Design and development of a LabVIEW-based LED-induced fluorescence spectroscopy system with applications in non-destructive quality assessment of agricultural products

Hamed Abbasi[1], Majid Nazeri[1] and Seyed Ahmad Mireei[2]

[1] Department of Photonics, Faculty of Physics, University of Kashan, Kashan 87317-51167, Iran
[2] Department of Biosystems Engineering, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

Email: hamed.abbasi@grad.kashanu.ac.ir

Abstract. Over the past several years, the demand for high quality agricultural products has been remarkably increased. Thus, it is important to use non-destructive methods for product quality monitoring. LED-induced fluorescence spectroscopy has proved its potential for non-destructive detection of some defects in agricultural products, such as tissue browning and bruising. Due to such defects, changes in the polyphenol and chlorophyll contents occur which can be considered as the visible marks of decreasing fruit quality. In the present work, a fluorescence spectrometer (spectrofluorometer) controlled by LabVIEW software was designed and developed. In this spectrometer, a consumer-grade webcam was used as an imaging sensor. The spectrometer was able to measure the fluorescence spectra directly from the fruit and vegetable surface in the desired regions. To do so, the spectrometer was equipped with a suitable fiber-optic probe. The hardware solution was based on data acquisition working on the USB platform and controlled by the application running on the PC. In this system, light emitting diodes with different wavelengths were used as the excitation sources for inducing fluorescence spectra of some famous fruits and vegetables.

1. Introduction

Nowadays, spectral measuring methods such as fluorescence, transmission, remission and diffuse reflection have been investigated for fruit quality control applications [1]. Fluorescence spectroscopy has proved its potential for various applications in agriculture, such as detection of tissue browning and bruising [2-4] and freshness control [5]. Also, it can be used to monitor water stress in plants [6]. Due to such defects, changes in molecular structure of the chlorophyll of products occur which can be considered as visible marks of decreasing fruit quality. Plants show a fluorescence emission in the visible spectral range that is excited by UV radiation and partially also by visible light [1]. The chlorophyll fluorescence of agricultural products mainly consists of two maxima in the red and far-red region. The intensity and shape of the chlorophyll fluorescence emission spectrum at room temperature are primarily dependent on the concentration of the fluorophore chlorophyll-α, and to a lower degree also on the structure, the photosynthetic activity, and the it's characteristics and arrangements of cells in the tissue. [7]. A Laser-induced fluorescence (LIF) spectroscopy system consists of two main parts: a sensitive spectrometer and a source for excitation. It is possible to use

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different sources to induce the fluorescence in fruits and vegetables [8-10]. 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 [11]. In this study, the power light emitting diodes (LEDs) rather than laser have been used as the excitation source, due to their lower price. There some attempts for developing cost-effective LIF spectroscopy system with application in agriculture sciences. Paul K Buah-Bassuah et al. developed a portable fibre-probe ultraviolet light emitting diode (LED)-induced fluorescence detection system by combining an Ocean Optics spectrometer and a 365 nm UV LED [12]. In this paper, first, a home-made spectrometer using a consumer-grade webcam was designed and developed. In the second step, the fluorescence spectra of the fruits and vegetables were collected with the five different excitation sources, including power UV and blue light emitting diodes (LEDs) with different wavelengths.

2. Materials and methods

In this study a prism-based spectrometer (which has the higher sensitivity compared to grating-based spectrometers) controlled by LabVIEW was developed in order to acquire the spectra in the interval of 571 to 1149 nm. Figure 1 shows the schematic diagram of the spectrometer.

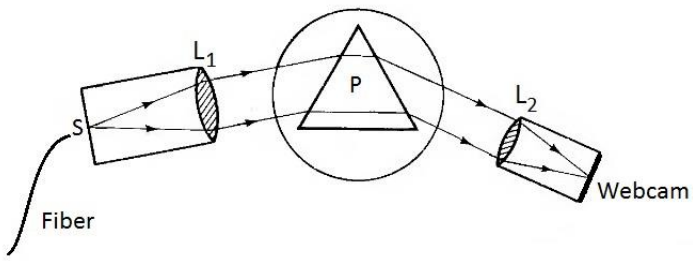

Figure 1. The schematic diagram of the spectrometer. “S”, “P”, “L1” and “L2” represent slit, prism, collimator lens and the imaging lens (camera lens), respectively.

The entrance light illuminates the slit, which was placed in the focal plane of the collimator lens. Behind the collimator lens, the parallel light beam passes through the prism, where it is diffracted depending on the wavelength λ. The imaging lens forms an image of the entrance slit on the webcam sensor. A consumer-grade webcam (Dell, 640 X 480 pixels) was used in this study. The position of the image of slit in the focal plane of imaging lens was a function of the wavelength λ. Figures 2 and 3 show the internal and external schematic of the developed spectrometer, respectively.

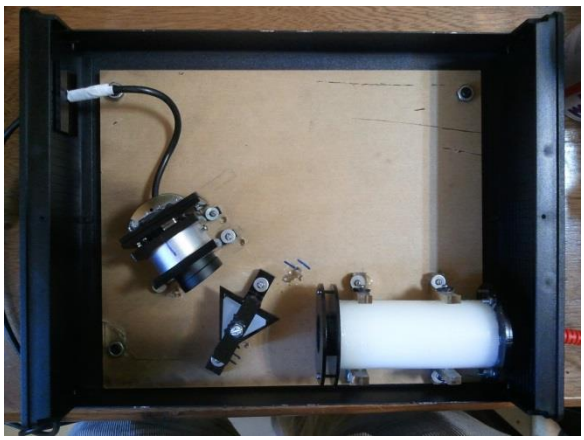

Figure 2. Internal schematic of the developed spectrometer.

Figure 3. External schematic of the developed spectrometer.

The first wavelength in the free spectral range (FSR) of the spectrometer was 571 nm, and the last one was 1149 nm. The triangle flint prism was used in this spectrometer. The prism was used at the minimum angle of deviation. In this angle, the wavelength of interest traveled parallel to the base of the prism, and the angle of incidence was equal to the angle of refraction. The refraction index of the used prism was 1.6216, 1.6207, 1.6005 and 1.6003 in the wavelengths of 571, 581, 1139 and 1149 nm, respectively. Therefore, for the first and last 10 nm of FSR, the spectral dispersion, dn/dλ, equals:

$$\frac{\mathrm{d}n}{\mathrm{d}\lambda} = \frac{1.6207 - 1.6216}{10\,\mathrm{nm}} = -0.9 \times 10^{-4}\ \mathrm{nm}^{-1} \tag{1}$$

$$\frac{\mathrm{d}n}{\mathrm{d}\lambda} = \frac{1.6003 - 1.6005}{10\,\mathrm{nm}} = -0.2 \times 10^{-4}\ \mathrm{nm}^{-1} \tag{2}$$

The spectral resolving power, R, of any triangle prism is defined as follows [13]:

$$R = \frac{\lambda}{\Delta\lambda} \le \frac{1}{3} g \left(\frac{\mathrm{d}n}{\mathrm{d}\lambda}\right) \tag{3}$$

In this formula “g” is base length of the prism. In this study, the base length of the prism was 25 mm, therefore for the first 10 nm of the spectrometer, the maximum value of R can be calculated as 750. Also, the smallest resolvable wavelength interval, Δλ, in 571 nm was calculated as follows:

$$\Delta\lambda_{\min} = \frac{571\,\mathrm{nm}}{750} = 0.76\,\mathrm{nm} \tag{4}$$

With similar method, the maximum value of R for the last 10 nm of the spectrometer and the smallest resolvable wavelength interval, Δλ, in 1149 nm were calculated as 166.6 and 6.8 nm, respectively.

Obviously, the operation of the spectrometer in the visible region was better than the near infrared region. The spectrometer was able to collect the fluorescence spectra directly from the fruit and vegetable surface in the desired regions. To do so, the spectrometer was equipped with a suitable fiber-optic probe. The hardware solution was based on the data acquisition working on the USB platform and controlled by the application running on the PC.

Generally, in prism spectrometers, the resolution varied at the different wavelengths. Therefore, a number of reference lines are required for calibration. In this study, lines of Mercury (Hg), Sodium (Na) and Cadmium (Cd) lamps were used as well as the first and second harmonic of Nd-YAG laser for calibration of the spectrometer. Figure 4 shows the lines of the Mercury lamps on the webcam sensor, in case of adjusting the spectrometer from 360 to 1100 nm.

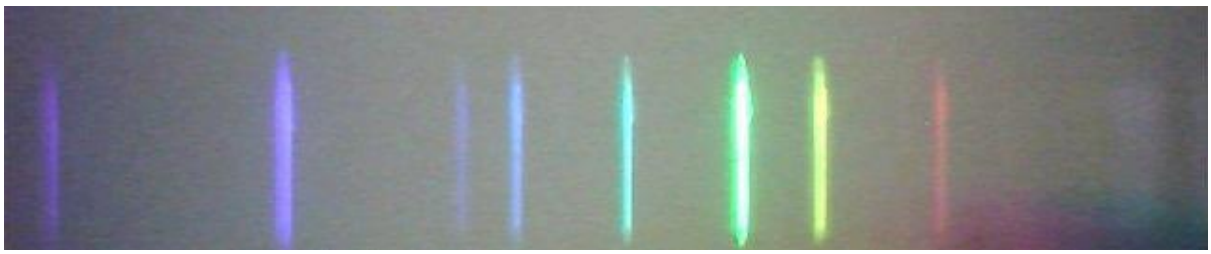

Figure 4. Lines of the Mercury lamps on the webcam sensor (360-1100 nm).

We improved the dynamic range of our system by means of using a large number of rows of webcam sensor (instead of using a single row of that) without going to saturation (by trying to have a homogenous vertical distribution of spectral lines). The webcam had a 640 pixel in length. Usually, the prism spectrometers are calibrated either by using the famous formula such as Sellmeier or Cauchy's equation [14] or applying a suitable formula that are able to fit in desired region. Figure 5 shows the fitted formula for calibration of the spectrometer (360-1100 nm) with R-squared (0 to 1) value very close to 1. R-squared is a statistical parameter of how close the data are to the fitted regression line which normally is defined between 0 to 1 or 0 to 100.

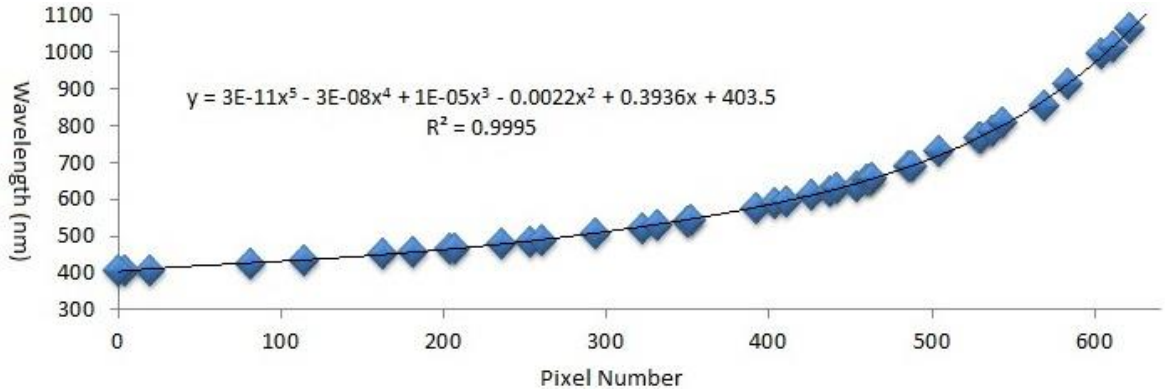

Figure 5. The trendline for calibration of the spectrometer with high R-squared value. The horizontal axis represents the pixel number and the vertical axis represents wavelength in nm.

LabVIEW software was employed to control the system. Figure 6 shows a print screen of the user interface of the LabVIEW program.

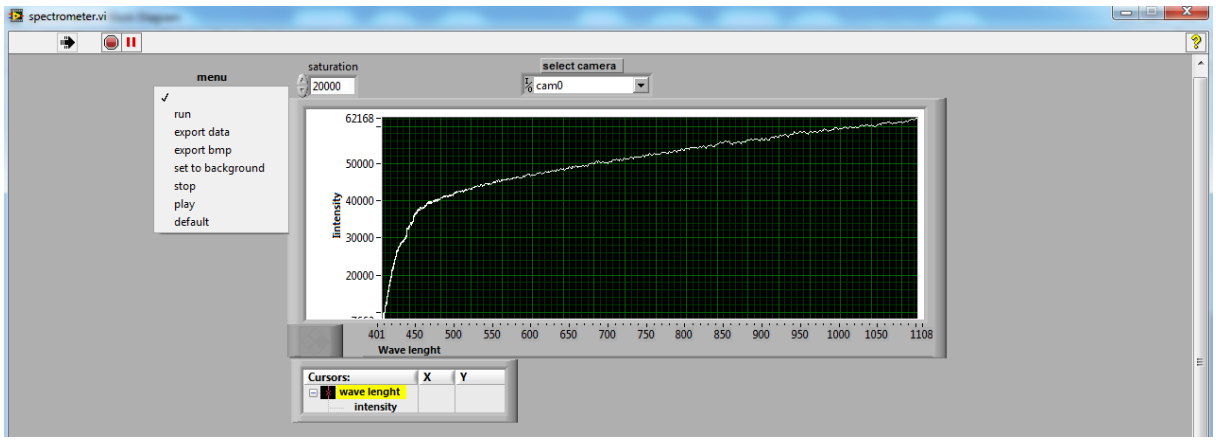

Figure 6. Print screen of the user interface of the LabVIEW program.

In LIF Spectroscopy, lasers are mainly used as the excitation source. In recent years, by developing the technology of producing LEDs, they have gradually taken the place of lasers in LIF spectroscopy. Due to the Kasha's rule, the fruits and vegetables have the excitation-wavelength-dependent fluorescence behaviour, and consequently, all of the used LEDs behave in the similar way [11]. Figure 7 shows the spectrum of the used LEDs.

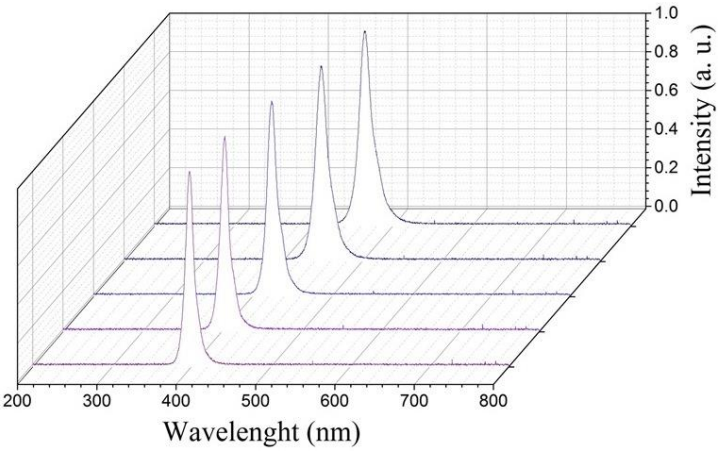

Figure 7. The spectrum of the used LEDs with the similar power values of 1 Watt and the central wavelengths at 397.5 nm, 403.5 nm, 424 nm, 448 nm, and 465.5 nm. The horizontal axis represents the wavelength in nm and the vertical axis represents the normalized intensity in arbitrary unit.

3. Results

One of the most important factors in designing and developing a laser or LED-induced fluorescence spectroscopy system for quality assessment of agricultural products is the capability of the instrument to induce and collect the fluorescence spectra of various types of fruits and vegetables. Therefore, it was necessary to test the system for various types of fruits and vegetables. Since the fruits and vegetables have the excitation-wavelength-dependent fluorescence behavior, it is possible to use the mentioned sources as the excitation source in LED-induced fluorescence spectroscopy. Figure 8 shows the fluorescence spectra of some famous fruits and vegetables including pear, peach, cherry, orange, red apple, yellow apple, water melon, carrot, potato, and basil.

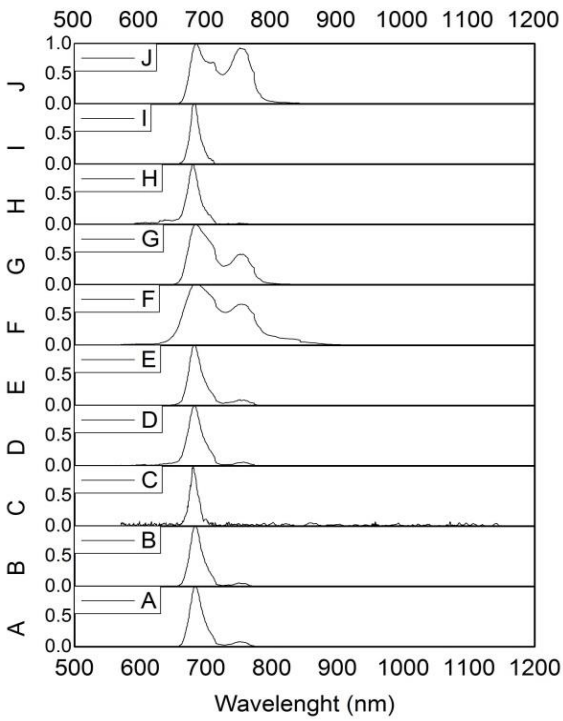

Figure 8. The collected fluorescence spectra of some famous fruits and vegetables. The first row, A, the second row, B, …, and the last row, J, represent the fluorescence spectrum of pear, peach, cherry, orange, red apple, yellow apple, water melon, carrot, potato, and basil respectively.

For the basil, water melon and Golden Delicious apple, three peaks were observed at 690 nm, 735 nm and 792 nm. The peaks at 690 nm (red fluorescence) and 735 nm (far red fluorescence) are related to the chlorophyll-α fluorescence and are the good indices to assess the chlorophyll-α concentration. The variation in the peak of 740 nm can be due to the inactive photosynthesis process [12, 15]. It

should be also be noted that the present of fluorophore in the basil leaf and other fruits which are found by the 690 nm and 740 nm fluorescence peaks, can be used to explain the chlorophyll degradation during the photosynthesis. The peak at 792 nm may be due to the unwanted wavelength of laser-light other wavelength than expected. For the other fruits and vegetables, including the Red Delicious apple, orange, peach, pear, potato, carrot and cherry, the peaks at 735 nm and 792 nm diminished drastically and only a strong peak at 690 nm can be observed. This can be due to the fact that the samples of this group had the lower active photosynthesis process and their chlorophyll status were significantly less than the first group.

4. Conclusion

In this paper, a spectrometer was designed and developed using commercial components which was controlled by the LabVIEW software and equipped with an appropriate fiber-optic probe. The 200 micrometer fiber optic was used as probe; the triangle prism was used as a diffraction element; and the consumer-grade webcam was applied as the imaging sensor. The lines of Mercury (Hg), Sodium (Na) and Cadmium (Cd) lamps as well as the first and second harmonic of Nd-YAG laser were used for calibration of the spectrometer. The light emitting diodes (LEDs) with different wavelengths in UV and blue regions were selected as the excitation source We successfully tested our system for different types of fruits and vegetables to make sure that it was able to work correctly, because different types of agricultural products have different amount of fluorescence emission and one system may be able to collect the fluorescence of one type of vegetable/fruit but not capable of collect the fluorescence spectra of another vegetable/fruit (because it may need another excitation source or more sensitive spectrometer).

Acknowledgments

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