Nondestructive detection and biochemical quantification of buckwheat leaves using visible (VIS) and near-infrared (NIR) hyperspectral reflectance imaging

Nedeštrukčná detekcia a biochemická kvantifikácia listov pohánky s využitím hyperspektrálneho zobrazovania s reflektanciou vo viditeľnej (VIS) a blízkej infračervenej (NIR) oblasti

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Abstract

The present experimental study has been investigated way of use methodology of nondestructive detection with hyperspectral reflectance imaging together with wet chemistry quantitative analysis based on correlation analysis of receiving data. Higher correlation from reflectance of hyperspectral imaging analysis and total polyphenol and anthocyanin content has been observed for carotenoid reflectance index-2 compared to the carotenoid reflectance index-1. It was found that high total polyphenols content is related with high values of hyperspectral indices which characterize chlorophyll concentration and parameters of vegetation. In experimental buckwheat cultivars presence of vanillic, methoxycinnamic, cinnamic acids can be expressing by anthocyanin reflectance index, modified anthocyanin reflectance index and carotenoid reflectance index-2 because high correlation coefficient. Changes of chlorogenic acid during the vegetative period of plant growth can be studied with normalized difference vegetation index and normalized difference vegetation index-2 which found to have a positive correlation with this phenolic acid content.

Keywords: anthocyanins, hyperspectral index, hyperspectral reflectance imaging, phenolic acids, polyphenols

Abstrakt

Predkladaná experimentálna práca skúmala možnosti uplatnenia metodológie nedeštrukčnej detekcie s hyperspektrálnym reflektančným zobrazovaním spolu s kvantitatívnou chemickou analýzou na základe korelačnej analýzy získaných údajov. V porovnaní s karotenoidovým reflektančným indexom-1 bola vyššia korelácia s obsahom celkových polyfenolov a celkových antokyánov pozorovaná s reflektanciou karotenoidového reflektančného indexu-2 z analýzy hyperspektrálneho zobrazovania. Vysoký obsah celkových polyfenolov súvisel s vysokými hodnotami hyperspektrálnych indexov charakterizujúcich koncentráciu chlorofylu a parametre vegetácie. V experimentálnych kultivaroch pohánky môže byť prítomnosť kyseliny vanilovej, metoxyškoricovej a škoricovej, vzhľadom k vysokému korelačnému koeficientu, vyjadrená pomocou antokyánového reflektančného indexu, modifikovaného antokyánového reflektančného indexu a karotenoidového reflektančného indexu-2. Zmeny obsahu kyseliny chlorogénovej počas vegetačného obdobia pohánky môžu byť sledované prostredníctvom normalizovaného diferenčného vegetačného indexu a diferenčného vegetačného indexu-2, ktoré preukázali pozitívnu koreláciu s obsahom fenolových kyselín.

Kľúčové slová: antokyány, fenolové kyseliny, hyperspektrálny index, hyperspektrálny reflektančný imaging, polyfenoly

Introduction

A novel nondestructive technique such as hyperspectral imaging (HSI) is a fast analytical tool for plant analysis which use spectral signals from plant object without wet chemical analysis (Pu et al., 2015). The many biochemical compounds accumulated in plant leaves can be estimated with biochemical reflectance indices which based on the specific reflectance peaks at given wavelength of visible (VIS), near-infrared (NIR) or short-wave infrared (SWIR) range. For example, the MCARI index (modified chlorophyll absorption ratio index) and the LCI (leaf chlorophyll index) are able to estimate content and distribution of chlorophyll in leaves. To estimate content of anthocyanins in plant material is used modified anthocyanin reflectance index (mARI) (Gitelson et al., 1995). Furthermore, content of carotenoids in leaves can be measured by carotenoid reflectance index (CRI) (Gitelson et al., 2002). The HSI analysis is also used for tissue water content (Kim et al., 2015), lignin, nitrogen (Serrano et al., 2002) and cellulose (Daughtry et al., 2004) estimation.

Therefore, non-destructive techniques can estimate wide range internal biochemical data in short time and in an urgent need. Nogales-Bueno et al. (2014) used HSI NIR in range 900 to 1,700 nm for total phenolic content estimation in grape skins with farther quantitative analysis with modification of partial least squares (MPLS) regression. A novel neural network method, called AdaBoost, was used for calculation of anthocyanin content after estimation in intact grape skins by VIS/NIR HSI (Fernandes et al., 2011).

Nowadays is not existing much information regarding capability of biochemical reflectance indices to the quantitative characteristics of some biochemical compounds. In proposed research is planned to estimate total polyphenols, anthocyanins and phenolic acids contents by known wet biochemical methods with aim to find correlation with reflectance indices after hyperspectral imaging analysis. The proposed research can make wider knowledge in methodology of use such non-destructive technique as HSI.

Materials and methods

Plant material

Plants of 7 buckwheat of Ukrainian (*Fagopyrum tataricum himalaicum*, *Faropyrum tataricum rotundatum* – red, *Fagopyrum esculentum* cv. Rubra - red, *Fagopyrum esculentum* cv. Karadag) and Chinese genotypes (*Fagopyrum esculentum* cv. SuQiao 1, *Fagopyrum esculentum* cv. YuQiao 4, *Fagopyrum esculentum* cv. NingQiao 1) were exposed to direct sunlight in open field conditions for 52 days. The leaves for hyperspectral reflectance imaging and biochemical quantitative analysis were collected in the flowering phase.

Hyperspectral imaging analysis

Hyperspectral imaging cameras used for buckwheat leaves analysis are part of Plant Screen^{TM} Conveyor System (PSI, Drásov, Czech Republic) of AgroBioTech Research Centre, Slovak University of Agriculture in Nitra. Technical specification VNIR camera were next: spectral range: 340 - 900 nm, band size: 400 nm, entrance slit width: 25 um, dispersion per pixel: 0.2 nm*pixel^{-1}, wavelength resolution: FWHM 0.8 nm, detector: silicon, spatial resolution: 480 pixels, spectral resolution: 640 pixels, image frequency: 12 - 60 fps, dynamic range: 68 db. Technical specification SWIR camera were next: spectral range: 1,100 – 1,700 nm, band size: 600 nm, entrance slit width: 25 um, dispersion per pixel: 0.95 nm*pixel^{-1}, wavelength resolution: 1.2 nm, detector: InGaAs, spatial resolution: 480 pixels, spectral resolution: 640 pixels, image frequency: 50 fps, bit depth: 16 bit.

Spectral images from leaves of different cultivars of buckwheat were acquired on plate (Figure 2). It was analyzed to determine if the reflectance spectra in visible (VIS) and near infrared (NIR) regions (400–1,000 nm) differed from one cultivar to another. Spectral acquisition of the plant leaves was carried out in duplicate for each aliquot, under the same conditions for all the samples. The next hyperspectral indices were estimated: NDVI (normalized difference vegetation index), NDVI-2, PRI (photochemical reflectance index), PSRI (plant senescence reflectance index), SIPI (structure insensitive pigment index), MCARI1(modified chlorophyll absorption ratio index 1), OSAVI (optimized soil-adjusted vegetation index), ARI (anthocyanin reflectance index), mARI (modified anthocyanin reflectance index), CRI-1 (carotenoid reflectance index-1), CRI-2 (carotenoid reflectance index-2), NPCI (normalized pigment chlorophyll index), SR (simple ratio), SGI (sum green index) and LCI (leaf chlorophyll index).

Anthocyanins estimation

0.1 - 0.5 g plant material was homogenized on ice with 3 ml of acidified methanol (1% HCl) and then incubated at 4 °C for 12 h with moderate shaking. The mixture was centrifuged for 10 min at 14,000 rpm at 4 °C. Absorbtion of the extracts at 530 and 657 nm wavelengths was determined spectrophotometrically. The blank was acidified methanol. The concentration of the anthocyanins was expressed as mg*g^{-1} dry weight and was calculated by formula:

anthocyanins = A_{530}-(0.25*A_{657})*V/(W*1,000),

where A is absorbance; V is total volume of the extract (ml) and W is weight of the dry leaf tissue (g).

Total polyphenols estimation and analysis of hydroxycinnamic acid derivatives

Total polyphenol content in the buckwheat leaves extracts was determined by standard spectrophotometric method of Lachman et al. (2003) by using Folin–Ciocalteu reagent (Singleton and Rossi, 1965). 0.25 g powdered samples (freeze-dried) was extracted for 16-18 hours with 20 ml of 80% ethanol. After the time of extraction a volume of 100 μl of the plant extract was pipetted into 50 ml volumetric flask. 2.5 ml of Folin-Ciocalteau reagent was added to the extract. Then after 3 minutes (agitation) 5 ml 20% Na_2CO_3 solution was mixed. After two hours at 25 °C the absorbance was measured on the spectrophotometer Jenway UV/Vis 6,405 (Jenway, UK) at wavelength λ = 765 nm against blank. Gallic acid was used as a reference standard for plotting calibration curve. Total polyphenol content was expressed as mg*kg^{-1} gallic acid equivalent of dry matter. Analysis of hydroxycinnamic acid derivatives has been previously developed (Mewis et al., 2010).

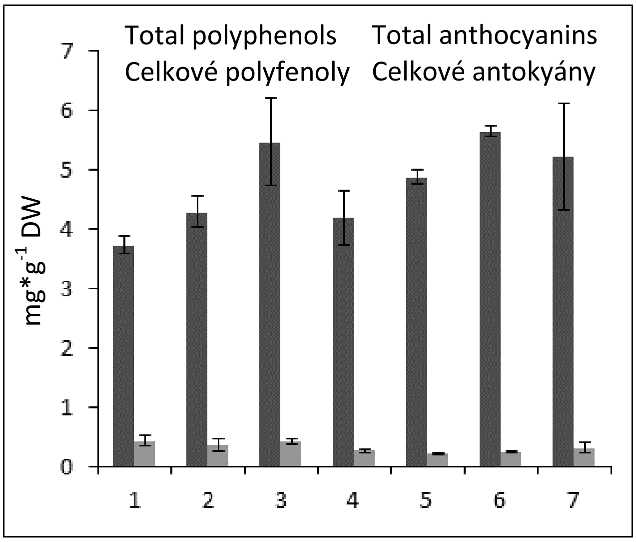
Statistical analysis

Means and standard deviations were calculated by the Microsoft Office Excel 2,010. Significant differences of these data were calculated using analysis of variance ANOVA Duncan’s multiple test (STATISTICA 10, StatSoft, Tulsa, USA). All results were expressed as mean ± standard deviations from replications n = 6. The correlation analysis between values of hyperspectral indices and results of phenolic acids content obtained by high-performance liquid chromatography (HPLC) method and total polyphenols, flavonoids determined spectrophotometrically was calculated by Pearson´s correlation coefficient.

Results and discussion

The aim of proposed research was to develop and improve knowledge's regarding nondestructive detection with using visible (VIS) and near-infrared (NIR) hyperspectral reflectance imaging based on correlation results of biochemical quantification.

The majority of open-source tools found in the Plant Image database (Lobet et al., 2013) focus on phenotyping of plants with rosette architecture (*Arabidopsis*) and specific plant organs such as excised leaves. In presented research were used buckwheat plants which possesses the advantages of a classical model species (short life cycle, high seed productivity) but it has some particular features which are not present either in *A. thaliana* or in the other classical model plant species (Quinet et al., 2004). The biochemical analysis found that highest total polyphenol and anthocyanin contents were in *Fagopyrum esculentum* cv.Rubra (Ukrainian cultivar) and Chinese cultivars (Figure 1).



* numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. Rubra, 4 – *F. esculentum* cv. Karadag, 5 – *F. esculentum* cv. SuQiao 1, 6 – *F. esculentum* cv. YuQiao 4, 7 – *F. esculentum* cv. NingQiao 1

* čísla predstavujú jednotlivé kultivary pohánky nasledovne: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. Rubra, 4 – *F. esculentum* cv. Karadag, 5 – *F. esculentum* cv. SuQiao 1, 6 – *F. esculentum* cv. YuQiao 4, 7 – *F. esculentum* cv. NingQiao 1

Figure 1. Content of total polyphenols and anthocyanins in the buckwheat leaves of different cultivars determined by HPLC method

Obrázok 1. Obsah celkových polyfenolov a antokyánov v listoch pohánky rôznych kultivarov stanovených HPLC metódou

HPLC prescreening buckwheat cultivars of different origin has been identified chlorogenic, *p*-coumaric, *p*-anisic, cinnamic, methoxycinnamic, ferulic and vanillic acids. It was found highest content of chlorogenic and *p*-coumaric acids in the Chinese cultivars *F. esculentum* cv. NingQiao 1 and *F. esculentum* cv. YuQiao 4 (Table 1). The highest *p*-anisic acid content was found for buckwheat cultivars of *F. tataricum himalaicum*. The data of HPLC analysis has been used for comparable characteristic and correlation calculation between biochemical estimation results and indices of hyperspectral imaging analysis.

Table 1. Content of phenolic acids identified via HPLC analysis in the experimental buckwheat leaves

Tabuľka 1. Obsah fenolových kyselín identifikovaných HPLC analýzou v skúmaných listoch pohánky

Leaves of buckwheat Listy pohánky	Chlor. acid Kys. chlor.	*p*-Coum. acid Kys. *p*-kumár.	*p*-Anis. acid Kys. *p*-aníz.	Cinn. acid Kys. škoric.	Methoxy. acid Kys. metoxy.	Fer. acid Kys. ferul.	Van. acid Kys. vanil.
F. tataricum rotundatum	0.4±0.1	8.4±0.2	0.6±0	0±0	0.8±0.2	0.2±0	0.1±0
F. tataricum himalaicum	0.3±0	0.2±0.1	0.8±0.1	0±0	2.6±0.1	0.2±0	0.1±0
F. esculentum cv. Rubra	0.1±0	9.4±0.3	0±0	0.4±0.1	11.2±2.8	0.5±0.1	0.5±0
F. esculentum cv. Karadag	0.2±0	9.4±0.8	0±0	0.1±0.1	4.8±0.6	2.2±0.3	0.2±0
F. esculentum cv. SuQiao 1	0.3±0.1	20.1±8.3	0.1±0	0±0	3±0.3	0.2±0.1	0.3±0
F. esculentum cv. YuQiao 4	0.4±0.1	21.2±5	0±0	0±0	2.4±0.1	0.2±0	0.2±0
F. esculentum cv. NingQiao 1	0.5±0	25.1±0.6	0.1±0.1	0±0	4±0.3	0.1±0	0.3±0

It is known that reflectance indices associated with physiological changes in plants – like in nitrogen and water-limited (Peñuelas et al., 1994). Leaves from stressed plants can have higher reflectances in the visible wavelengths and lower in the near-infrared than leaves of control plants. The only clear trend across canopy levels was the higher reflectance at all wavelengths but especially in the visible of the lower (oldest) leaves. It’s typical for NDVI, NDVI-2 indices. The leaf color is governed both by cellular structure and biochemical components, the concentration and composition of pigments, including chlorophylls *a* and *b*, carotenoids and anthocyanins are the primary determinants of leaf color which can be seen on Figure 2. Especially it is visible for reflectance indices imaging like ARI, CRI-2 and MCARI for buckwheat cultivar Rubra, which is characterized by highest total polyphenol and anthocyanin contents from results of biochemical measurements.

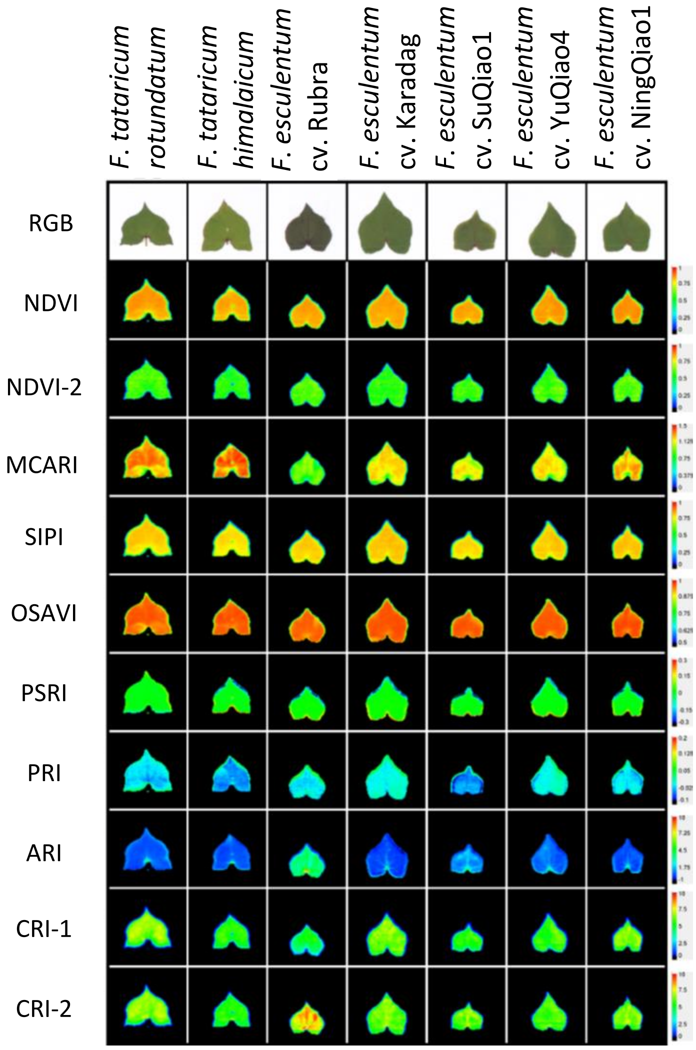


Figure 2. Reflectance indices of individual leaves of experimental buckwheat plants obtained from hyperspectral imaging

Obrázok 2. Reflektančné indexy jednotlivých listov skúmaných rastlín pohánky získané z hyperspektrálneho zobrazovania

Values of hyperspectral indices (NDVI, NDVI-2, PRI, PSRI, SIPI, MCARI, OSAVI, ARI, mARI, CRI-1, CRI-2, NPCI, SR and LCI) of leaves of experimental buckwheat plants obtained from hyperspectral imaging are presented in Table 2.

Table 2. Values of hyperspectral indices (HI) of individual leaves of experimental buckwheat plants

Tabuľka 2. Hodnoty hyperspektrálnych indexov (HI) jednotlivých listov skúmaných rastlín pohánky

HI	1	2	3	4	5	6	7
NDVI	0.8±0^{C}	0.8±0^{AB}	0.8±0^{BC}	0.8±0^{A}	0.8±0^{AB}	0.8±0^{CD}	0.9±0^{D}
NDVI-2	0.6±0^{B}	0.5±0.1^{A}	0.6±0^{AB}	0.5±0^{A}	0.6±0^{AB}	0.6±0^{B}	0.6±0^{B}
PRI	-0±0^{B}	-0.1±0^{A}	-0±0^{B}	-0±0^{B}	-0.1±0^{A}	-0±0^{AB}	-0±0^{B}
PSRI	-0±0^{A}	-0±0^{A}	0±0^{D}	0±0^{C}	-0±0^{AB}	-0±0^{B}	-0±0^{AB}
SIPI	1±0^{A}	1±0^{B}	1±0^{A}	1±0^{AB}	1±0^{AB}	1±0^{A}	1±0^{A}
MCARI	1.3±0^{B}	1.3±0^{C}	1.1±0^{A}	1.3±0^{B}	1.3±0.1^{B}	1.3±0^{B}	1.3±0^{B}
OSAVI	1±0^{A}	1±0^{AB}	1±0^{BC}	1±0^{ABC}	1±0^{ABC}	1±0^{ABC}	1±0^{C}
ARI	-0.3±0.2^{A}	-0.1±0.2^{A}	4.2±0.8^{C}	0.2±0.4^{AB}	0.5±0.6^{B}	0.1±0.2^{AB}	-0.2±0.4^{A}
mARI	-0.2±0.2^{A}	-0.1±0.2^{A}	3.5±0.7^{C}	0.2±0.3^{AB}	0.4±0.5^{B}	0.1±0.2^{AB}	-0.2±0.3^{A}
CRI-1	2.4±0.1^{CD}	2.4±0.1^{D}	1.4±0.1^{A}	2.2±0.1^{B}	2.1±0.2^{B}	2.3±0.2^{BCD}	2.2±0.1^{BC}
CRI-2	5.9±0.5^{AB}	5.5±0.3^{A}	8.1±0.6^{C}	6.2±0.7^{AB}	5.9±0.4^{AB}	6.4±0.6^{B}	6.1±0.5^{AB}
NPCI	-0.2±0^{AB}	-0.2±0.1^{BC}	-0.2±0^{BC}	-0.1±0^{C}	-0.2±0^{AB}	-0.2±0^{AB}	-0.2±0^{A}
SR	11.8±0.7^{BC}	10.8±0.7^{A}	11.3±0.4^{AB}	10.6±0.5^{A}	11±0.3^{A}	11.9±1^{BC}	12.2±0.5^{C}
LCI	0.5±0^{ABC}	0.4±0^{A}	0.5±0^{D}	0.5±0^{BCD}	0.5±0^{AB}	0.5±0.1^{ABCD}	0.5±0^{CD}

* different letters indicate the difference between genotypes significant at the P ≤ 0.05

** numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. Rubra, 4 – *F. esculentum* cv. Karadag, 5 – *F. esculentum* cv. SuQiao 1, 6 – *F. esculentum* cv. YuQiao 4, 7 – *F. esculentum* cv. NingQiao 1

* odlišné písmená indikujú rozdiely medzi genotypmi významné na hladine preukaznosti P ≤ 0.05

** čísla predstavujú jednotlivé kultivary pohánky nasledovne: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. Rubra, 4 – *F. esculentum* cv. Karadag, 5 – *F. esculentum* cv. SuQiao 1, 6 – *F. esculentum* cv. YuQiao 4, 7 – *F. esculentum* cv. NingQiao 1

The algorithms for estimating total carotenoid and anthocyanin concentrations from the spectral reflectance signature have been developed (Gitelson et al., 2006), but still discussed if results of optical measurements can be used for prescreening or can be similar with invasive biochemical measurements. In this case is useful to build correlations between hyperspectral indices and results of total polyphenols, phenolic acids and anthocyanin content measured by biochemical methods.
The normalized difference vegetation index (NDVI) is a simple graphical indicator that can be used to analyze advantage of NDVI over a simple infrared/red ratio is therefore generally limited to any possible linearity of its functional relationship with vegetation properties (e.g. biomass). The simple ratio (unlike NDVI) is always positive, which may have practical advantages, but it also has a mathematically infinite range (0 to infinity), which can be a practical disadvantage as compared to NDVI (Crippen, 1990).

NDVI-like parameters were useful in distinguishing stress and control leaves over the growing season. Correlation between hyperspectral indices NDVI and NDVI-2 to the total polyphenol content is positive in range 0.42-0.46 (Table 3). Optimized soil-adjusted vegetation index (OSAVI) has also positive correlation to the total polyphenol content (value 0.57). OSAVI is strongly sensitive to chlorophyll concentration and highly resistant to the variations of leaf area index and solar zenith angle at the canopy level (Rondeaux et al., 1996). Correlation between LCI (leaf chlorophyll index) and total polyphenol content was positive (value 0.54). The high concentration of total polyphenols occurs high values of NDVI, NDVI-2, OSAVI and LCI indices which can be connected with chlorophyll concentration too. Although single leaf analysis (Devadas et al., 2009) indicated that indices like NDVI, NPCI, NRI, PSRI and SIPI could be useful in discriminating stripe rust infected plants from healthy plants, correlation analysis of canopy data demonstrated that these indices were relatively ineffective in quantifying the changes due to stripe rust infection in the field.

Negative correlation of SIPI index (structure insensitive pigment index), MCARI1 (modified chlorophyll absorption ration index 1) and NPCI (normalized pigment chlorophyll index) to total polyphenol content has been found. Correlation between hyperspectral indices (SIPI, MCARI1) and total polyphenol content can evidence that high concentration of total polyphenols can decrease values of SIPI, MCARI1 and NPCI indices during hyperspectral imaging analysis.

Different results of correlation between two different carotenoid reflectance indices (CRI-1 and CRI-2) and total polyphenol and anthocyanin contents have been observed (Table 3). CRI-2 index has been shown positive correlation to the total polyphenol and anthocyanin contents (0.55 and 0.37, respectively). CRI-1 has been shown negative correlation to the total polyphenol and anthocyanin contents. Higher CRI-2 values mean greater caratenoid concentration relative to chlorophyll. High values of CRI-2 index are a modification to CRI-1 that provides better results in areas of high carotenoid concentration. Based on received results CRI-2 index can show also total polyphenol and anthocyanin concentrations because with their high concentration will be also high values of CRI-2.

Recently, combinations of indices based on the modified chlorophyll absorption in reflectance (MCARI) (Daughtry et al., 2000) and the optimized soil-adjusted vegetation index (OSAVI) (Rondeaux et al., 1996), such as MCARI/ OSAVI, have been demonstrated to successfully minimize soil background and LAI variation in crops, providing predictive relationships for precision agriculture applications with hyperspectral imagery in closed crops (Haboudane et al., 2002) and open tree canopy orchards (Zarco-Tejada et al., 2004).

Biochemical analysis of anthocyanin content and ARI (anthocyanin reflectance index) from hyperspectral imaging analysis has been shown positive correlation but not high. ARI measures non-chlorophyll pigment concentration and obtained results can evidence about presence not just anthocyanins among non-chlorophyll pigments which measured by ARI. Weakening vegetation contains higher concentration of anthocyanins, so this index is one measure of stressed vegetation. In a previous laboratory study (Devadas et al., 2009) the vegetation index ARI, which measures non-chlorophyll pigment concentration, was found to be most reliable in discriminating healthy leaves from those infected with stripe, stem or leaf rust.

Table 3. Correlations between hyperspectral indices and total polyphenol (TPC) and anthocyanin content (TAC)

Tabuľka 3. Korelácie medzi hyperspektrálnymi indexmi a obsahom celkových polyfenolov (TPC) a antokyánov (TAC)

Hyperspectral index Hyperspektrálny index	TAC	TPC
NDVI	0.16	0.42
NDVI-2	0	0.46
PRI	0.4	0.13
PSRI	0.12	0.26
SIPI	-0.07	-0.63
MCARI1	-0.27	-0.5
OSAVI	-0.17	0.57
ARI	0.39	0.47
mARI	0.39	0.47
CRI-1	-0.25	-0.53
CRI-2	0.37	0.55
NPCI	0.06	-0.37
SR	0.16	0.37
LCI	0.11	0.54

The high positive correlation between hyperspectral indices ARI, mARI, CRI-2 and content of vanillic, methoxycinnamic and cinnamic acids was recorded (Table 4). ARI measures non-chlorophyll pigments, especially anthocyanins. Increased CRI-2 values showed higher carotenoid concentration relative to chlorophyll. Both indices can measure of stressed vegetation concentration. So these indices can evidence also about presence of vanillic, methoxycinnamic and cinnamic acids in the experimental leaves.

At the same time negative correlation of MCARI1 index to content vanillic (-0.94), methoxycinnamic (-0.90) and cinnamic (-0.94) acids has been estimated. CRI-1 index and content of vanillic, methoxycinnamic and cinnamic acids has been shown negative correlation too.

Chlorogenic acid is an important biosynthetic intermediate (Wout et al., 2003). The chlorogenic acid content strongly correlated with total phenols (Sytar et al., 2016). Role of chlorogenic acid in the plant growth and development has been confirmed by positive correlations of NDVI, NDVI-2 and SR (simple ratio) indices (0.69, 0.73 and 0.71, respectively). SR is vegetation index like NDVI and NDVI-2 but it is indicates amount of vegetation and normally high for vegetation. Negative correlation of NPCI (-0.83) to content of chlorogenic acid has been observed.

Among estimated correlations between hyperspectral indices and identified phenolic acids the highest positive correlation (0.95) was found for PSRI to catechin compounds content. PSRI (plant senescence reflectance index) is used for quantity plant senescence (Hatfield and Prueger, 2010). An increase in PSRI indicates increased canopy stress (carotenoid pigments), the onset of canopy senescence, and plant fruit ripening.

Table 4. Correlations between hyperspectral indices (HI) and identified phenolic acids content

Tabuľka 4. Korelácie medzi hyperspektrálnymi indexmi (HI) a obsahom identifikovaných fenolových kyselín

HI	Chlor. acid Kys. chlor.	*p*-Coum. acid Kys. *p*-kumár.	*p*-Anis. acid Kys. *p*-aníz.	Cinnam. acid Kys. škor.	Methoxy. acid Kys. metoxy.	Ferul. acid Kys. ferul.	Vanil. acid Kys. vanil.	Catechin compounds Katechín. zlúčeniny
NDVI	0.69	0.48	-0.2	-0.07	-0.1	-0.54	0.18	-0.09
NDVI-2	0.73	0.58	-0.27	-0.15	-0.19	-0.57	0.17	-0.12
PRI	-0.02	0.08	-0.45	0.56	0.48	0.26	0.55	0.72
PSRI	-0.62	-0.19	-0.58	0.85	0.86	0.66	0.71	0.95
SIPI	-0.32	-0.58	0.7	-0.47	-0.48	0.15	-0.76	-0.55
MCARI1	0.48	-0.05	0.59	-0.94	-0.9	-0.15	-0.94	-0.86
OSAVI	0.14	0.47	-0.7	0.46	0.67	0.22	0.72	0.56
ARI	-0.62	-0.13	-0.39	0.97	0.93	0.05	0.87	0.74
mARI	-0.62	-0.13	-0.39	0.97	0.93	0.05	0.87	0.74
CRI-1	0.5	-0.07	0.56	-0.94	-0.94	-0.1	-0.96	-0.81
CRI-2	-0.46	-0.06	-0.51	0.95	0.9	0.08	0.89	0.8
NPCI	-0.83	-0.63	0.03	0.38	0.37	0.79	0.01	0.46
SR	0.71	0.45	-0.13	-0.14	-0.17	-0.57	0.1	-0.16
LCI	0.11	0.4	-0.74	0.62	0.67	0.17	0.81	0.75

Conclusions

The obtained results have been shown that known proposed indices can evidence about presence of some specific phenolic acids which connected with plant metabolism and growth. Optical methods are able to show right content of polyphenols and anthocyanins when possible to choose for them right indices.

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