

## Estimating leaf carotenoid contents of shade-grown tea using hyperspectral indices and PROSPECT-D inversion

メタデータ	言語: eng 出版者: 公開日: 2019-09-27 キーワード (Ja): キーワード (En): 作成者: 佐野, 智人, 堀江, 秀樹 メールアドレス: 所属:
URL	<a href="https://repository.naro.go.jp/records/2732">https://repository.naro.go.jp/records/2732</a>

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**Estimating leaf carotenoid contents of shade grown tea using  
hyperspectral indices and PROSPECT–D inversion**

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# Estimating leaf carotenoid contents of shade grown tea using hyperspectral indices and PROSPECT–D inversion

Quantifying carotenoid contents has many applications in agriculture, ecology, and health science. Hyperspectral reflectance has been one of the promising tools for this purpose. However, previous studies were based on measurements under relatively low light–stress conditions. Therefore, assessing its robustness by using measurements under various levels of stress is required. In this study, the measurements of reflectance and carotenoid contents were carried out with four shading treatments including open–0 %, 35 %, 75 %, and 90 % shading to generate various chlorophyll/carotenoid ratios. Then the performances of fifteen published hyperspectral indices and PROSPECT–D inversion were evaluated based on our dataset for estimating leaf carotenoid contents. According to the ratio of performance to deviation,  $R_{NIR}/R_{510}$ ,  $R_{720}/R_{521}-1$ , and PROSPECT–D inversion were applicable for this purpose, although calibration of the absorption coefficients was required for PROSPECT–D. Using them, root mean square percentage errors of 4.53–5.46 % were achieved. Given that total chlorophyll/carotenoid ratios could be a good indicator for evaluating environmental stress in plants, PROSPECT–D, which also estimates total chlorophyll and anthocyanin contents, could be a strong tool for controlling the qualities of shade grown tea.

Keywords: carotenoid; hyperspectral; PROSPECT–D; reflectance; shading treatment; tea

## 1. Introduction

Carotenoid pigments, which include two carotenes and five xanthophyll cycle pigments, are important photosynthetic pigments and play important roles for plant survival.

Carotenoid compositions are determined by the developmental stage, tissue type, and environmental stimuli (Cazzonelli *et al.*, 2009). Furthermore, carotenoid rich foods decrease the risk of developing certain types of cancer (Giovannucci *et al.*, 1995, Wright *et al.*, 2003) and atherosclerosis (Klipstein–Grobusch *et al.*, 2000, Abbott *et al.*,

2003). Therefore, quantifying carotenoid contents has many applications in agriculture, ecology, and health science.

Although ultraviolet and visible (UV–VIS) spectroscopy or high performance liquid chromatography (HPLC) (Thayer and Bjorkman, 1990) have been used for assessing carotenoid contents, these approaches require destruction of samples, and they are time consuming and expensive. On the other hand, hyperspectral remote sensing offers some non–destructive methods that could be alternatives. Moreover, field spectroscopy integrated with hyperspectral remote sensing has been applied in forestry, vegetation, and environmental monitoring (Prasad *et al.*, 2015).

Two approaches including the numerical inversion of radiative transfer models (RTM) and hyperspectral indices have been widely used to estimate carotenoid contents. However, the similarity between chlorophyll and carotenoid over the wavelengths of 400–700 nm sometimes makes it difficult to estimate carotenoid concentrations from hyperspectral reflectance (Féret *et al.*, 2011). As a result, the methods for remote estimation of carotenoid contents are still not well developed compared with those for chlorophyll content (Yi *et al.*, 2014). Therefore, we evaluated these approaches for assessing carotenoid contents.

The PROSPECT model (Jacquemoud and Baret, 1990) is one of the most famous RTMs and has been widely used for simulating reflectance from given vegetation properties (Hernandez–Clemente *et al.*, 2014, Gu *et al.*, 2016, Hunt *et al.*, 2016, Sonobe and Wang, 2017) and retrieving chlorophyll, carotenoid (Féret *et al.*, 2008, Hernandez–Clemente *et al.*, 2014), or dry matter content (Romero *et al.*, 2012). Furthermore, PROSPECT–D, which makes it possible to simulate leaf optical properties through a complete lifecycle, has been released and is superior to the previous version for the estimation of pigment content, especially carotenoid contents (Féret *et al.*, 2017).

Hyperspectral indices have also been widely used to estimate the growth conditions of vegetation and are a synthesis of leaf area index (LAI), coverage, chlorophyll content, biomass, and photosynthetically active radiation (Zou *et al.*, 2015, Huang *et al.*, 2017). To assess carotenoid contents, several hyperspectral indices have been developed, and most of them are based on a small peak of reflectance around 470–530 nm, which is called the ‘green peak’(Chappelle *et al.*, 1992, Blackburn, 1998, Gitelson *et al.*, 2002, Gitelson *et al.*, 2006, Hernandez–Clemente *et al.*, 2012, Fassnacht *et al.*, 2015). Besides them, some indices were proposed to assess the epoxidation state (EPS) of xanthophyll cycle pigments, which are also included in the carotenoid (Gamon *et al.*, 1992, Hernandez–Clemente *et al.*, 2011, Sonobe and Wang, 2016), and some of them have been used for estimating carotenoid contents (Gitelson *et al.*, 2002, Zhou *et al.*, 2017).

Generally, these studies were based on measurements under relatively low light–stress environments except for the studies focusing on EPS. However, the highest quality green tea is cultivated using shading treatments, which changes the balance between chlorophyll content and carotenoid contents, and shading has sometimes led to early mortalities in tea plants. Although Féret *et al.* (2008) reported that the coefficients of linear regression models for estimating chlorophyll *a* content from carotenoid contents were 2.99 (for LOPEX dataset) to 3.45 (for HAWAII dataset), those of the dataset used in this study ranged from 3.61 to 6.35 due to environmental stress. Even though some assumptions in the previous studies for estimating carotenoid contents are not valid for assessing the carotenoid contents of shade grown tea, a knowledge of carotenoid contents from remote sensing is required because the ratio of chlorophyll to carotenoid could be a good indicator for evaluating environmental stress in plants.

The purpose of this analysis is to evaluate the potentials of PROSPECT-D inversion and hyperspectral indices for estimating the leaf carotenoid contents of shade grown tea.

## **2. Materials and Methods**

### ***2.1. Measurements and datasets***

Tea leaf samples were collected from the Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organisation, Shimada, Japan. Shading treatment which is conducted for the top grades (Figure 1) makes tea leaves synthesise higher levels of chlorophyll and amino acids. Therefore, tea leaves were shaded for approximately 2 weeks before harvesting to produce high quality green tea. Four shading treatments (open–0 % shading, 35 % shading, 75 % shading, and 90 % shading) were conducted using a Dio Chemicals shading net #410 (35 % shading), #1210 (75 % shading), and #1220 (90 % shading) (Dio Chemicals, Ltd., Japan) from 21 April to 11 May. Figure 2 represents the weather conditions during the experiment. Averages of daily temperature were 12.5–19.2 °C and daily precipitations were 0–17.5 mm during the experiment (Japan Meteorological Agency, 2017).

<Figure 1>

<Figure 2>

The spectral reflectance and carotenoid contents of leaves were measured on 11 May. A FieldSpec spectrometer (Analytical Spectral Devices Inc., USA) was used with a leaf clip for acquiring reflectance data. This device is composed of three detectors including VNIR, SWIR 1, and SWIR 2. However, some inherent variation in detector sensitivities can cause differences in the spectral drifts at two wavelength locations (1000 and 1800 nm). These drifts were corrected by applying the splice correction

function of ViewSpec Pro Software (Analytical Spectral Devices Inc., USA) (Prasad *et al.*, 2015). On the other hand, a dual-beam scanning ultraviolet–visible spectrophotometer (UV–1280, Shimadzu, Japan) was utilised, and Wellburn’s method (Wellburn, 1994) was applied for quantifying carotenoid contents. The equations used in this method for quantifying chlorophyll *a* (Chl*a*,  $\mu\text{g ml}^{-1}$ ), chlorophyll *b* (Chl*b*,  $\mu\text{g ml}^{-1}$ ), and carotenoid (Car,  $\mu\text{g ml}^{-1}$ ) in dimethyl–formamide extracts are as follows:

$$\text{Car} = (1000.00A_{480.0} - 1.12\text{Chl}a - 34.07\text{Chl}b)/245.00 \quad (1)$$

$$\text{Chl}a = 12.00A_{663.8} - 3.11A_{646.8} \quad (2)$$

$$\text{Chl}b = 20.78A_{646.8} - 4.88A_{663.8} \quad (3)$$

where *A* is the absorbance and the suffixes are the wavelength (nm).

The hyperspectral reflectance and carotenoid contents of sixty samples were measured, and then a stratified random sampling approach was applied to select 28 samples (7 samples  $\times$  4 treatments) as the training samples. The remaining 32 samples (8 samples  $\times$  4 treatments) were used to perform the accuracy assessment.

## ***2.2. Hyperspectral indices for assessing carotenoid***

In this study, the fifteen reported hyperspectral indices (Table 1) were evaluated for their correlations with carotenoid contents based on the aforementioned four datasets.

The ratio of reflectance at 760 nm to 500 nm (Chappelle) (Chappelle *et al.*, 1992) was developed based on the measurements from soybean leaves, whereas  $R_{\text{NIR}}/R_{510}$  (Gitelson1),  $\text{CRI}_{550}$ , and  $\text{CRI}_{700}$  (Gitelson *et al.*, 2002) were based on the experiment using juvenile, mature, and senescent leaves of Norway maple (*Acer platanoides*) and horse chestnut (*Aesculus hippocastanum*). Furthermore, Gitelson *et al.* (2002) evaluated their advantages by comparing Chappelle with PRI (Gamon *et al.*, 1992), Datt, which is expressed as  $R_{672}/(R_{550} \times R_{708})$  (Datt, 1998), and Blackburn’s

indices including Blackburn1, which is expressed as  $R_{800}/R_{470}$ , and Blackburn2, which is expressed as  $(R_{800}-R_{470})/(R_{800}+R_{470})$  (Blackburn, 1998). Furthermore, Gitelson *et al.* (2006) developed the two indices  $(1/R_{510-520}-1/R_{560-570}) \times R_{NIR}$  and  $(1/R_{510-520}-1/R_{690-710}) \times R_{NIR}$  using anthocyanin-free juvenile, mature, and senescent leaves. Hernandez-Clemente *et al.* (2012) proposed the ratio of reflectance at 515 nm to 570 nm (Hernandez-Clemente) using measurements from a conifer forest and simulation data.

Besides them, angular vegetation indices (AVI) were considered, and three AVIs and merged vegetation indices were developed by Fassnacht *et al.* (2015) based on the dataset obtained from Norway maple (*Acer platanoides*), horse chestnut (*Aesculus hippocastanum*), and European beech (*Fagus sylvatica*). Zou *et al.* (2017) proposed the Carotenoid index (CARI) based on the ANGERS dataset (Féret *et al.*, 2008) and experimental data acquired in field experiments in China.

<Table 1>

### **2.3. Model inversion**

Inversion of PROSPECT-D was carried out as another approach for quantifying leaf carotenoid concentrations using hyperspectral data. In the PROSPECT model, a leaf is assumed to be a stack of plates composed of absorbing and diffusing constituents, and leaf optical properties are simulated from pigment content (chlorophyll, carotenoid, and anthocyanin for PROSPECT-D), leaf dry mass, leaf water mass, and effective number of leaf layers. To estimate these parameters, model inversion was conducted. Inversion of PROSPECT-D was applied using MATLAB and Statistics Toolbox Release 2016a (MathWorks, Inc., USA). The source codes (PROSPECT-D\_Matlab.rar) were downloaded from the portal site (Institut de physique du globe de Paris, 2017), and the code for inversion of PROSPECT-5 was modified for application of PROSPECT-D.



The absorption coefficient of this model was conducted according to the calibration algorithm of Féret *et al.* (2008) using the training data to avoid potential systematic bias and error propagation in the inversion process.

#### ***2.4. Statistical criteria***

For revealing the wavelengths in which significant differences were observed among the four shading treatments, stepwise linear discriminant analysis was applied (Draper, 1998). This technique has been applied for selecting suitable wavelengths to be included in a multiple regression model with a combination of forward and backward stepwise regression (Sonobe and Wang, 2016). The addition or removal of wavelengths was determined by a significance level of 5% in this study.

Regression models were generated based on linear or exponential regression using the training data. Next, the regression models were applied for the test data, and their performances were evaluated.

The ratio of performance to deviation (RPD, Equation (1)) (Williams, 1987) was applied to evaluate the performances of the hyperspectral indices, and the indices were classified into three categories (Chang *et al.*, 2001): Category A (RPD > 2.0), Category B ( $1.4 \leq \text{RPD} \leq 2.0$ ), and Category C (RPD < 1.4). RPD was calculated using equation (4):

$$\text{RPD} = \text{SD} / \text{SEP} \quad (4)$$

where SEP is the standard error of prediction, which is calculated as the root mean square error, and SD is the standard deviation of the carotenoid contents. The indices ranked as Category A or B were assumed to have the potential to estimate carotenoid contents.

Besides RPD, the root mean square percentage error (RMSPE, Equation (5)) and the determination coefficient ( $R^2$ ) were also calculated to evaluate the fit between the index values and carotenoid contents.

$$\text{RMSPE} = \sqrt{\frac{1}{n} \sum_{i=1}^n \left( \frac{\hat{y}_i - y_i}{y_i} \right)^2} \quad (5)$$

where  $n$  is number of samples,  $y_i$  is the measured value, and  $\hat{y}_i$  is the estimated value.

### 3. Results and Discussion

#### 3.1. Carotenoid contents after each treatment

Table 2 summarises the carotenoid contents after the different shading treatments. The carotenoid contents ranged from 6.16 to 11.29  $\mu\text{g cm}^{-2}$ , and there were no significant differences among the treatments ( $p > 5.0\%$ , based on the Tukey–Kramer test).

However, significant differences were confirmed against the chlorophyll contents except for the treatments of 90 % shading and 35 or 75 % shading. The shading treatments contributed to increasing the chlorophyll  $a$  content of leaves to harvest more light and nitrogen (Suzuki and Shioi, 2003). However, excessive shading can sometimes prevent leaves from producing chlorophyll, and the opposite tendencies have been reported (Rozali *et al.*, 2016). Indeed, hakuyocha (white leaf) green tea is produced in Japan by completely shading the leaves. Thus, 90 % shading might have prevented the leaves from synthesising chlorophyll pigments during 21 April to 11 May, and this treatment made this tendency obscure.

Figure 3 represents the relationships between carotenoid contents and total chlorophyll content ( $a$ ) or chlorophyll  $a$  content ( $b$ ). Positive correlations were confirmed between them, and their  $R^2$ s were increased with levels of shading.

For the relationship between carotenoid contents and chlorophyll  $a$  content, their confidents increased with levels of shading except for the relationship between 0 % and

35 % shading. Whereas the coefficients of linear regression models for estimating chlorophyll *a* content from carotenoid contents was 2.99 (for LOPEX dataset) to 3.45 (for HAWAII dataset) (Féret *et al.*, 2008), those of our measurements ranged from 3.61 to 6.35. As a result, we concluded that various combinations of chlorophyll and carotenoid contents were generated by the four shading treatments.

<Table 2>

<Figure 3>

### ***3.2. Spectral reflectance of different treatments***

The mean reflectance of each treatment is shown in Figure 4. Generally, the reflectance after 0 % shading was the highest, and that of 75 % shading was the lowest over 400 – 900 nm. In particular, the green peak of shading of 0 % was significantly higher compared with the other treatments. Although clear trends related to shading treatments were not confirmed regarding reflectance, the four shading treatments could be identified with an overall accuracy of 65 % using the reflectance values at 564 nm and 701 nm with a stepwise linear discriminant analysis.

<Figure 4>

### ***3.3. Accuracy validation***

The accuracies from the test data of the 15 indices including the RPD, RMSPE, and  $R^2$  are presented in Table 3. Gitelson1 and CARI were classified as ‘A’ category, and their estimated values are compared in Figure 5 with the measured values. CARrededge, Fassnacht1, and Fassnacht2 were categorised as ‘B’ based on RPD, and the results revealed that they also have the potential for assessing carotenoid contents; however, they were inferior to Gitelson1 and CARI.

Although Gitelson1, CRI<sub>550</sub>, and CRI<sub>700</sub> were developed from the same dataset, the differences in performance for our dataset were obvious. Figure 6 shows the correlations between reflectance at each wavelength and carotenoid contents. The reflectance near 510 nm was negatively correlated with carotenoid contents ( $r = -0.893$ ,  $p < 0.1\%$ ), whereas the reflectance at 760–800 nm had no significant correlation, and it was reported to be almost constant by Gitelson *et al.* (2002). Thus, the reflectance at 760–800 nm could be a reference wavelength to clarify the change of reflectance at 510 nm. These features also caused the high performance of CARI, but the correlation coefficient of reflectance at 521 nm ( $r = -0.755$ ,  $p < 0.1\%$ ) was inferior to that of reflectance at 510 nm.

<Table 3>

<Figure 5>

<Figure 6>

Next, the performance of PROSPECT–D inversion was evaluated. The absorption coefficients for carotenoid pigments are shown in Figure 7, and the calibrated values were smaller than their original values. Figure 8 shows the relationship between estimated and measured carotenoid contents of the test data. Although a high  $R^2$  was confirmed between the measured carotenoid contents and that estimated by the original PROSPECT–D, the calibration of absorption coefficients made the RMSPE value smaller (from 9.86 % to 4.90 %) and RPD value larger (from 1.05 to 2.23). Although the statistics of the calibrated PROSPECT–D were inferior to those of Gitelson1, there were no significant differences in the estimated values among Gitelson1, CARI, and calibrated PROSPECT–D ( $p > 5.0\%$  based on a Tukey–Kramer test). Furthermore, they were categorised as ‘A’ according to RPD, and the results revealed that they could be good indicators for assessing carotenoid contents.

Carotenoid content could be a good indicator for assessing stress of green tea because carotenoid is involved in photoprotection and light collection in photosynthesis (DemmigAdams *et al.*, 1996) and they help to protect unsaturated fatty acids, phospholipids and galactolipids from damage (Edge *et al.*, 1997). However, a combination use of content of chlorophyll, which also absorbs sunlight and synthesizes carbohydrates from CO<sub>2</sub> and H<sub>2</sub>O is more useful for evaluating environmental stress in plants (Hendry and Price, 1993). Therefore, PROSPECT-D inversion was more of an ideal choice because it also estimated total chlorophyll content with a high accuracy (RMSPE = 6.73 %). However, chlorophyll pigments consist of two main types, namely chlorophyll *a* and *b*, and chlorophyll *a/b* ratio can also be influenced on the change of the physiological state of the plant (Kouril *et al.*, 1999). Chlorophyll *a/b* ratios increase sharply in a linear manner at low light intensity, but increase gradually and linearly at higher light intensities (Leong and Anderson, 1984), and the ratio is positively correlated with the ratio of PSII cores to light harvesting chlorophyll-protein complex (LHCI) (Terashima and Hikosaka, 1995). Therefore, assessing individual influence on leaf reflectance could improve the models.

<Figure 7>

<Figure 8>

#### **4. Conclusions**

The relationship between carotenoid contents and fifteen published hyperspectral indices has been assessed using the measurements from shade grown tea, which resulted in various combinations between chlorophyll and carotenoid contents. Among the indices, Gitelson1 and CARI had the highest performances, achieving the RMSPEs of 4.53 and 5.46 %, respectively, and they were categorised as 'A' based on RPD.

Inversion of PROSPECT-D was also considered for quantifying leaf carotenoid concentrations in this study. Although calibration of the absorption coefficients was required, it could estimate carotenoid contents with an RMSPE of 4.90 %, and it was categorised as ‘A’ based on RPD. Thus, both approaches were applicable for estimating carotenoid contents of shade grown tea from hyperspectral data, although not all hyperspectral indices were applicable.

Although the dataset used in this study included measurements under high stress environments, these treatments are normal for green tea production. Thus, the applicable approaches can help to improve our ability to monitor agricultural fields.

## **Funding**

This work was supported by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and Food Industry [grant number 27015C].

## **Disclosure statement**

No potential conflicts of interest are reported by the authors.

## **References**

- Abbott, R.D., Ando, F., Masaki, K.H., Tung, K.H., Rodriguez, B.L., Petrovitch, H., Yano, K. & Curb, D.J., 2003. Dietary magnesium intake and the future risk of coronary heart disease (The Honolulu Heart Program). *American Journal of Cardiology*, 92, 665–669.
- Blackburn, G.A., 1998. Quantifying chlorophylls and carotenoids at leaf and canopy scales: An evaluation of some hyperspectral approaches. *Remote Sensing of Environment*, 66, 273–285.
- Cazzonelli, C.I., Cuttriss, A.J., Cossetto, S.B., Pye, W., Crisp, P., Whelan, J., Finnegan, E.J., Turnbull, C. & Pogson, B.J., 2009. Regulation of Carotenoid Composition and Shoot Branching in Arabidopsis by a Chromatin Modifying Histone Methyltransferase, SDG8. *Plant Cell*, 21, 39–53.
- Chang, C.W., Laird, D.A., Mausbach, M.J. & Hurburgh, C.R., 2001. Near-infrared reflectance spectroscopy–principal components regression analyses of soil properties. *Soil Science Society of America Journal*, 65, 480–490.
- Chappelle, E.W., Kim, M.S. & McMurtrey, J.E., 1992. Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of

- chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. *Remote Sensing of Environment*, 39, 239–247.
- Datt, B., 1998. Remote sensing of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+b*, and total carotenoid content in eucalyptus leaves. *Remote Sensing of Environment*, 66, 111–121.
- Demmigadams, B., Gilmore, A.M. & Adams, W.W., 1996. Carotenoids .3. In vivo functions of carotenoids in higher plants. *Faseb Journal*, 10, 403–412.
- Draper, N., H, 1998. *Applied regression analysis (Wiley Series in Probability and Statistics)*: Wiley–Interscience.
- Edge, R., Mcgarvey, D.J. & Truscott, T.G., 1997. The carotenoids as anti–oxidants – a review. *Journal of Photochemistry and Photobiology B–Biology*, 41, 189–200.
- Fassnacht, F.E., Stenzel, S. & Gitelson, A.A., 2015. Non–destructive estimation of foliar carotenoid content of tree species using merged vegetation indices. *Journal of Plant Physiology*, 176, 210–217.
- Féret, J.–B., Francois, C., Asner, G.P., Gitelson, A.A., Martin, R.E., Bidel, L.P.R., Ustin, S.L., Le Maire, G. & Jacquemoud, S., 2008. PROSPECT–4 and 5: Advances in the leaf optical properties model separating photosynthetic pigments. *Remote Sensing of Environment*, 112, 3030–3043.
- Féret, J.–B., Francois, C., Gitelson, A., Asner, G.P., Barry, K.M., Panigada, C., Richardson, A.D. & Jacquemoud, S., 2011. Optimizing spectral indices and chemometric analysis of leaf chemical properties using radiative transfer modeling. *Remote Sensing of Environment*, 115, 2742–2750.
- Féret, J.–B., Gitelson, A.A., Noble, S.D. & Jacquemoud, S., 2017. PROSPECT–D: Towards modeling leaf optical properties through a complete lifecycle. *Remote Sensing of Environment*, 193, 204–215.
- Gamon, J.A., Penuelas, J. & Field, C.B., 1992. A narrow–waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sensing of Environment*, 41, 35–44.
- Giovannucci, E., Ascherio, A., Rimm, E.B., Stampfer, M.J., Colditz, G.A. & Willett, W.C., 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. *Journal of the National Cancer Institute*, 87, 1767–1776.
- Gitelson, A.A., Keydan, G.P. & Merzlyak, M.N., 2006. Three–band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. *Geophysical Research Letters*, 33.
- Gitelson, A.A., Zur, Y., Chivkunova, O.B. & Merzlyak, M.N., 2002. Assessing carotenoid content in plant leaves with reflectance spectroscopy. *Photochemistry and Photobiology*, 75, 272–281.
- Gu, C.Y., Du, H.Q., Mao, F.J., Han, N., Zhou, G.M., Xu, X.J., Sun, S.B. & Gao, G.L., 2016. Global sensitivity analysis of PROSAIL model parameters when simulating Moso bamboo forest canopy reflectance. *International Journal of Remote Sensing*, 37, 5270–5286.
- Hendry, G.A.F. & Price, A.H., 1993. *Stress Indicators: Chlorophylls and Carotenoids*.
- Hernandez–Clemente, R., Navarro–Cerrillo, R.M., Suarez, L., Morales, F. & Zarco–Tejada, P.J., 2011. Assessing structural effects on PRI for stress detection in conifer forests. *Remote Sensing of Environment*, 115, 2360–2375.
- Hernandez–Clemente, R., Navarro–Cerrillo, R.M. & Zarco–Tejada, P.J., 2012. Carotenoid content estimation in a heterogeneous conifer forest using narrow–band indices and PROSPECT plus DART simulations. *Remote Sensing of Environment*, 127, 298–315.

- Hernandez–Clemente, R., Navarro–Cerrillo, R.M. & Zarco–Tejada, P.J., 2014. Deriving Predictive Relationships of Carotenoid Content at the Canopy Level in a Conifer Forest Using Hyperspectral Imagery and Model Simulation. *IEEE Transactions on Geoscience and Remote Sensing*, 52, 5206–5217.
- Huang, J., Sun, Y.H., Wang, M.Y., Zhang, D.D., Sada, R. & Li, M.C., 2017. Juvenile tree classification based on hyperspectral image acquired from an unmanned aerial vehicle. *International Journal of Remote Sensing*, 38, 2273–2295.
- Hunt, E.R., Daughtry, C.S.T. & Li, L., 2016. Feasibility of estimating leaf water content using spectral indices from WorldView–3 's near–infrared and shortwave infrared bands. *International Journal of Remote Sensing*, 37, 388–402.
- Institut de physique du globe de Paris. 2017. “PROSPECT+SAIL=PROSAIL”. Accessed 27 May 2017.<http://teledetection.ipgp.jussieu.fr/prosail/>
- Jacquemoud, S. & Baret, F., 1990. PROSPECT: A Model of leaf optical properties spectra. *Remote Sensing of Environment*, 34, 75–91.
- Japan Meteorological Agency, 2017. Accessed 4 September 2017. <http://www.jma.go.jp/jma/index.html>
- Klipstein–Grobuch, K., Launer, L.J., Geleijnse, J.M., Boeing, H., Hofman, A. & Witteman, J.C.M., 2000. Serum carotenoids and atherosclerosis – The Rotterdam Study. *Atherosclerosis*, 148, 49–56.
- Kouril, R., Ilik, P., Naus, J. & Schoefs, B., 1999. On the limits of applicability of spectrophotometric and spectrofluorimetric methods for the determination of chlorophyll a/b ratio. *Photosynthesis Research*, 62, 107–116.
- Leong, T.Y. & Anderson, J.M., 1984. Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll–protein complexes. *Photosynthesis research*, 5, 105–15.
- Prasad, K.A., Gnanappazham, L., Selvam, V., Ramasubramanian, R. & Kar, C.S., 2015. Developing a spectral library of mangrove species of Indian east coast using field spectroscopy. *Geocarto International*, 30, 580–599.
- Romero, A., Aguado, I. & Yebra, M., 2012. Estimation of dry matter content in leaves using normalized indexes and PROSPECT model inversion. *International Journal of Remote Sensing*, 33, 396–414.
- Rozali, S.E., Rashid, K.A. & Farzinebrahimi, R., 2016. Effects of Shading Treatments on Pigmentation and Inflorescence Quality of *Calathea crotalifera* Bracts. *International Journal of Agriculture and Biology*, 18, 549–556.
- Sonobe, R. & Wang, Q., 2016. Assessing the xanthophyll cycle in natural beech leaves with hyperspectral reflectance. *Functional Plant Biology*, 43, 438–447.
- Sonobe, R. & Wang, Q., 2017. Towards a universal hyperspectral index to assess chlorophyll content in deciduous forests. *Remote Sensing*, 9, 191.
- Suzuki, Y. & Shioi, Y., 2003. Identification of chlorophylls and carotenoids in major teas by high–performance liquid chromatography with photodiode array detection. *Journal of Agricultural and Food Chemistry*, 51, 5307–5314.
- Terashima, I. & Hikosaka, K., 1995. Comparative ecophysiology of leaf and canopy photosynthesis. *Plant Cell and Environment*, 18, 1111–1128.
- Thayer, S.S. & Bjorkman, O., 1990. Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research*, 23, 331–343.
- Wellburn, A.R., 1994. The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, 144, 307–313.



- Williams, P., 1987. Variables affecting near-infrared reflectance spectroscopic analysis. In P. Williams & K. Norris (eds.) *Near-Infrared Technology in the Agricultural and Food Industries*. American Association of Cereal Chemists Inc., 143–167.
- Wright, M.E., Mayne, S.T., Swanson, C.A., Sinha, R. & Alavanja, M.C.R., 2003. Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri Women's Health Study (United States). *Cancer Causes & Control*, 14, 85–96.
- Yi, Q.X., Jiapaer, G., Chen, J.M., Bao, A.M. & Wang, F.M., 2014. Different units of measurement of carotenoids estimation in cotton using hyperspectral indices and partial least square regression. *ISPRS Journal of Photogrammetry and Remote Sensing*, 91, 72–84.
- Zhou, X.F., Huang, W.J., Kong, W.P., Ye, H.C., Dong, Y.Y. & Casa, R., 2017. Assessment of leaf carotenoids content with a new carotenoid index: Development. and validation on experimental and model data. *International Journal of Applied Earth Observation and Geoinformation*, 57, 24–35.
- Zou, X.C., Hernandez-Clemente, R., Tammeorg, P., Torres, C.L., Stoddard, F.L., Makela, P., Pellikka, P. & Mottus, M., 2015. Retrieval of leaf chlorophyll content in field crops using narrow-band indices: effects of leaf area index and leaf mean tilt angle. *International Journal of Remote Sensing*, 36, 6031–6055.

## Table

Table 1. Published hyperspectral indices.

Index	Formula	Reference
Chappelle	$R_{760} / R_{500}$	Chappelle <i>et al.</i> (1992)
PRI	$(R_{531} - R_{570}) / (R_{531} + R_{570})$	Gamon <i>et al.</i> (1992)
Blackburn1	$R_{800} / R_{470}$	Blackburn (1998)
Blackburn2	$(R_{800} - R_{470}) / (R_{800} + R_{470})$	Blackburn (1998)
Datt	$R_{672} / (R_{550} \times R_{708})$	Datt (1998)
Gitelson1	$R_{NIR} / R_{510}$	Gitelson <i>et al.</i> (2002)
CRI550	$1 / R_{510} - 1 / R_{550}$	Gitelson <i>et al.</i> (2002)
CRI700	$1 / R_{510} - 1 / R_{700}$	Gitelson <i>et al.</i> (2002)
CARrededge	$(1 / R_{510-520} - 1 / R_{690-710})R_{NIR}$	Gitelson <i>et al.</i> (2006)
CARgreen	$(1/R_{510-520}-1/R_{560-570})R_{NIR}$	Hernandez-Clemente <i>et al.</i> (2012)
Hernandez-Clemente	$R_{515} / R_{570}$	Hernandez-Clemente <i>et al.</i> (2012)
AVIcar	$AVI2(R_{410}, R_{530}, R_{550})$	Hernandez-Clemente <i>et al.</i> (2012)
Fassnacht1	$scale(AVIcar) + scale(Chappelle)$	Fassnacht <i>et al.</i> (2015)
Fassnacht2	$scale(AVIcar) + scale(CARred-edge)$	Fassnacht <i>et al.</i> (2015)
Carotenoid index (CARI)	$R_{720} / R_{521} - 1$	Zhou <i>et al.</i> (2017)

Note. The  $R_{wavelength}$  indicate the reflectance at this wavelength. The use of scaled index values from 0–1(scale) and the angular vegetation index (AVI) were proposed by Fassnacht *et al.* (2015). Gitelson *et al.* (2006) defined NIR as the reflectance at 760–800 nm.

Table 2. Carotenoid content ( $\mu\text{g cm}^{-2}$ ) for each treatment.

Treatment	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum
0% shading	7.37	8.25	8.51	8.53	8.86	9.86
35% shading	7.30	8.54	8.86	9.07	9.43	11.29
75% shading	7.68	9.10	9.36	9.35	9.68	10.27
90% shading	6.16	8.27	8.80	8.78	9.70	10.22
All	6.16	8.34	8.86	8.93	9.46	11.29

Table 3. Performance of 15 published indices for test data ( $n=32$ ).

Index	Regression Type	RPD	RMSPE (%)	RMSE ( $\mu\text{g cm}^{-2}$ )	$R^2$
Chappelle	Linear	1.15	8.84	0.85	0.489
PRI	Linear	1.04	10.25	0.93	0.125
Blackburn1	Linear	1.00	10.38	0.97	0.101
Blackburn2	Linear	1.02	10.10	0.95	0.161
Datt	Linear	1.06	10.17	0.92	0.257
Gitelson1	Exponential	2.33	4.53	0.42	0.833
CRI550	Linear	0.98	10.32	0.99	0.085
CRI700	Linear	0.99	10.34	0.98	0.088
CARrededge	Exponential	1.62	6.53	0.60	0.689
CARgreen	Linear	0.98	10.49	0.99	0.034
HernandezClemente	Linear	1.03	10.40	0.94	0.139
AVIcar	Exponential	1.14	9.53	0.85	0.343
Fassnacht1	Linear	1.73	6.63	0.56	0.739
Fassnacht2	Exponential	1.96	5.53	0.50	0.751
CARI	Exponential	2.06	5.46	0.47	0.782

## Figures

Figure 1. Shading treatments conducted in this study.

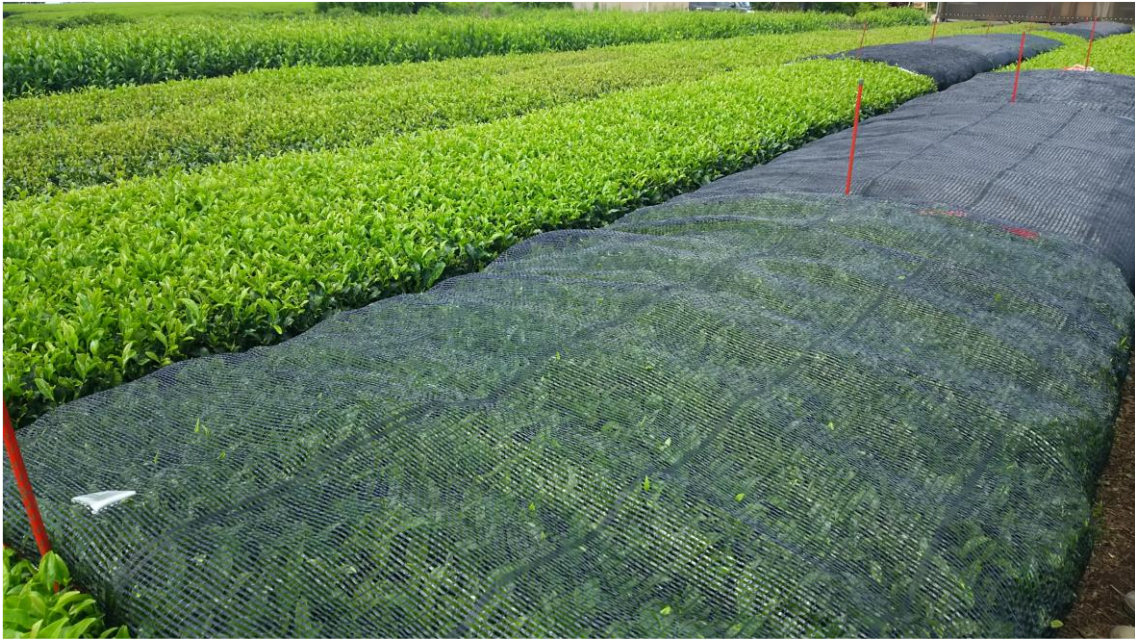


Figure 2. Weather conditions during the experiment.

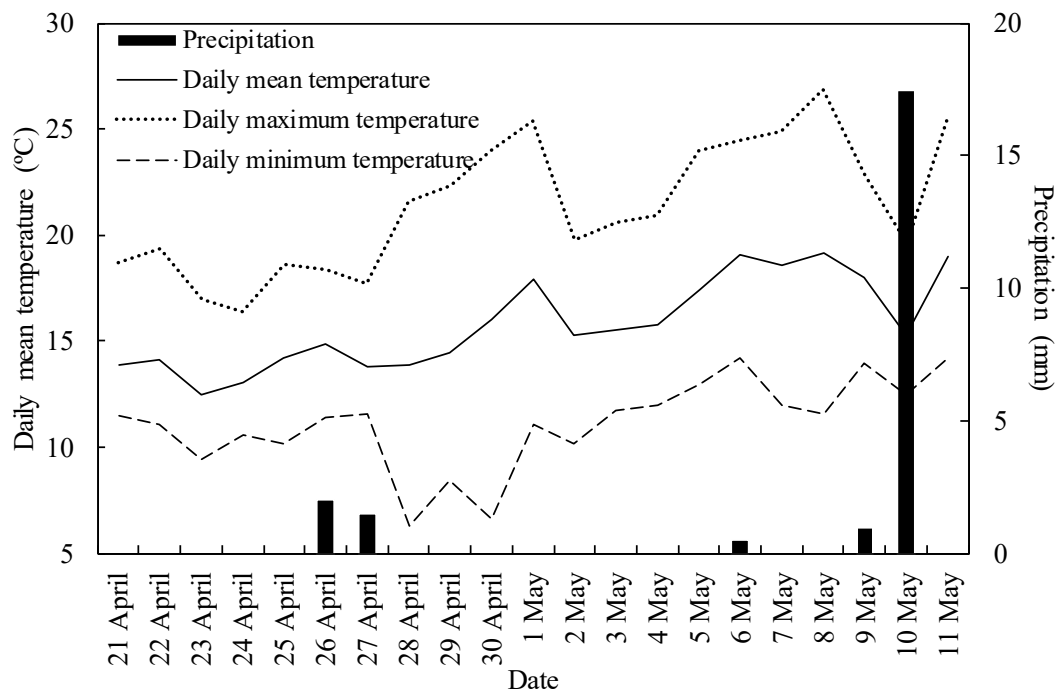


Figure 3. Relationship between carotenoid content and total chlorophyll content (a) or chlorophyll *a* content (b). \*\*\* indicates  $p < 0.1\%$  and \*\* indicates  $p < 1\%$ .

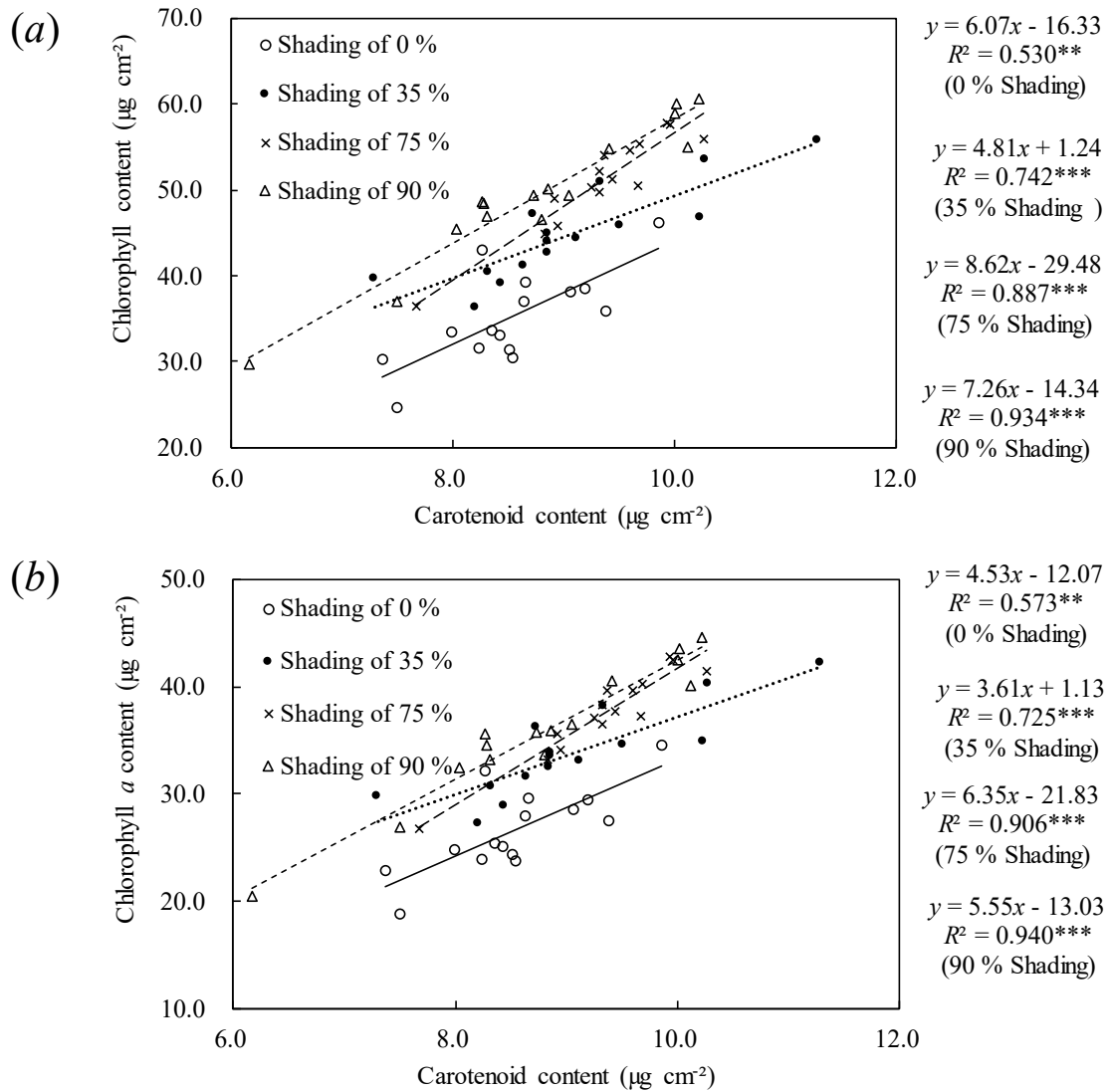


Figure 4. Mean reflectance spectra of different shading treatments.

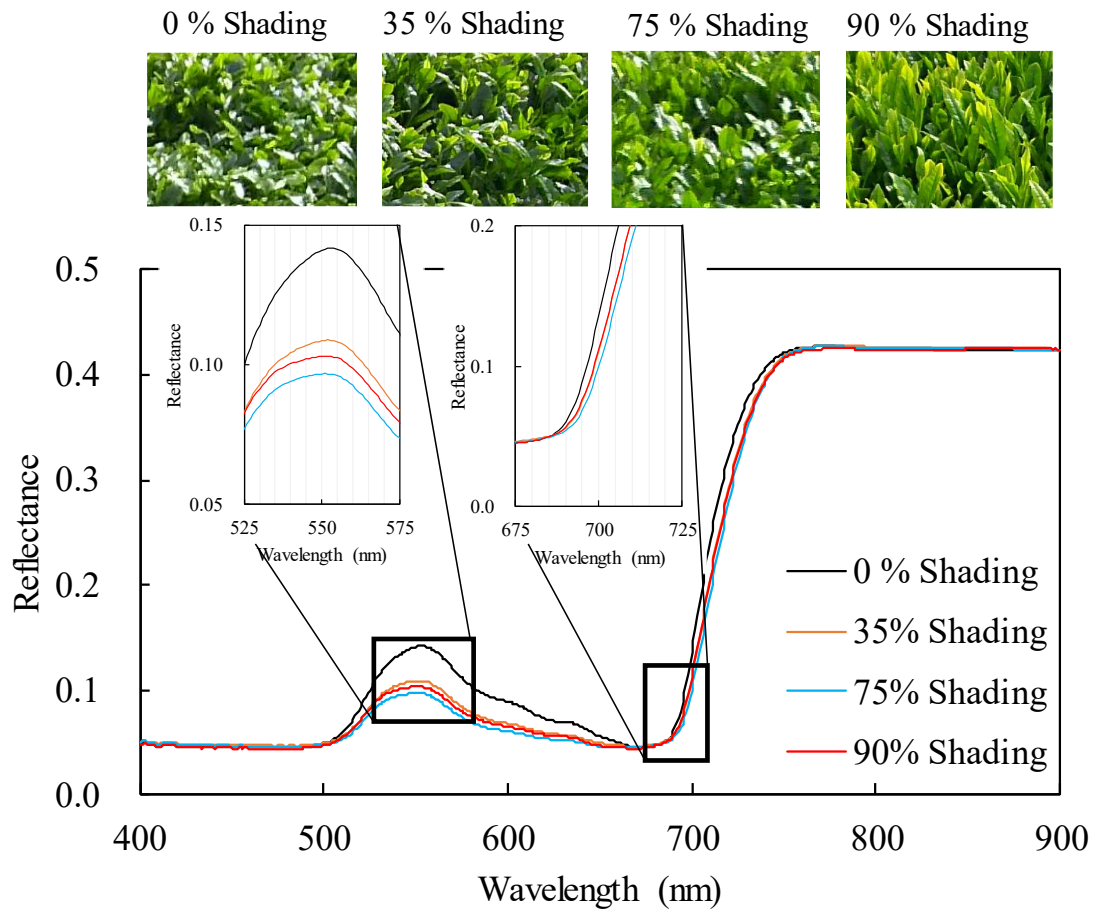




Figure 5. Relationships between estimated and measured carotenoid contents using Gitelson1 and CARI. \*\*\* indicates  $p < 0.1\%$

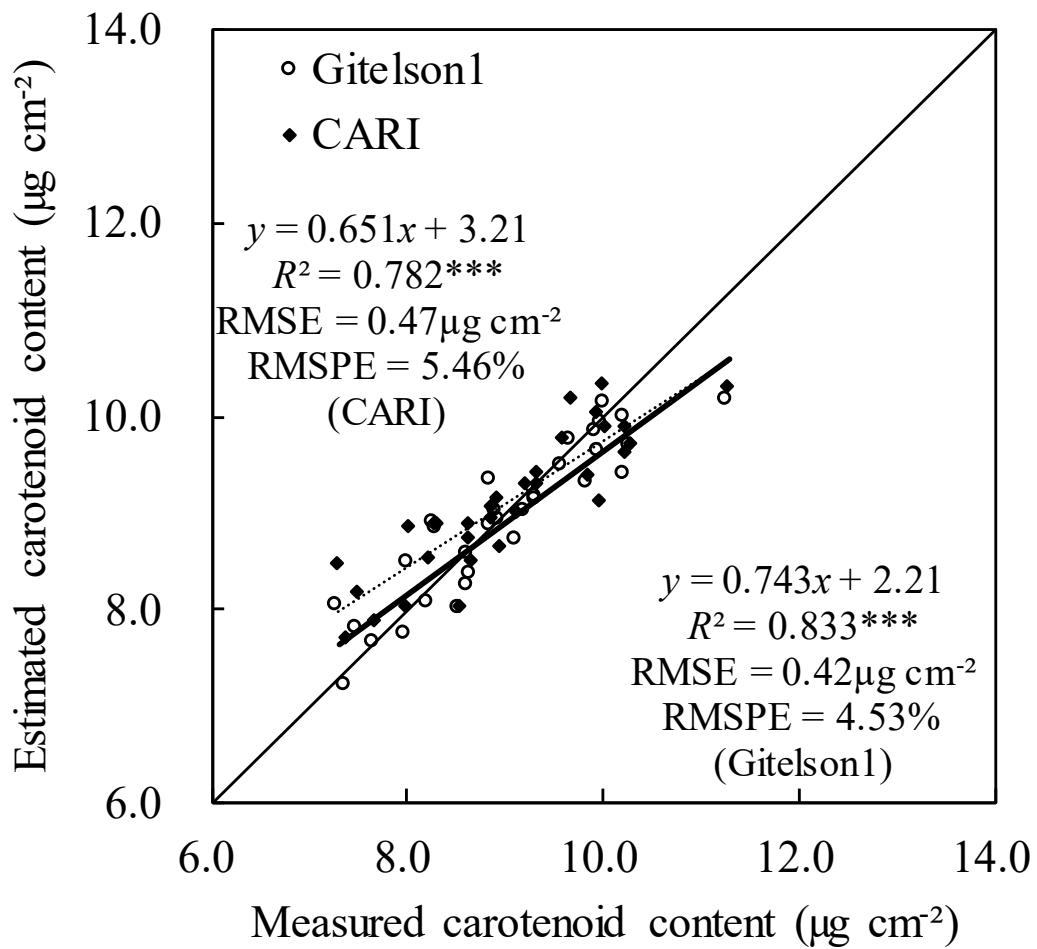


Figure 6. Correlations coefficient between reflectance at each wavelength and carotenoid content.

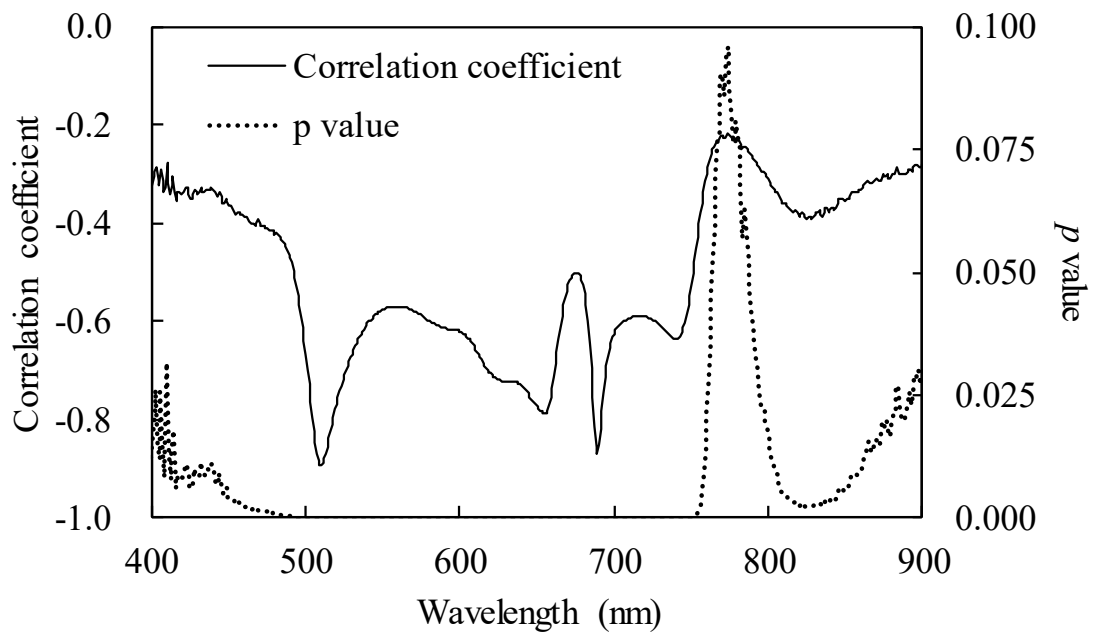


Figure 7. Absorption coefficients for carotenoid pigments.

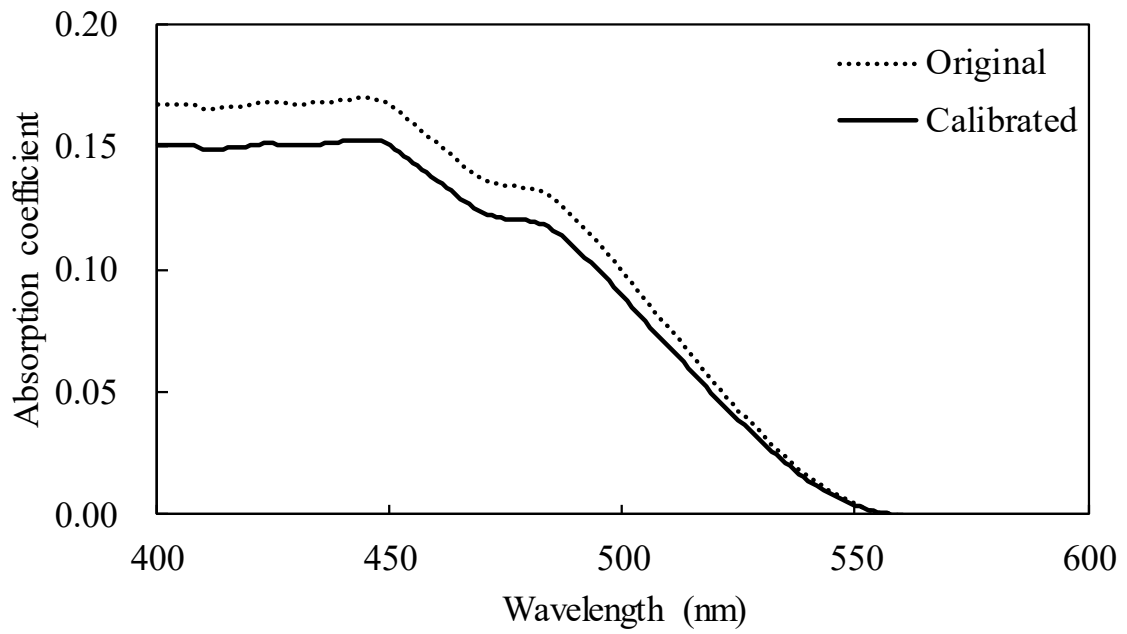


Figure 8. Relationship between estimated and measured carotenoid contents using original and calibrated PROSPECT-D. \*\*\* indicates  $p < 0.1\%$

