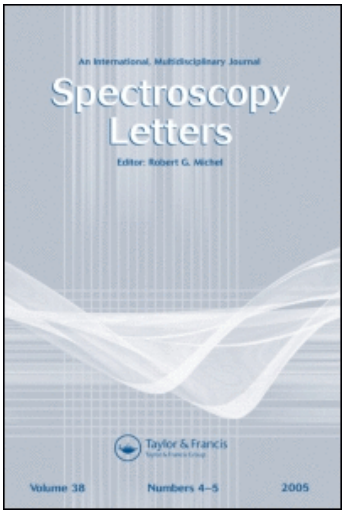

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ABSTRACT Steady-state and time-resolved fluorescence measurements of the seeds of chilli in raw and ripe stages have been carried out. The 440-nm excited fluorescence emission spectrum from raw seeds has given rise to bands in the green and red regions. The same wavelength excitation of ripe seeds has shown the strong green band only—the weaker red band being conspicuous by its absence. Time-resolved spectra of the raw and ripe seeds, excited by a 450-nm femtosecond Ti:sapphire laser pulse have revealed absolutely no difference in their fluorescence decay times.

KEYWORDS raw seed, ripe seed, steady-state fluorescence, time-resolved fluorescence

INTRODUCTION

Chilli is cultivated over large areas in all Asian countries, Africa, South and Central America, parts of the United States, and southern Europe, both under tropical and subtropical conditions. The chilli pepper has been found to be the oldest spice in use in the Americas and one of the oldest in the world.[1] The composition and other properties of chillis can be found in a volume of collected articles by De.[2] Elemental and vibrational band analyses of the seeds of chilli have been performed by Gohain Barua et al.[3] In this work, fluorescence emission spectra of raw and ripe chilli seeds have been recorded. It could be mentioned here that no fluorescence spectroscopic investigation specifically on the seeds of a crop or a fruit has been carried out until now. Also, an attempt has been made here to find out the fluorescence lifetimes in the two stages of the seeds by recording their time-resolved spectra. It has recently been found that under ultraviolet light, a ripe yellow banana fluoresces blue, due to chlorophyll breakdown in the banana peels, which disappears once the banana is too ripe.[4]

MATERIALS AND METHODS

Chiles cultivated inside the campus of Gauhati University were collected for the experiment. A photograph of the species was shown elsewhere.[3] The raw chiles were (mostly) light green and (a few) dark green, and the fully ripe ones were dark red in color. Their skins were removed by clean hands, and the seeds were washed with distilled water. After drying, these

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were crushed to powder in agate mortars. Two days were spent in the process, because after the first crush the seeds have to be dried to remove the natural stickiness. Only after that could a fine powder be made from the seeds. Utmost precaution was taken to avoid any contamination in this process. Three different lots, for raw and ripe ones each, were prepared for recording of fluorescence.

A commercial spectrofluorometer (SPEX, Fluorolog II, DSS Imagetech Pvt. Ltd., New Delhi, India) was used to record static fluorescence from the powdered chilli seeds. The powdered samples were kept in a quartz cuvette with path length of 10 mm. The excitation light, from a 450-W xenon lamp, was focused on the sample surface to a spot size of approximately 1 mm. Fluorescence was collected at a 20° angle (front face geometry) with respect to the excitation light. Band-pass for both the excitation and emission monochromator was 1.7 nm. The offset was set at 30 nm. The integration time was 0.2 s and the scan step was 1 nm. All spectra were corrected for the system response with the correction curve provided for the instrument.

For recording time-resolved spectra, the second harmonic wavelength of the 900-nm output of a Ti:sapphire laser (Coherent Mira, 76 MHz repetition rate, Laser Science Services (I) Pvt. Ltd., Mumbai, India) having $sech^2$ pulses of 100 fs duration was used. $Sech^2$–shaped pulses are pulses with a temporal intensity profile having the shape of a $sech^2$ function. A beam expander (5×) was used to completely overfill the back aperture of a 0.32 NA microscope objective, which focused the laser inside the rectangular cuvette. Fluorescence was collected at right angles after it was passed through a band-pass filter (transmission range 450–700 nm, Laser Components, Germany). Time-resolved fluorescence was recorded in a time-correlated single-photon counting (TCSPC) system (LifeSpec Red, Edinburgh Instruments, Olching). The emission was collected at magic angle through a monochromator equipped with a holographic grating blazed at 450 nm with 1200 grooves/mm. The emission slits were kept at 0.5 mm to provide a resolution of 4 nm. The detector was a thermoelectric cooled Hamamatsu photomultiplier (H7422, DSS Imagetech Pvt. Ltd., New Delhi, India) tube and the instrument response function (IRF) of the TCSPC system was about 180 ps. The fluorescence decays were deconvoluted from the IRF using the iterative software provided by the manufacturer. Although the kinetic traces had some signatures of RF noise, this was not serious in view of the rather significant differences in the profiles. The experiments, both steady-state and time-resolved, were performed in the months March–July 2008, keeping the room temperature at 25°C.

RESULTS AND DISCUSSION

Excitation scans, performed after carrying out synchronous scans from 300 to 700 nm, showed peaks for both the samples at 397 and 440 nm. For the raw seeds, the 397-nm excitation reveals fluorescence emission peaks around 502 and 676 nm, and the 440 nm excitation shows the same around 505 and 662 nm. The bands excited by 397- and 440-nm wavelengths appear approximately at the same positions in the spectra of ripe seeds. Emission peaks for the three lots of raw and ripe seeds differ from one another by a maximum of 15 nm. The three sets of fluorescence emission of the raw seed excited by the 440-nm light are shown in Fig. 1a. The broad band around 505 nm is primarily due to riboflavin; for riboflavin $\lambda_{em} = 520$ nm for $\lambda_{ex} = 360$–460 nm.[5] The red band is missing in the case of the ripe sample for both 397- and 440-nm excitations. Figure 1b shows the 440-nm excited fluorescence spectra from the three lots of ripe seeds of chilli. The peak around 510 nm, again, could be attributed to riboflavin. The weaker band at 662 nm in the spectrum of raw seeds could be due to a chlorophyll-like compound, as attributed to the similar type of band around the same wavelength region in the fluorescence emission spectra of Swiss cheese–like samples exposed to light of different colors.[6] The involvement of phycobiliproteins could be ruled out, because even though they emit around the same wavelength region for exciting wavelengths from 470 to 650 nm, these are intensely fluorescent proteins.[7] It is worthwhile to mention here that protochlorophyll has been found in roots of dark-grown plants of seven species and one pea species, displaying low temperature *in vivo* fluorescence emission maxima at 633 and 642 nm when the wavelengths of the excitation light were 440 and 460 nm, respectively.[8]

The time-resolved spectrum of the raw seeds excited by the 450-nm second harmonic Ti:sapphire

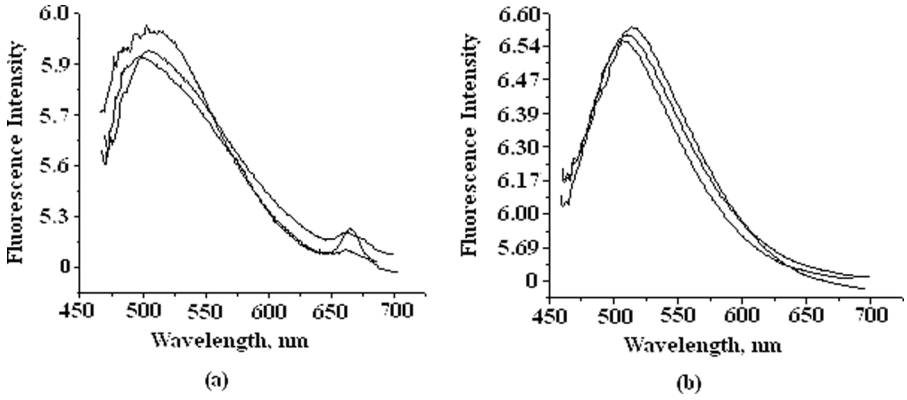

FIGURE 1 **440-nm Excited fluorescence emission spectra of three lots of chilli seeds. (a) Raw seeds display fluorescence peaks around 505 nm and 662 nm. (b) Ripe seeds display a strong fluorescence band around 510 nm.**

laser pulse of duration 100 fs is shown in Fig. 2. The same for the ripe seeds is a replica of the raw ones, showing identical decay time. Using the curve-fitting technique, the decay time is determined as 952 ± 27 ps from

$$\tau_{\text{mean}} = \tau_1 A_1 + \tau_2 A_2 + \tau_3 A_3$$

($\tau_1 = 350 \pm 27$, $\tau_2 = 1670 \pm 2$, $\tau_3 = 599 \pm 10$, $A_1 = 0.32$, $A_2 = 0.41$, $A_3 = 0.26$). The fit is

$$A + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) + B_3 \exp(-t/\tau_3),$$

where $A = 8.9$, $B_1 = 0.003 \pm 0.00005$, $B_2 = 0.057 \pm 0.0005$, $B_3 = 0.013 \pm 0.0001$.

The disappearance of the weak red band in the spectrum of ripe seeds raises curiosity. Because the seed is at its most complex in the ripe stage,[9] we could attribute the probable reason for the disappearance of the red band in the fluorescence emission spectrum of the ripe seeds to the fluorophore being used up in the process of growth or formation of a ground state complex.[7] The fluorescence intensity ratio F670/F510 could be taken as an indicator of ripening. This disappearance, however, causes no change in their decay times, supporting the conclusion drawn on the basis of the well-known Stern-Volmer equation that static quenching does not decrease the lifetime.[7] Further research is required on the changes taking place in the seeds when a crop or a fruit ripens, which causes the red fluorescence band to disappear. Similar studies on the fluorescence of seeds of other crops or fruits at different stages of growth would help us in drawing a general conclusion.

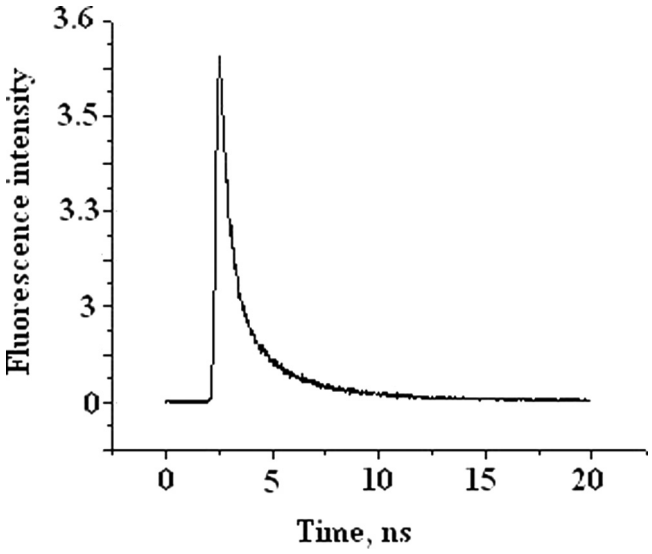

FIGURE 2 **Time-resolved spectra of raw chilli seeds, excited by 450-nm Ti:sapphire laser pulses of duration 100 fs.**

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