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LIF Spectroscopy of Fruits: Study of Excitation Wavelength Independence

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Abstract—**In fluorescence spectroscopy, fluorescence emission generally occurs from the excited state with the lowest energy. This is known as "Kasha's rule" and is a general principle with only a few rare exceptions. As a consequence, the emission wavelength is independent of the excitation wavelength. In order to select an appropriate excitation source in fluorescence spectroscopy, it is very important to know the relation between the excitation source wavelength and the emission wavelength. The aim of this paper is to study the excitation-wavelength-independent fluorescence emission of some fruits. To do so, a fluorescence spectrometer controlled by labVIEW software was developed for collecting the fluorescence spectra of some famous fruits. A semiconductor laser and light emitting diodes with different wavelengths was used as the excitation sources.**

Keywords-component; fluorescence spectroscopy; fruits; Kasha's rule; laser; LED

I. INTRODUCTION

Laser-induced fluorescence (LIF) spectroscopy has proved its potential for various applications in agriculture, such as detection of tissue browning and bruising [1-3] and freshness control [4]. Also, it can be used to monitor water stress in plants [5]. Due to such defects, changes in molecular structure of the chlorophyll of products occurs which can be considered as visible marks of decreasing fruit quality. A LIF spectroscopy system consists of two main parts: a sensitive spectrometer and a source for excitation. It is possible to use different sources to induce the fluorescence in fruits and vegetables [6-9]. However, in order to select an appropriate excitation source, it is very important to know the relation between the excitation source wavelength and the emission wavelength [10]. There are a large body of research which has studied the relation between excitation wavelength and emission wavelength in LIF spectroscopy for various materials. Some of them reported the excitation-wavelength-dependent fluorescence behavior [11-13], while others investigated the excitation-wavelength-independent fluorescence behavior [14, 15]. This paper examines the relation between the excitation wavelength and the emission wavelength in LIF spectroscopy for some famous fruits. In the first step, a home-made spectrometer was designed and developed. In the second step, the fluorescence spectra of fruits were collected with six different excitation sources, including a semiconductor UV laser and UV and blue light emitting diodes (LEDs) with different wavelengths.

II. JABLONSKI DIAGRAM AND KASHA'S RULE

The processes which occur between the absorption and the emission of light are usually represented by the Jablonski diagram. Jablonski diagrams are often used as the starting point for studying absorption and emission of light. They are used in different forms, to illustrate various molecular processes that can occur in excited states. These diagrams are named after the Ukrainian scientist, Alexander Jablonski, who is regarded as the father of fluorescence spectroscopy, because of his many accomplishments, including descriptions of concentration depolarization and defining the term "anisotropy" to describe the polarized emission from solutions [16].

A typical Jablonski diagram is shown in Fig. 1. In this Fig., the first singlet electronic state (ground) is depicted by S_0 and the second singlet electronic state is shown by S_1. At each one of these electronic energy levels, the fluorescent compound can exist in a number of vibrational energy levels, depicted by horizontal lines. The upward vertical lines, "a" and "b", represent absorption of light and the downward vertical line, "c", represents fluorescence emission of light.

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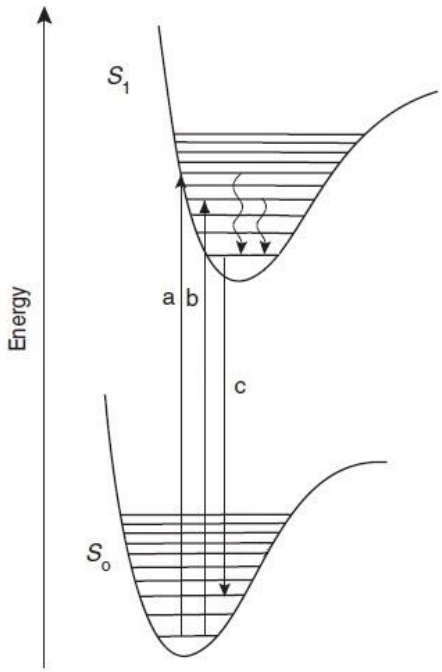

Figure 1. A typical Jablonski diagram.

Following the absorption of light, several processes usually occur in excited states. A fluorescent compound is usually excited to some higher vibrational level of S_1. With a few rare exceptions, molecules in condensed phases rapidly relax to the lowest vibrational level of S_1. This process is called internal conversion. Internal conversation generally occurs within 10^{-12} s or less, while the fluorescence lifetimes are typically near to 10^{-8} s. Typically, return to the ground state occurs to a higher excited vibrational ground state level, which then quickly (10^{-12} s) reaches thermal equilibrium (Fig. 1). Return to an excited vibrational state at the level of the S_0 state is the reason for the vibrational structure in the emission spectrum of fluorescent compounds [16]. Fluorescence emission is generally shifted to longer wavelengths (lower energy) relative to the re-emission of the light (red shift).

In 1950, Michael Kasha reported that the same fluorescence emission spectrum is generally observed irrespective of the excitation wavelength [17] (due to the internal conversion mentioned in the previous paragraph). Kasha's rule is a general principle with only a few rare exceptions. The result is that the emission wavelength is independent of the excitation wavelength.

III. Materials and Methods

In this step, the kasha's rule in LIF spectroscopy of fruits has been examined. A laser induced fluorescence spectroscopy system consists of two main parts: a sensitive spectrometer and a source for excitation. In this study, a home-made spectrometer was first designed and developed in order to acquire the spectra in the interval of 571 to 1149 nm. The software solution was based on the labVIEW program. The spectrometer was able to measure the fluorescence spectra directly from the fruit and vegetable surface in the desired regions. It was equipped with a suitable fiber-optic probe to do so. The hardware solution was based on the USB platform and controlled by the application running on the computer. Fig. 2 shows the schematic of the developed spectrometer.

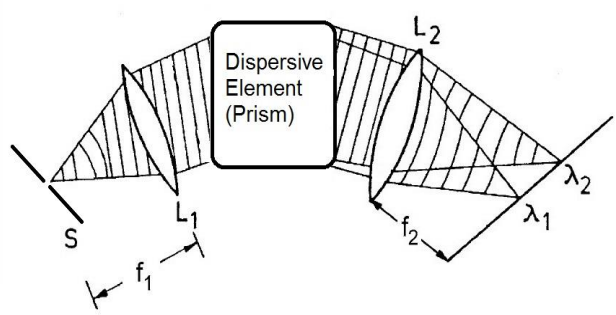

Figure 2. Schematic of the developed spectrometer. "S", "L1" and "L2" represent entrance slit, collimator lens and the imaging lens (camera lens), respectively.

The entrance light illuminates the slit, S, placed in the focal plane of the first lens, L_1. Behind L_1 the parallel light beam passes through the dispersive element (prism), where it is diffracted depending on the wavelength λ. The second lens, L_2, forms an image of the entrance slit on the camera. The position of this image in the focal plane of L_2 is a function of the wavelength λ.

IV. Results and Discussions

Fig. 3 shows the emission spectra of the applied excitation sources. In this Fig., the narrowband spectrum shown in row B is related to the spectrum of semiconductor UV laser with a peak at 402.5 nm. While, the rows C to G are related to the broadband spectra of different light emitting diodes (LEDs) with the central wavelengths at 397.5 nm, 403.5 nm, 424 nm, 448 nm, and 465.5 nm, respectively.

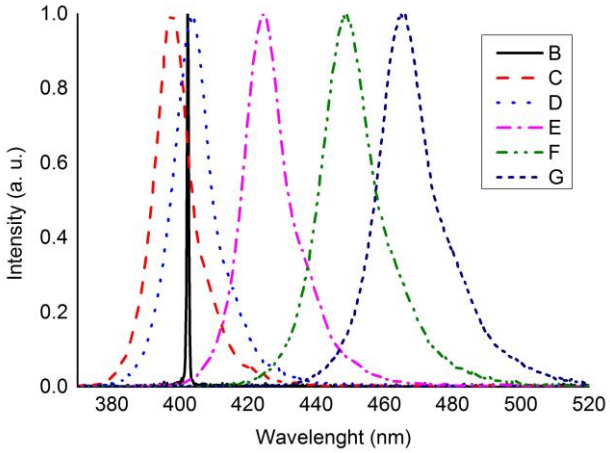

Figure 3. The emission spectra of applied excitation sources, the vertical axis is intensity, normalized to 1, and the horizontal axis is wavelength in nm.

The developed LIF spectroscopy system was successfully tested in four various fruits and vegetables including lemon, peach, pear and potato.

Figs. 4 to 7 illustrate the fluorescence spectra of lemon, peach, pear and potato, respectively.

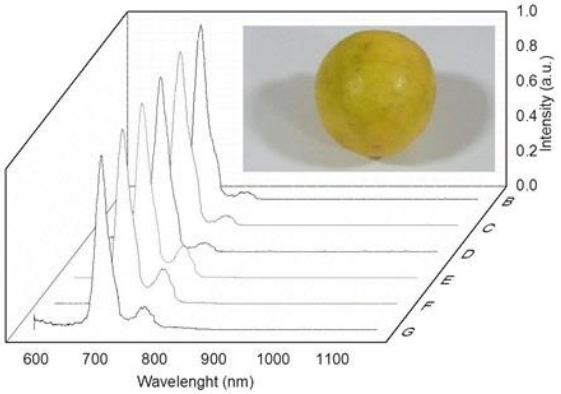

Figure 4. Fluorescence emission of lemon excited with various sources.

In all of fluorescence spectra shown through the Figs. 4 to 7, the vertical axis is intensity which is normalized to 1, the horizontal axis is wavelength in nm, and the row B is related to the fluorescence emission of fruits excited by a semiconductor laser with a peak at 402.5 nm. Also, rows C to G are related to the fluorescence emissions of fruits excited by light emitting diodes (LEDs) with central wavelengths at 397.5 nm, 403.5 nm, 424 nm, 448 nm and 465.5 nm, respectively.

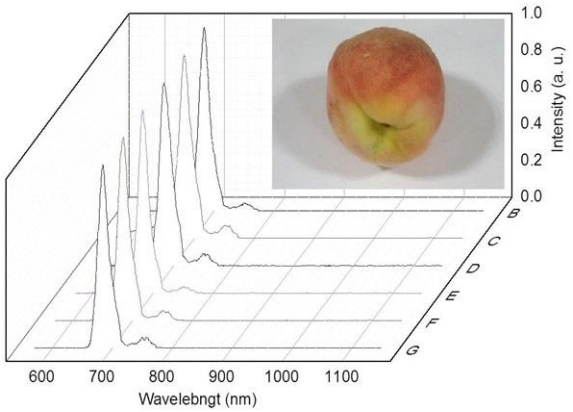

Figure 5. Fluorescence emission of peach excited with various sources.

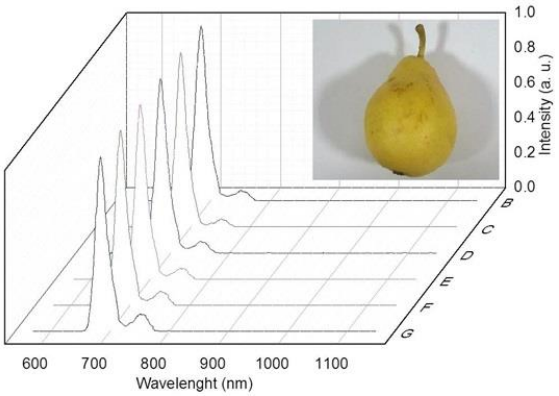

Figure 6. Fluorescence emission of pear excited with various sources.

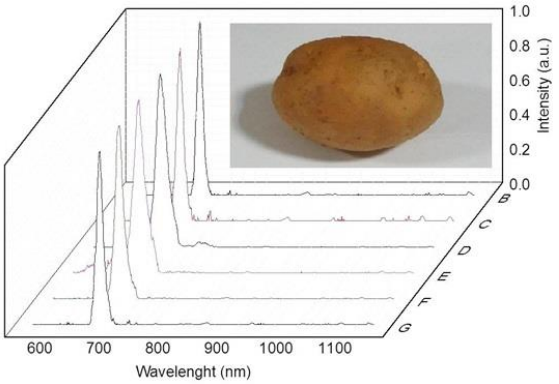

Figure 7. Fluorescence emission of potato excited with various sources.

As shown in Figs. 4 to 7, the fruits have an excitation-wavelength-independent fluorescence behavior. Although the difference between the peaks of excitation sources was 68 nm, no shift was observed in fluorescence emission of the fruits.

V. CONCLUSIONS

A general property of fluorescence is that the same fluorescence emission spectrum is generally observed irrespective of the excitation wavelength. This is known as Kasha's rule. Upon excitation into higher electronic and vibrational levels, the extra energy is quickly dissipated, leaving the fluorescent compound in the lowest vibrational level of the second electronic state. This relaxation occurs in about pico seconds, and is probably a result of a strong overlap among numerous states of nearly equal energy. Because of this non-radiative relaxation, emission spectra are usually independent of the excitation wavelength. In this paper, a spectrometer was designed and developed which was controlled by the labVIEW software and equipped with an appropriate fiber-optic probe. Light emitting diodes (LEDs) with different wavelengths in UV and blue regions were selected as excitation sources as well as a UV semiconductor laser. The developed LIF spectroscopy system was successfully tested in four fruits and vegetables. Finally, it was observed that the fruits have excitation-wavelength-independent fluorescence behavior. Although the difference between the peaks of excitation sources was 68 nm, no shift was observed in fluorescence emission of fruits. This result indicates that it is possible to use the mentioned sources as excitation source in LIF spectroscopy of fruits.

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