors.

Biochemical analyses shoved that chlorophyll content in vegetable leaves varied from 0.119 mg g^{-1} (0.221 mg dm^{-2}) fixed in lettuce 'Lollo Bionda' till 1.181 mg g^{-1} (3.146 mg dm^{-2}) determined in cabbage. Results showed that additional red lighting decreased chlorophyll content in vegetable leaves (except cabbage seedlings). There are reports about significant effects of LED illumination on plant pigment content (Olle &

Viršile, 2013) In research done with hydroponically grown lettuce significant differences in pigment content as result of LED lighting was not found (Lin et al., 2013). Similar result was obtained with cucumber transplants (Brazaitytė et al., 2009).

Table 1. Content chlorophylls in plant leaves detected biochemically

Plant	Light Variant	Pigment content, mg g^{-1}			Pigment content, mg dm^{-2}		
		Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b
Lettuce Lollo Bionda	Blue	0.257	0.108	0.365	0.454	0.190	0.644
	Red	0.077	0.042	0.119	0.143	0.077	0.221
	Mix	0.142	0.066	0.208	0.295	0.137	0.432
	Control	0.314	0.139	0.453	0.524	0.232	0.756
Lettuce Lollo Rosa	Blue	0.182	0.099	0.281	0.290	0.157	0.447
	Red	0.117	0.066	0.184	0.201	0.113	0.314
	Mix	0.113	0.069	0.182	0.191	0.117	0.308
	Control	0.182	0.100	0.283	0.308	0.170	0.479
Cabbage	Blue	0.666	0.330	0.996	1.558	0.771	2.329
	Red	0.806	0.375	1.181	2.147	0.999	3.146
	Mix	0.686	0.308	0.994	1.919	0.861	2.779
	Control	0.729	0.365	1.094	1.641	0.823	2.464
Leaf mustard	Blue	0.804	0.320	1.124	1.487	0.591	2.077
	Red	0.594	0.249	0.843	1.259	0.529	1.789
	Mix	0.601	0.242	0.842	1.247	0.502	1.749
	Control	0.920	0.409	1.329	1.454	0.646	2.100
Radish leaves	Blue	0.649	0.311	0.959	1.467	0.701	2.168
	Red	0.619	0.261	0.879	1.722	0.725	2.447
	Mix	0.602	0.259	0.861	1.522	0.653	2.175
	Control	0.756	0.349	1.105	1.621	0.748	2.369
	$RS_{0.05}$	0.059	0.028	0.086	0.128	0.057	0.182

Calculated coeficients of correlation between single wavelengths and biochemically determinated pigment content are showed (Fig. 2). In the visible light spectrum the maximum sensitivity of reflectance to variation in pigment content is at 605 nm and 696 nm. It fits to data described by other authors (Datt, 1998; Daughtry, et al., 2000; Blackburn, 2007). For detection of chlorophylls content reflectance at near infrared region (740–930 nm) can be used. At the visible light region better correlation was observed with chlorophyll content calculated as mg g^{-1} (w), but in NIR region if calculated as mg dm^{-2}. Similar results are obtained with low cost handheld chlorophyll meter atLEAF+ (Fig. 2).

Majority of investigated wavelengths, calculated indices and readings of chlorophyll meter atLEAF+ are more sensitive to chlorophyll a content calculated per unit area (Fig. 2, Table 2). It is not surprising, because chlorophyll a constitute a majority of the total chlorophylls. The content of chorophyll a may be up to 85.5% in total content of chlorophylls (calculated from Kitajima & Hogan, 2003).

Evaluating chlorophyll detection methods for the individual plant species, it is recognized that the use of reflectance of single wavelength is less suitable in comparison with calculated indices. At the region of visible light the best correlation between results obtained with non destructive methods and biochemical analyses was stated for radish

leaves, but in NIR region for leaf mustard. Lower correlation was obtained for cabbage seedlings.

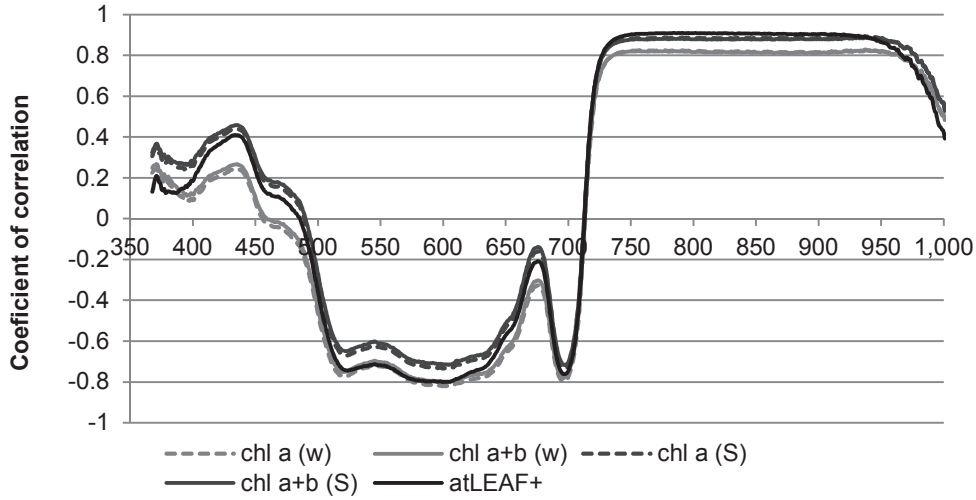

Figure 2. Coeficients of correlation between single wavelenght and biochemically detected content of chlorophyll a and sum of chlorophylls detected as mg g^{-1}(w) or mg dm^{-2} (S), and readings of chlorophyll meter atLEAF+.

Table 2. Coeficients of correlation

Index	Pigment content, mg g^{-1}			Pigment content, mg dm^{-2}		
	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b
atLEAF+	0.86	0.84	0.86	0.94	0.92	0.93
SIPI	0.82	0.79	0.81	0.88	0.85	0.87
CNDVI	0.90	0.86	0.89	0.90	0.86	0.89
CTR1	-0.76	-0.79	-0.77	-0.85	-0.87	-0.86
CTR2	-0.88	-0.83	-0.87	-0.86	-0.81	-0.84
G	-0.46	-0.46	-0.46	-0.48	-0.48	-0.48
GM1	0.66	0.60	0.64	0.57	0.52	0.56
GM2	0.88	0.83	0.87	0.87	0.82	0.86
LIC1	0.66	0.58	0.64	0.56	0.49	0.54
LIC2	0.82	0.84	0.83	0.93	0.94	0.94
NDVI	-0.90	-0.91	-0.90	-0.97	-0.97	-0.97
NPCI	-0.59	-0.52	-0.57	-0.66	-0.59	-0.64
NPQI	0.66	0.58	0.64	0.56	0.49	0.54
SRPI	0.88	0.88	0.88	0.96	0.96	0.96
TVI	0.64	0.60	0.63	0.64	0.61	0.64
ZMI	0.89	0.85	0.88	0.91	0.86	0.89
MRESRI	-0.28	-0.19	-0.25	-0.12	-0.04	-0.10
RENDVI	0.90	0.86	0.89	0.90	0.86	0.89
VREI1	0.89	0.85	0.88	0.92	0.88	0.91
VREI2	-0.86	-0.82	-0.85	-0.91	-0.86	-0.90
VREI3	0.68	0.68	0.68	0.74	0.74	0.74
PRI	-0.87	-0.83	-0.86	-0.91	-0.87	-0.90

Assessing separately green leafed and red leafed vegetables it can be concluded that the non destructive determination methods is better suited for first ones. For example coeficient of correlation between measurments with chlorophyll meter atLEAF+ and biochemical methods for green leafed vegetables (lettuce cv. 'Lollo Bionda', radish leaves) is 0.990, but for red leafed ones 0.942. Similar results were obtained also with Miniature Leaf Spectrometer CI-710. All indices excluding SIPI and NDVI have higher values for green leafed vegetables. There are references in literature that the presence of other pigments did not significantly affect estimation of chlorophyll from spectral reflectance (Sims & Gamon, 2002).

Correlation coefficient between chorophyll a content and biochemical analyses for nine indices (Cl-710) was equal or higher than 0.90, for four between 0.81–0.89. If different indices are compared then the best results was detected for Normalized Difference Vegetation Reflectance index (NDVI), coefficient of correlation for chlorophyll a –0.971, for sum of chlorophylls -0.972. NDVI was calculated using equation (W680-W430) / (W680+W430), were W680 and W430 light reflectance at 680 and 430 nm respectively. The next better result was obtained using Simple Ratio Pigment Index (SRPI), were the same wave length reflectance was used for calculations. SRPI=W430/W680.

CONCLUSIONS

Majority of investigated wavelengths, calculated indices and readings of chlorophyll meter atLEAF+ are more sensitive to chlorophyll a content calculated per unit area.

The maximum sensitivity of reflectance to variation in pigment content is found at 605 nm and 696 nm and near infrared region (740–930 nm).

Higher correlation between non – destructive methods and biochemical analyses was observed in radish and leaf mustard leaves. Lower correlation was obtained for cabbage seedlings.

The non destructive determination methods are better suited for green leafed vegetables.

The highest correlation coefficient is obtained with Difference Vegetation Reflectance index (NDVI) and Simple Ratio Pigment Index (SRPI) was reflectances at wavelength 680 and 430 nm are used for calculations.

Nondestructive chlorophyll determination with chlorophyll meter atLEAF+ and Miniature Leaf Spectrometer CI-710 can completely replace biochemical analyses.

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