Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 4 Issue 10 October 2020

Review Article

Laser Induced Fluorescence Spectrum of "Sweet Shrub" Stevia

LK Rajkhowa[1]*, R Mahanta[2], A Hazarika[1], J Saikia[3] and GD Baruah[1]

[1]*Center for Laser and Optical Science, New Uchamati, India*

[2]*Department of Physics, Dibru College, Dibrugarh, India*

[3]*Department of Physics, Jaganath Baruah College, Jorhat, India*

***Corresponding Author:** LK Rajkhowa, Center for Laser and Optical Science, New Uchamati, India.

Received: August 24, 2020

Published: September 30, 2020

Abstract

Laser Induced Fluorescence spectrum of acetone extract of stevia has been excited with the help of a blue diode laser (415 nm, 10 mW) and recorded on a mini spectrometer. The spectrum exhibits three prominent bands at 444, 474 and 503 nm along with the well known chlorophyll bands at 685 nm and 730 nm.

Keywords: Stevia; LIF

Introduction

The plant *Stevia rebaudiana* is a perennial herb of significant economic value due to its high content of natural, dietetically valuable sweeteners in its level [1,2]. The sweet taste of stevia is mainly due to diterpene glycoside. It is also calorie free and does not metabolize. Thus, it is established as a natural sweet herb. Stevia possesses many beneficial properties as compared to other sweeteners. As an example stevia has caloric value 2.7 kcal/gm [2] whereas glucose has caloric value 3.80kcal/gm [2]. It is thermo stable and withstands a temperature of about 200°C [2]. It is used in cooking and baking as it is non-fermentable. Stevia is used in the treatment of diabetes and obesity by suppressing appetite and reduces urge for sweet. Further it is helpful in the management of weight [3,4]. It is worthy to remark here that stevia is also called "Honey Tulsi" in Assam and elsewhere as "Sweet Weed", "Sweet Leaf/Meethi Patti" and "Sweet Herb" which is estimated to be 300 times sweeter than sugar while dry leaves of this plant are 30 times sweeter than sugar [5]. *Stevia rebaudiana* has a long history of use by the tribal people [6]. In the 1930's and 1940's stevia was introduced in UK and Russia as sugar substitute and Japan has been using it extensively since early 1970 [7].

As historical perspective it is worthwhile to mention that stevia (family-Asteracese) was botanically classified by Dr. Moises Santiago Bertoni in 1889 by name *Eupatorlum rebaudianum*, but later its name was changed to stevia rebaudi (bertoni) in honour of Paraguayan chemist Dr. Rebaundi. Then its sweet component was isolated which was purified as stevioside, the main sweet component in the form of an extremely sweet white crystalline compound. In the year 1970's an important rebaudioside-A was isolated, with a sweetening potential even higher than stevioside [8] and it is about 250 to 400 times higher than the sucrose.

From what has been said above, as an introduction to the sweet shrub stevia it follows that the shrub is well known since many decades due to its potential applications in several fields. In the present work we are concerned with the laser induced fluorescence (LIF) spectra of stevia. From a survey of available literature it is reasonable to believe that it is first report of this kind. The characteristic features of this LIF of stevia have been described in detail and discussed in the section which follows.

In this connection it is worthwhile to remark that the excitation of fluorescence with the help of laser radiation has become a procedure that has attracted research workers in various fields

and disciplines. As an example LIF of green plants detection of vegetation stress has been initially proposed by Chappelle., *et al*. [9]. Later these studies have been used to explore the possibility of using laser as remote means of measuring the various characteristics of vegetation such as vigour, plant type identification and natural mineral deficiency [10-12]. The LIF spectra have been studied to investigate some meteorite samples by our group [13,14]. The LIF technique has also been used to study polluted water of the Brahmaputra River by Mahanta., *et al* [15].

Experimental

Figure 1: Shows the photograph of the plant leaves "sweet shrub" stevia which is used for the experiment.

In the present case immersion of leaves in a glass vessel containing a suitable organic solvent such as acetone, enables the pigments responsible for their colour to be easily extracted. The extract may then be transferred to an observation tube of suitable length which is held against a brilliant source of blue laser light. The tube with the sample is held in front of the opening end of the optical fibre which is connected to a mini USB spectrophotometer with spectral range 120 - 1200 nm. The spectrophotometer is connected to a laptop and necessary software is installed. Figure 2 shows the LIF spectrum of the acetone extract of the sample stevia and figure 3 shows the LIF spectrum of the acetone extract of the well known plant leaves "Tulsi" for direct comparison.

Figure 2: Laser induced fluorescence spectrum of the acetone extract of the leaves of stevia.

Figure 3: Laser induced fluorescence spectrum of the acetone extract of the leaves of tulsi.

Result and Discussion

We shall now consider the LIF spectrum of the specimen stevia and its characteristic features which come to notice when compared to the LIF spectra other green leaves.

As may be seen from figure 2, the LIF spectrum of the acetone extract of the green leaves of stevia shows three well-defined peaks almost at equal intervals in the blue green sector of the spectrum (444 nm, 474 nm and 730 nm) and two prominent peaks at 685 nm and 730 nm. It is well known that the bands at 685 and 730 nm are due to chlorophyll-A.

Chlorophyll-B shows fluorescence only after the separation and not *in vivo* leaf or the chloroplast. The two bands in the red sector of the spectra are always present in the LIF spectra of green leaves, and there are no fluorescence bands in the blue green sector of the spectrum as shown in figure 3 where there are two distinct bands in red sector but in the blue green sector there are no fluorescence bands. Thus, we find that the triplet bands at 444, 474 ad 503 nm may be considered as characteristic feature of the compound stevia and the bands are due to diterpene glycoside. As described earlier this is the first report of LIF of stevia and we believe that the present work will supplement the works which have already been established during last many decades.

Conclusion

From what has been discussed above it appears appropriate to make some concluding remark which should be generally applicable. In the present work laser induced fluorescence has been used to characterize a perennial branched bushy shrub "stevia". The result of which shows a triplet emission peak in the blue green sector of the spectrum may be used to characterize a green leaf of stevia. It is quite reasonable to believe that LIF technique is a simple and efficient technique for characterizing any specimen in the domain of agriculture. These are instances where complex phenomenon like spatial holes burning is appearing in the quantum physics are applied to non-physics contexts.

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