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Spectrophotometric Analysis

Quantitative Analysis of Chlorophyll a, b and Carotenoids in Cabbage : Use of Evaluation Function of LabSolutions™ UV-Vis

The color of plants and vegetables is one indicator of their freshness and quality. The color of plants is expressed by four pigments, chlorophylls, carotenoids, flavonoids, and betalains, and ultraviolet-visible (UV-Vis) spectrophotometer is frequently used as a method for evaluating the contents of these pigments.

This article reports the results of measurement of the contents of chlorophyll a and b and carotenoids in cabbage by UV-Vis spectroscopy. The article also introduces a method for efficiently advancing the chlorophyll/carotenoid content calculation process using polynomials by utilizing the Spectra Evaluation Function of LabSolutions UV-Vis, which is the standard software program of Shimadzu UV-Vis spectrophotometers.

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Chlorophylls and Carotenoids

Five types of chlorophylls (a, b, c, d, f) are known. Among these, the leaves, stems, and other green tissues of vegetables contain large amounts of chlorophyll a and b. Since the content of chlorophylls differs depending on the condition of nutrition, light environment, and whether herbicides are used or not, the chlorophyll content is an important indicator for management of vegetables. In particular, the ratio of chlorophyll a and b (a/b ratio) is an index showing the efficiency of photosynthesis, and it is known that vegetables grown in a strong light environment have a high a/b ratio [1].

An extremely large number of carotenoids exist, but β-carotene in carrots and lycopene in tomatoes are well-known representative examples. The carotenoids have an anti-oxidative effect and are known to prevent damage of vegetables by preventing generation of active oxygen and limiting its oxidizing power, particularly after harvest. Thus, the freshness of vegetables can be managed by evaluating their carotenoid content. Moreover, because carotenoids are expected to have an antiaging effect when consumed by humans, the carotenoid content of vegetables is also important from the viewpoint of nutritional science.

Sample Preparation

Samples with different light environments were prepared by cutting 0.1 g of cabbage leaves from three locations (top, middle, bottom parts) each of the first leaf (outer leaf) and third leaf (inner leaf) of a cabbage. These cut leaf samples were frozen with liquid nitrogen and crushed in a mortar, and 20 ml of 80 % acetone (acetone : ultrapure water = 8 : 2) was added. The fat-soluble material was then extracted by shaking for 3 min, and its supernatant was used in the measurements. Fig. 1 shows the appearance of the samples.

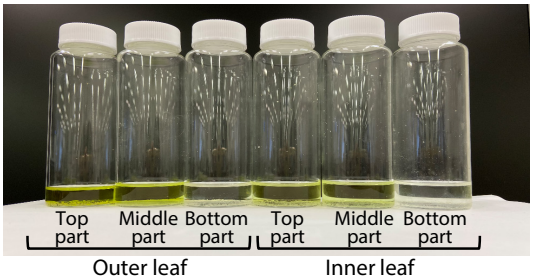


Fig. 1 Cabbage Extract Samples

Quantitation of Chlorophylls and Carotenoids

Various formulas are used in quantitation of chlorophylls and carotenoids. In measurement of the 80 % acetone extracts in this experiment, quantitation was also done by the following method [2], which was proposed by Lichtenthaler & Welburn and enables simultaneous calculation of chlorophylls and carotenoids. Table 1 shows the measurement conditions.

Chlorophyll a;

$$Chl\ a = 12.21A_{663} - 2.81A_{645} \quad (1)$$

Chlorophyll b;

$$Chl\ b = 20.13A_{645} - 5.03A_{663} \quad (2)$$

Carotenoids;

$$Carotenoids = \frac{1000A_{470} - 3.27[Chl\ a] - 104[Chl\ b]}{227}$$

$$= 4.405A_{470} - 9.182A_{645} + 2.129A_{663} \quad (3)$$

Chl a, *b*: concentration of chlorophyll a and b in extract [µg/ml]

Carotenoids: concentration of carotenoids in extract [µg/ml]

A_x: absorbance value at x nm

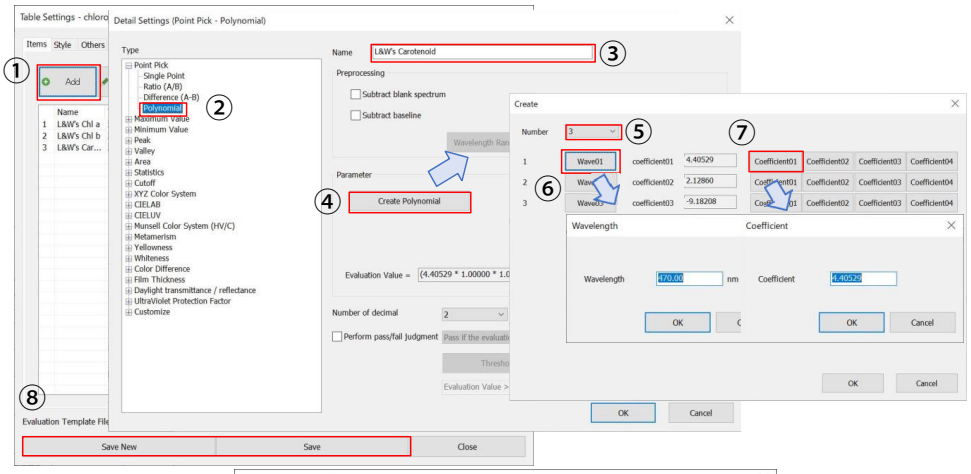
Table 1 Measurement Conditions

Instrument	: UV-1900i UV-Vis spectrophotometer
Type of photometric value	: Absorbance
Wavelength range	: 400 - 700 nm
Scanning speed	: High
Sampling pitch	: 0.1 nm
Light source switching wavelength	: 340 nm
Slit width	: 1 nm

As a representative example of the method of setting polynomials in the evaluation table in Spectra Evaluation Function, Fig. 2 shows the setting procedure for quantitation of carotenoids (Eq. (3) above).

By selecting "Polynomial" (② in the following figure), polynomials with a maximum of 10 terms can be prepared (⑤). Convenient and efficient calculation is also possible when the same evaluation is to be carried out repeatedly, because evaluation tables which have been set once can be saved as templates (⑧).

In addition, if the user checks "After measurement, data is automatically added to the evaluation table" (⑨) under the "Others" tab in the table settings, the evaluation results will be reflected in the table immediately after the spectrum measurement. Spectrum data which have already been stored in your folders can also be added to the evaluation table afterwards, making it possible to evaluate those data in comparison with the previous measured data.



① Click "Add" at Table settings
② Select "Point Pick"- "Polynomial"
③ Fill in the Name field
④ Click "Create Polynomial" to set Parameters
⑤ Select the number of terms in the Polynomial between 1 and 10
⑥ Enter the appropriate wavelength into each term
⑦ Enter the appropriate coefficient into each term
⑧ Click "OK" at each window and save the Evaluation Template File

Fig. 2 Procedure for Setting Polynomials in Evaluation Table (①-⑧) and Setting Automatic Reflection of Results in Evaluation Table (⑨)

		L&W's Chl a	L&W's Chl b	L&W's Carotenoid
	File Name	Value	Value	Value
1	Cabbage_outer_top.vspd	2.572669	0.525731	0.85
2	Cabbage_outer_middle.vspd	1.370171	0.441541	0.40
3	Cabbage_outer_bottom.vspd	0.149676	0.063691	0.05
4	Cabbage_inner_top.vspd	0.869273	0.239698	0.26
5	Cabbage_inner_middle.vspd	0.783447	0.199222	0.21
6	Cabbage_inner_bottom.vspd	0.091275	0.066899	0.03

Fig. 3 Evaluation Result Display Screen

Results

Fig. 3 shows the evaluation results displayed in the evaluation table. The contents of chlorophyll a and b are both highest in the top part of the outer leaf and lowest in the bottom part of the inner leaf. The chlorophyll a/b ratio is approximately 4.9 in the top part of the outer leaf and approximately 1.3 in the bottom part of the inner leaf. These results are consistent with the light environment described above. A tendency in which the carotenoid content was high in the top part of the outer leaf, which is a strong light environment, and low in the bottom part of the inner leaf was also observed.

Conclusion

The contents of chlorophyll a and b and carotenoids were calculated from absorbance values by using the Spectra Evaluation Function of the LabSolutions UV-Vis software, and results consistent with the light environment were obtained.

The Spectra Evaluation Function is also useful for improving work efficiency because results can be confirmed simultaneously with the completion of spectrum measurement and conversion equations can be stored as templates.

<References>
(1) Sonoike, K., Photosynthesis and Response to Light Environment, Protein, Nucleic Acid and Enzyme, Vol. 53, No. 9 (2008)
(2) Lichtenthaler, K. & Welburn, A.R., Biochem. Soc. Trans. 11: 591-592 (1983)

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