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Laser-Induced Fluorescence of Chlorophyll Concentration

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ABSTRACT

The chlorophyll fluorescence of the Amaranthus leaf has been irradiated with a He-Ne laserof peak wavelength 632.8 nm. Chlorophyll is a natural pigment found in green plants. It is the primary pigment that absorbs light energy from the sun for photosynthesis. This energy is then used by the plant to synthesize glucose from carbon dioxide and water. The extraction of chlorophyll from Amaranthus leaves was done using a cold/solvent extraction method. Amaranthus leaves were dried at room temperature and pounded in a mortar with pestle into powdered form. The chlorophyll solution was poured into seven different test tubes for the analysis. The cuvette was filled with the most diluted sample and irradiated with He-Ne laser light. The fluorescence intensity was measured with the help of a photocell connected to a multimeter.It has been observed that the fluorescence intensity depends on the concentration of the sample but at the same time, it also depends on the absorption of the sample. When the chlorophyll concentration is minima the fluorescence intensity is maxima. The result obtained in this work can be used to estimate the fluorescence intensity in various chlorophyll concentrations of the Amaranthus leaf.

Introduction

Amaranthus leaves are a stockroom of essential phytonutrients and antioxidants that help reduce inflammation in the body and provide an extra boost of nutrition for one's health. Amaranthus leaves, are less popular relative of spinach, are incredibly nutrient-dense and far superior to most other greens. Chlorophyll plays an important role in making plants green and healthy. It also has vitamins, antioxidants, and therapeutic properties that have the potential to benefit the body. To the best of my knowledge, however, research results are mixed about whether chlorophyll can boost your health. Dietary supplements containing chlorophyll and chlorophyllin are available and generally considered safe, with no reported adverse side effects over several decades of human use (MacKeen, 2021). Despite the potential health benefits associated with chlorophyll, a significant number of chlorophyll-rich vegetables, leafy materials, and fruits are lost throughout the food supply chain (FAO, 2011)

Chlorophyll is a natural pigment found in green plants. It is the primary pigment that absorbs light energy fromthe sun for photosynthesis. This energy is then used by the plant to synthesize glucose from carbon dioxide and water.

The structure of the chlorophyll molecule consists of several conjugated nitrogen-containing rings surrounding a magnesium ion by coordinated covalent bonds. Chlorophyll is the component in photosynthesis that traps solar energy so that it can be used to drive the production of carbohydrates from carbon dioxide and water. Chlorophyll a and b absorb strongly in the red region of the spectrum and have peak absorptions near 650 nm. The absorption spectra for chlorophylls a and b differ somewhat, and these two types of chlorophyll complement each other in absorbing sunlight.

During photosynthesis, once a photon is absorbed by a chlorophyll molecule, the energy from that absorption is quickly transferred to the chloroplasts, where it is used to drive chemical processes. When the chlorophyll is extracted from the leaves, however, the absorbed energy can no longer be trapped, and the chlorophyll re-emits the energy by fluorescence.

Fluorescence is the absorption of light of one colour and emission of light of a different colour. Since each colour of light corresponds to specific photon energy, fluorescence may be described as the absorption of a photon energy and emission of a Photon of a lower energy.

The chlorophyll solutions prepared in this experiment strongly absorb the 632.8nm light from a He-Ne laser, and the fluorescence maximum lies at approximately 685nm. Fluorescence can be distinguished from scattered laser light by the use of a filter. In contrast to many artificial pigments, chlorophyll is abundant, inexpensive, and benign.For these reasons, chlorophyll is an ideal substance for studying absorption and fluorescence phenomena in the laboratory (Zare. et al.,1995). When chlorophyll is extracted from leaves, light energy cannot be transferred to the chloroplasts. Instead, the light is re-emitted and/or absorbed as heat. The emission of light isknown as fluorescence and occurs between 675 and 685 nm (in the red region of visible light) (Hall. *et al*., 1994).

One of the pigment molecule's electrons is raised to an orbital with greater potential energy when the molecule absorbs a photon. When the electron is in its normal orbital, the pigment molecule is said to be in its ground state. After absorption of a photon boosts an electron to an orbital of higher energy, the pigment molecule is said to be excited.

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Photons that have an energy exactly equal to the difference between their ground state and excited state are the only ones that are absorbed; the energy difference varies depending on the type of atom or molecule. Thus, a particular compound absorbs only photons corresponding to specific wavelengths, which is why each pigment has a unique absorption spectrum.

The energy of an absorbed photon is converted to the potential energy of an electron raised from the ground state to an excited state. But the electron cannot remain there long, that is, the excited state, like all high-energy states, is unstable. Typically, when pigments absorb light, their excited electrons return to the ground-state orbital, dissipating their surplus energy as heat. Some pigments, including chlorophyll, emit light as well as heat after absorbing photons. The electron jumps to a state, of greater energy, and as it falls back to the ground state, a photon is given off and after-glow is called fluorescence (Lakowicz. et al., 1983). Fluorescence is affected by changes in temperature. As temperature increases, fluorescence decreases (Tuner, 1985). Several bioactive compounds in vegetables include vitamins minerals, antioxidants, as well as the pigment (chlorophylls and carotenoids). Amaranthus leaf is a green leaf which contains chlorophyll is an essential part of a healthy diet and is widely eaten by the people in the Northern part of Ghana. Chlorophyll which is found in plants when eaten, gives the human body some health benefits; Enhances energy, Detoxifies the liver, Eliminates body odour, and Cleans the digestive tract(Blankenship, 2002).

Fluorescent effects have been observed for thousands of years but it was not until recently that we analyzed it. Stokes used a prism to disperse the solar spectrum and illuminated asolution of quinine. He observed that no effect occurred until the solution was positioned within the UV spectrum.This finding prompted Stokes to assert that fluorescence possesses a longer wavelength than the stimulating light, resulting in this phenomenon being termed the Stokes shift. So, Stokes fluorescence is the re-emission of longer wavelength photons (energy) by a molecule that has absorbed photons of shorter wavelengths (higher frequency).

Fluorescence has mostly been used for studies on pigment-protein interactions, thylakoid membrane integrity, and the determination of chlorophyll concentration to mention but few. However, the relationship between chlorophyll and in vivo fluorescence varies with a wide range of time and space(Guilbault, 1990).In summary, the main purpose of this article is to provide a comprehensive understanding of the current knowledge on chlorophyll and its potential applications in the context of antioxidant activity and health benefits. The results and conclusions can be applied by readers and researchers to deepen their knowledge, guide future studies, explore applications in food and pharmaceutical industries, and stimulate further research in this field.

In this work, the quantitative form of the concentration dependence of fluorescence for chlorophyll was observed for many different molecules. The relationship between fluorescence intensity and concentration can be modelled for many systems according to the equation:

$$\mathbf{F = \phi I_o\left(1 - e^{-\epsilon bc}\right)} \qquad (1)$$

Where F is the intensity of fluorescenceis$\boldsymbol{I_0}$ the intensity of the incident light,$\boldsymbol{\epsilon}$ is the molar absorptivity (a constant for any particular compound at a particular wavelength), b is the path length of the cell, and c is the molar concentration. The dimensionless constant ϕ is the quantum efficiency which is defined as the number of quanta of radiation fluoresced by a sample divided by the number of quanta absorbed. Thus, the quantum efficiency represents the fraction of molecules that fluoresce from the excited state as opposed to decay by non-radiative processes. This formula accurately predicts that the fluorescence intensity will approach a maximum value $\emptyset\mathbf{I_0}$ as c becomes very large. Equation (1) can also predict the linear relationship between fluorescence and concentration at low concentrations, this can be shown using Taylor's series expansion;

$\boldsymbol{e^x \approx 1 + x + \cdots}$, for small $\boldsymbol{x}$ in terms of low concentrations, applying this expansion in equation (1) we find;

$$\mathbf{F = \emptyset I_0 \epsilon bc} \qquad (2)$$

Equation (2) is the modelled form of equation (1) for the relationship to model the chlorophyll fluorescence.

MATERIALS AND METHODS

Green leafy vegetables were collected from three different farms in Navrongo, Ghana. Early in the morning, all samples were acquired and brought into the lab. The specimens were refrigerated for additional examination. The materials used in the experiment are;Amaranthus leaves, mortar and pestle, round-bottom flask, weighing balance, acetone, and distilled water. The extraction of chlorophyll from Amaranthus leaves is by cold/solvent extraction method. Amaranthus leaves were dried at room temperature and pounded in a mortar with pestle into powdered form. 100g of the powdered sample was measured with the weighing balance and then poured into a round-bottom flask. 500 ml of acetone was added and the solution was stirred using a stirring rod and then left for three days, this was to extract the chlorophyll from the powdered sample.Theprepared Chlorophyll concentrations are from $\mathbf{1.3 \times 10^{-3}}$ **M to** $\mathbf{4.0 \times 10^{-5}}$ **M** and the solution was filtered into a round-bottom flask.

We prepared at least 3 mL of each chlorophyll solution and filtered the solutions with a 0.45 μm filter. The chlorophyll solution was poured into seven different test tubes for the analysis. The cuvette was filled with the most diluted sample and irradiated with He-Ne laser light. The fluorescence intensity was measured with the help of a photocell connected to a multimeter as shown in Figure 1. The procedure was repeated using other chlorophyll concentrations. When changing solutions, empty the cuvette, and rinse it with a small amount of the new solution from a clean pipette before filling the cuvette with the new solution. The whole experiment was accomplished from May 2024 until June 2024.

The optoelectronic components of the experimental set-up comprise of the following: He-Ne laser consists of a sealed tube filled with helium gas and neon gas, has a typical current of 10mA at 1000V, and the typical power of 10W, which produces a 1mW output beam for an overall efficiency of only 0.01% for converting electrical to coherent radiant energy and has a wavelength of 632.8nm. Photocell (2921 quadrant cell Photo receiver series) measures small deviations in the range of 300 to 1050 nm laser beam, sensor size 10×10 mm, and power ±15 V. Multimeter (OMM-6810B series) measures wide wavelength range from 300 to 1650 nm, and power measurement up to 10 W.

RESULTS AND DISCUSSION

Temperature and heat transmission from the system are directly correlated with reactant concentration. As the reactant's concentration increases, the reaction's temperature increases, and more energy is released as observed in Figure 2.

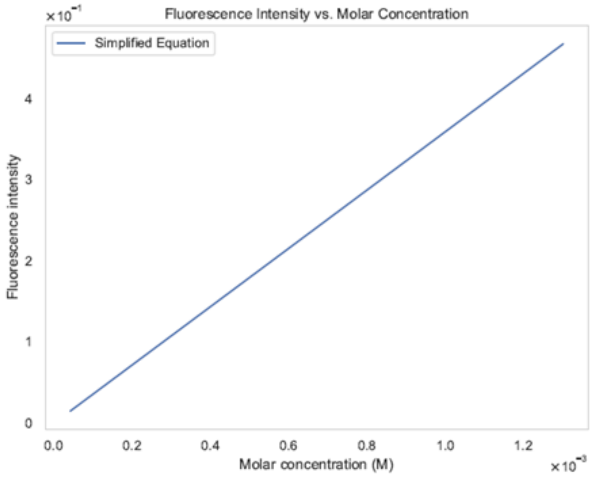

Figure 2: Model Fluorescence intensity and concentration

Figure 3 is a plot of fluorescence intensity as a function of chlorophyll concentration. At low concentrations, the intensity rises rapidly and nearly linearly with concentration, whereas at higher concentrations, the intensity approaches a constant value.

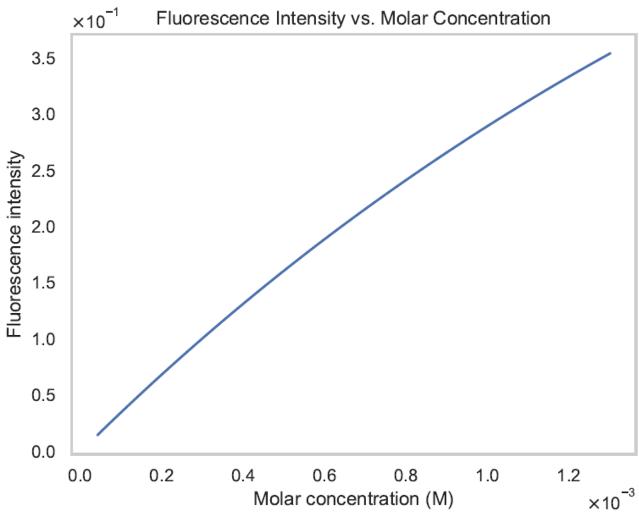

Figure 3: Non-model Fluorescence intensity and concentration

When the concentration is at maximum, the fluorescence intensity as measured from the digital meter was also minimum.

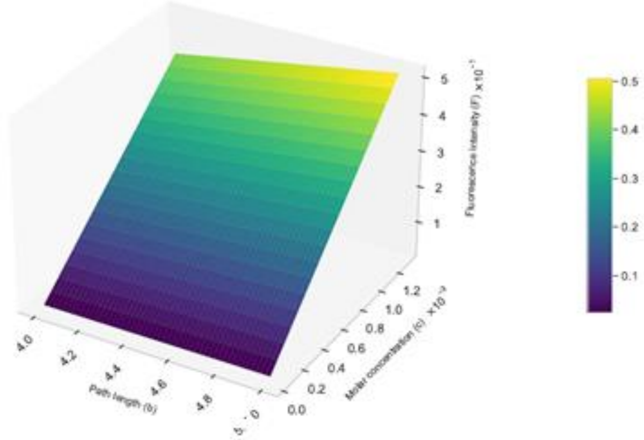

Figure 4: Model Fluorescence intensity and concentration in 3D

It may be noted that, from the graph in Figure 3, the fluorescence intensity at a particular stage does not rise but increases even (that means it gets to its threshold where there is no change in fluorescence intensity) as chlorophyll concentration decreases as shown in figure 4. This occurs primarily because of self-absorption, which always occurs whenever a beam of light penetrates a certain distance of the medium. Furthermore, as the concentration of the medium is maxima the process of self-absorption is complete under this condition no light transmission occurs.

CONCLUSION

A significant number of chlorophyll-rich vegetables, leafy green materials and antioxidants, coupled with this therapeutic property that has the potential to benefit the human body. When chlorophyll concentration is at maximum, fluorescence intensity is at minima, and when chlorophyll concentration is at minimum, fluorescence intensity is at maxima.

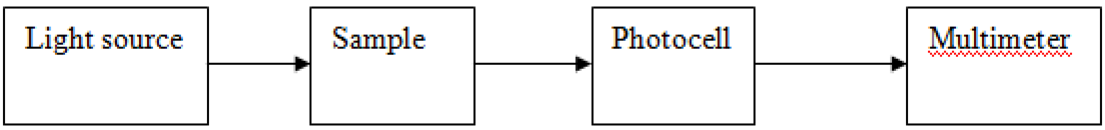

Figure 1: shows the experiment set-up

Author Contributions: The authors' responsibilities were as follows: PKM and SKF designed the research; PKM and SKF conducted the field study; PKM and SKF carried out laboratory-, data and analysis; PKM wrote the manuscript and SKF provided significant advice and critically edited the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest

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