<ELCAS 活動報告>Analysis of Colors in Nature

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CITATION:

Yasuoka, Rito. <ELCAS 活動報告>Analysis of Colors in Nature. ELCAS Journal 2018, 3: 111-113

ISSUE DATE:

2018-03

URL:

http://hdl.handle.net/2433/230607

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Analysis of Colors in Nature

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1. Introduction

Color is an important attribute of plants. The colors serve to attract pollinators to receptive flowers and signal fruit to seed dispersers. In addition, the colors serve to warn potential predators of poisonous or toxic substances contained in plant tissues. Pigments are the source of the color. Pigment is a molecule that absorbs and reflects light. Different pigments appear in different colors because they have different abilities to absorb and reflect the various colors of light. Previous studies revealed that plants have some typical pigments, such as chlorophyll, carotenoids, and flavonoids. The object of our study is an analysis of plant pigments by chromatography.

2. Methods and Materials

The plants for the experiments were harvested from a private vegetable garden or bought at a nearby supermarket. We divided the plants into three groups. Each group chosen had the same plants for the experiments, and we conducted three experiments.

(1) Simple separation of pigments by thin layer chromatography sheet

My group chose three plants: yellow zucchini, pumpkin skin, and nori seaweed.

①Cut plants into small pieces and break in a mixer. In addition, grind plants by a mortar.

And then add hexane to the plants. Using this method, the cells of plants are broken, and pigments come out of the cell wall. So we can extract pigment from plants.

②Using a pipette and a spoon, put a sample in a sample tube. Add hexane to the sample tube and let stand for a while.

The liquid in the sample tube is the extract for analysis.

③The white sheet in the picture on the right is a thin–layer chromatography (TLC) sheet, which is used for the separation of pigments.

Spot the sample on one of the check marks on the TLC sheet as in Figure 1.

④Place the TLC sheet in the developing chamber with the sample spot toward the bottom.

Using this method, pigments separate.

⑤After that, remove the sheet from the chamber.

Consider that what pigments the plant contains.

We selected nori for further experiments.

We extracted a solution that contained only one pigment from the sample solution and carried out a detailed analysis to identify the pigments in the nori. So, in the next experiment, we separated the solution that contained one pigment from the

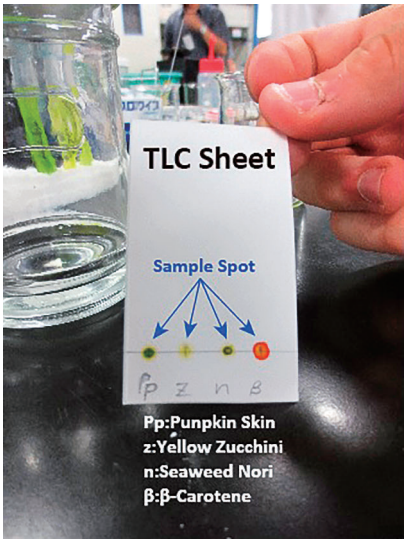

Fig. 1. TLC sheet.

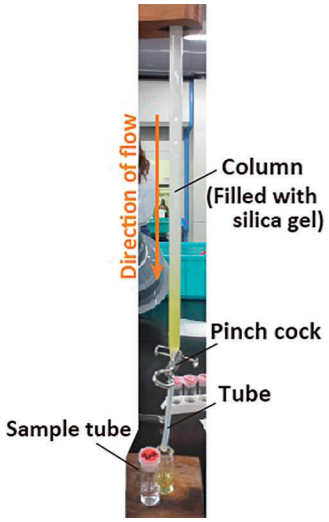

Fig. 2. Equipment used for column chromatography.

sample solution.

(2) Separation and sampling of solution that contains one pigment by column chromatography as in Figure 2
① Add the sample solution into the column.
② Open the pinch cock to let solvent flow down.
③ Draw off about 2 ml into each sample tube.
④ Arrange the sample tubes in a line to observe the pigment in the solutions.

Using experiment (2), we got a solution that contained one pigment. We analyzed the solution in the next experiment.

(3) Analysis of pigments by ultraviolet–visible absorption spectroscopy
① Among the pigment sample solutions obtained by elution, transfer the colored ones into optical cells (Figure 3 (b)) to measure the absorption spectra using a spectrophotometer (Figure 3 (a)) in the wavelength range from 350 to 750 nm.
② Save the measurement results in a data file.
③ Show the results in a graph.

3. Result

Figure 4 is the result of (1). It shows the pigments of each plant: pumpkin skin, yellow zucchini, and nori seaweed. A zucchini contains the yellow pigment xanthophyll. Pumpkins contain the dark green pigment chlorophyll a and the light green pigment chlorophyll b. The results of the nori are very interesting. Nori contains chlorophyll a, but it does not contain chlorophyll b. Also, nori contains a yellow pigment that we did not know. So we decided to carry out a detailed analysis to identify the pigments in nori.

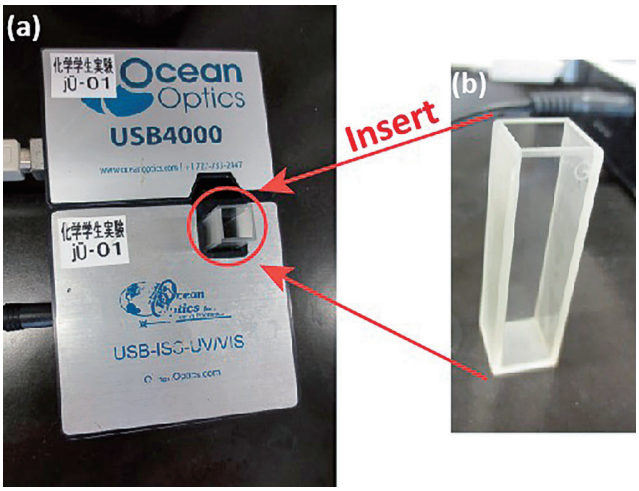

Fig. 3. Equipment used for spectrum analysis.
(a) Spectrophotometer. (USB4000, Ocean Optics, Inc.)
(b) Optical cell used for the spectrophotometer.

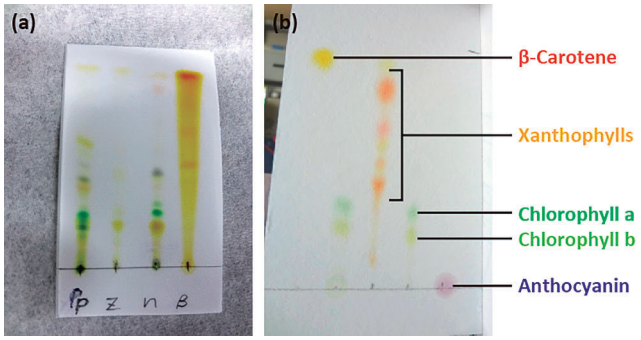

Fig. 4. Experimental result. (a) Result of TLC. (Pp: Pumpkin Skin, z: Yellow Zucchini, n: Seaweed Nori, β: β–Carotene), (b) Reference result of TLC at Kyoto University.

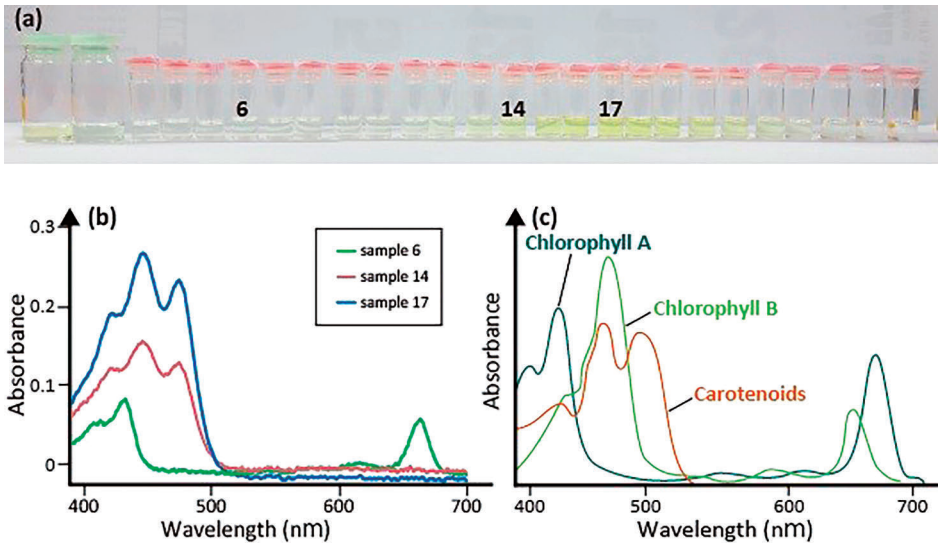

Fig. 5. Experimental result. (a) Experimental results of separating pigments by column chromatography. (b) Experimental results confirming the absorption spectrum of pigment solutions. (c) Reference spectrum for sample identification. (Figure (c) was drawn as a figure with reference to other materials. [1])

Figure 5 (a) shows the results of experiment (2). The solution in each sample tube contains one pigment. In particular, we can recognize yellow pigment. Figure 5 (b) shows the absorption spectra of each solution in the sample tubes. Compared with the reference material graph (Figure 5 (c)) that shows the absorption spectra of each pigment, nori contains chlorophyll a but does not contain chlorophyll b. The yellow pigments that we did not know are carotenoids.

4. Conclusion

Each plant contains pigments, and the pigments in vegetables are different from the pigments in seaweed. Vegetables that carry out photosynthesis contain chlorophyll a and b. The seaweed nori contains chlorophyll b and carotenoid. Some vegetables contain carotenoids. There are many types of carotenoids. Maybe, the type of carotenoid in vegetables and seaweed is different. So I want to study the types of carotenoids in vegetables and seaweeds.

5. Keywords

Pigments, thin layer chromatography, column chromatography, absorption spectroscopy, chlorophyll, carotenoids

6. Reference

1. http://ressources.unisciel.fr/tp_virtuels/Pigment_Extraction_Lab/co/module_Virtual%20Experiment_1.html