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Extracting, separating, and measuring retention factor values of pigments in spinach

Author: Sarah Marcus

Group Members: Alex Fox, Jay Fickes

8/30/2023

Introduction

Plants are able to perform photosynthesis because they contain specialized pigment molecules which can absorb different wavelengths of light. The wavelengths of light that can be absorbed by a pigment molecule are referred to as the absorption spectrum. These pigments are found in the chloroplasts of plant cells. The major pigments found in plants are chlorophyll a, chlorophyll b, and carotenoids. Chlorophylls absorb blue and red wavelengths of light and reflect green wavelengths. Carotenoids absorb violet and blue-green wavelengths of light and reflect red, orange, or yellow light. Chlorophylls are mainly involved in turning light energy into chemical energy through the process of photosynthesis, while carotenoids help protect the plant by absorbing excess light energy and releasing it as heat (Light and Photosynthetic Pigments 2023).

Paper chromatography is a laboratory technique that is used to separate mixtures such as ink, dye, or plant pigments. Different dissolved substances in a mixture will move at different rates through an absorbent paper as a solvent travels through the paper carrying the dissolved substances. Some of the substances will move slower because they are more attracted to the paper, while other substances will move faster because they are more attracted to the solvent. R_f values are calculated by dividing the distance a substance travels by the distance the solvent travels. R_f values range from 0 to 1, with 0 representing a substance that is entirely attracted to the paper and not at all attracted to the solvent and a 1 representing a substance that is entirely attracted to the solvent and not at all attracted to the paper. Chromatography can also be used to determine whether or not two substances are the same based on the number of components that are separated from the mixture and the R_f value of each component (BBC 2023).

The purpose of this investigation was to extract pigments from plants, then separate those pigments by paper chromatography and determine the R_f value of each pigment.

Methods

The height and diameter of a 400 ml beaker were measured with a ruler. The diameter was used to calculate the circumference of the beaker. A piece of filter paper was cut to a height of 1 cm less than the height of the beaker and a width of 1 cm less than the circumference of the beaker. A pencil was used to draw a line across the entire width of the filter paper 1.5 cm above the bottom edge of the paper. A large spinach leaf was blotted dry with paper towels to prevent water from being transferred onto the filter paper during the extraction process. This was important because water would cause a small amount of diffusion and create a blurry origin line. The leaf was placed on top of the pencil line. A clear plastic ruler was lined up over the leaf to create a guide along the pencil line. A quarter was rolled along the edge of the ruler and over the leaf taking care to avoid crushing other parts of the leaf and smearing the pigments on other parts of the paper. The pigment was allowed to dry for 4 minutes and the leaf was moved to another section of the pencil line where the extraction process was repeated. This was repeated until a continuous pigment line was created over the top of the pencil line. The prepared filter paper was formed into a cylinder and stapled in a way that prevented the edges from overlapping and allowed the extracted pigments to face the outside of the cylinder.

A solvent mixture of 3 parts petroleum ether, 1 part acetone, and 1 part water was poured into a beaker to a depth of approximately 1 cm. The paper containing pigments extracted from spinach was placed in the prepared beaker. Care was taken to ensure that the band of pigment was above the solvent line in order to prevent the pigments from being dissolved into the liquid at the bottom of the container. The beaker was covered with aluminum foil and allowed to sit until the solvent rose to approximately 1 cm within the upper edge of the paper. The paper was removed from the beaker using forceps and the solvent front was traced with a pencil. The color of each band of pigment was recorded and the paper was allowed to dry in a well ventilated area. The staples were removed from the paper cylinder and the distance from the origin to the solvent front was measured. The distance from the origin to the center of each band of pigment was also measured. The retention factor of each pigment was calculated using the following formula:

$$R_f = \frac{distance\ traveled\ by\ pigment}{distance\ traveled\ by\ solvent\ front}$$

The bands were tentatively identified based on their color. The data collected were the colors and distances traveled by each pigment. The colors were recorded because each plant pigment has a slightly different appearance. The distances traveled by each pigment were recorded so the pigments could be further identified by their R_f value. The data were analyzed by calculating the experimental retention factor of each pigment and comparing the retention factors of the pigments to known R_f values. This was helpful because each pigment has a unique retention factor which could be used to quantify the observed differences and confirm the identity of the pigments.

Argument

Spinach leaves contain chlorophyll a, chlorophyll b, and carotenoids. This claim agrees with the claims made by other laboratory groups.

Table 1. R_f values and colors of pigments in spinach

Leaf	**Color**	**Pigment**	**R_f Value**
Spinach	yellow/orange	carotenoid	0.99
	yellow	carotenoid	0.68
	bright green	chlorophyll a	0.30
	dark green	chlorophyll b	0.13

Table 1 shows the carotenoids and chlorophylls found in spinach leaves

The evidence in table 1 supports the claim that spinach leaves contain both chlorophylls and carotenoids because both green and yellow or yellow/orange pigments were identified. If the evidence did not support the claim there would have either been only one color present, or there would have been an additional color besides the two associated with chlorophylls and carotenoids.

Photosynthesis occurs in the green leaves of plants because of the presence of chlorophyll in the chloroplasts. Other pigments are present in the leaves of plants but they are masked by the chlorophyll. This explains why leaves change color in the fall when chlorophyll production decreases. Another field of study that could benefit from understanding plant pigments is the fashion industry. Plant based products are becoming increasingly popular among consumers as evidenced by the emergence of plant based milk and meat alternatives in the food industry. New plant based dyes for fabrics could be developed through understanding pigment molecules that are naturally present in different plants.

Works Cited

BBC. Separation and Purification. BBC; [accessed 2023 Aug 30]. https://www.bbc.co.uk/bitesize/guides/zqc6w6f/revision/4

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Plain Version

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