A Fluorescence Spectroscopic Study of Honey and Cane Sugar Syrup

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Fluorescence spectroscopic properties of honey and cane sugar syrup were investigated in order to explore the use of optical techniques for detection of adulteration of honey with cane sugar syrup. Measurements showed that while the major contributor to the fluorescence of cane sugar syrup is the reduced form of nicotinamide adenine dinucleotide, the fluorescence of honey is dominated by flavins. The difference in the synchronous luminescence spectra of honey and cane sugar syrup could be used to monitor adulteration of honey by cane sugar syrup.

Keywords: Adulteration, Fluorescence, Synchronous Luminescence

Introduction

Honey has been used as a food and medicinal product since ancient times (Ransome, H.M., 1937). Apart from carbohydrates (fructose, glucose and sucrose) that are its main constituents, honey also has small quantities of vitamins, minerals, proteins and amino acids (White and Doner, 1980). The high sugar concentration, low pH and the presence of flavonoids, hydrogen peroxide, phenolics and terpenes make it a powerful antiseptic and anti-microbial agent (Dunford *et al,* 2000). For unscrupulous economic gain, honey is often adulterated with cheaper sweeteners such as beet sugar, cane sugar or corn syrup. Conventional methods for detecting adulteration of honey are based on monitoring the ratio of the relative concentrations of the different forms of carbohydrates which are different for honey and the adulterates. Generally, enzymatic and chromatographic methods such as high performance liquid chromatography (HPLC) are used to measure the ratio of these carbohydrates. The other method used for monitoring the authenticity of honey is by measuring the ratio of carbon isotopes using mass spectrometry (Martin *et al,* 1998). The ratio of carbon isotopes ($^{13}C/^{12}C$) is known to be different in honey and several other cheaper sweeteners because of the difference in photosynthesis pathways for sugar in honey (C3 pathway) and other sweeteners (C4 pathway) (Korth and Ralston, 2002). As these methods are tedious and time consuming, there has been interest in exploring the use of optical spectroscopic techniques for easier and faster monitoring of adulteration in honey. Recently, Fourier transform infrared—attenuated total reflectance spectroscopy (FTIR-ATR) (Sivakesava and Irudayaraj, 2000) and Fourier transform Raman spectroscopy (De Oliveira *et al,* 2002) of honey and other sweeteners (cane sugar, corn syrup) have been carried out and subtle differences in the FTIR-ATR spectra of these samples were

observed (Sivakesava and Irudayaraj, 2000). Use of multivariate linear discrimination analysis on the differences in spectral features showed that the method can be used for nondestructive detection of adulteration of honey by these sweeteners with a standard error of prediction ∼4% w/w. Since flavonoids are fluorescent and known to provide honey its characteristic color, use of fluorescence spectroscopy may provide another more convenient spectroscopic approach for monitoring adulteration in honey. Therefore this paper examines the validity of fluorescence spectroscopy for monitoring adulteration of honey by cane sugar syrup, which is most often used to adulterate honey in India and neighboring countries.

Materials and Methods

A commercial spectrofluorometer (SPEX, Fluorolog II) was used to measure fluorescence from samples of honey and cane sugar syrup. Samples of pure honey and cane sugar syrup were kept in a quartz cuvette with path length of 10 mm. The excitation light, from a 450W xenon lamp, was focused on the sample surface to a spot size of 2 mm × 4 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. Integration time was 0.2 sec. and the scan step was 1 nm. All spectra were corrected for the system response with the correction curve provided with the instrument.

Five samples of honey extracted from Apis florea hives located at different places in Indore (Malwa region, Central India) were included in the study. The ten commercial cane sugar samples used for the study were procured from different sources located in Indore. All honey samples used were in liquid form and were pale yellowish in appearance. The honey samples were used in the native form without any further processing. The cane sugar syrup was prepared by adding a suitable amount of water to the cane sugar samples and by subsequently
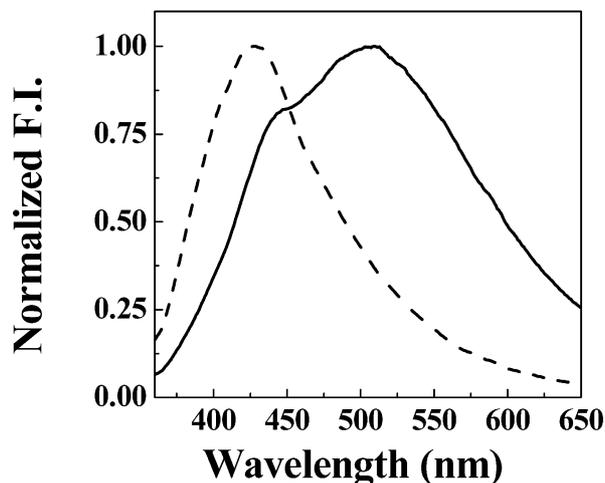
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Fig. 1. 340 nm excited fluorescence spectra recorded from pure honey (solid line) and cane sugar syrup (dashed line).
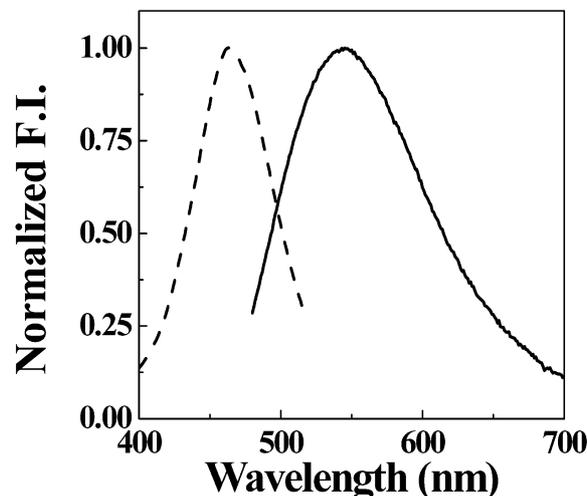

Fig. 2. 460 nm excited fluorescence spectra (solid line) and excitation spectra corresponding to emission wavelength of 540 nm (dotted line) from pure honey.

heating the solution in a water bath (∼50℃) until all the sugar crystals were fully dissolved. The amount of water in the solution was adjusted such that the density of the cane sugar syrup was ∼ 1.42 gm-cm^{-3}, the measured value for the density of the honey samples used. This cane sugar syrup was added to pure honey samples in weight ratios varying from 8.6% to 43.0%, and the mixtures were heated in a water bath to prepare homogeneous cane sugar adulterated honey samples.

Results and Discussion

Figure 1 shows typical 340 nm excited fluorescence spectra from pure honey and cane sugar syrup samples. Both the spectra have been normalized with respect to peak fluorescence intensity. All spectra from pure honey samples were characterized by two prominent features, a shoulder around 440 nm and a broad band around 510 nm. In contrast, a single prominent band around 430 nm characterized the spectra from cane sugar syrup. The excitation spectra corresponding to 440 nm emission from pure honey and 430 nm emission from cane sugar syrup showed a single prominent band around 340 nm (not shown here) that is characteristic of NADH absorption peak (Richards, Kortum and Sevick-Muraca, 1996). This suggests that these fluorescence bands in the spectra of cane sugar and honey samples are due to NADH. The broad band around 510 nm seen in fluorescence spectra of pure honey samples is primarily due to flavins. This follows because the emission spectra of pure honey samples with 460 nm excitation (shown as solid line in Figure 2) as well as excitation spectra corresponding to 540 nm emission (shown as dashed line in Figure 2) are quite similar to the corresponding emission and excitation spectra of flavins (Richards Kortum and Sevick-Muraca, 1996). In contrast, with 460 nm excitation the cane sugar syrup showed no appreciable fluorescence.

The excitation and emission spectra of honey and cane sugar samples show that a significant difference exists in the fractional contribution of NADH and flavins to the

fluorescence from honey and cane sugar syrup. While the major contributor to the fluorescence of cane sugar syrup is NADH, the fluorescence of honey is dominated by flavins. The ratio of the fluorescence intensities of the two bands can therefore be exploited for monitoring cane sugar adulteration of honey. This can be facilitated by recording synchronous luminescence (SL) spectra where the fluorescence signal is recorded by simultaneously scanning both the excitation and emission monochromators at the same speed with a fixed wavelength interval ($\delta\lambda$) between the excitation and emission wavelengths (Vo-Dinh, 1981). Since SL spectra take advantage of the absorption as well as the emission characteristics of a given compound, sharper spectral features representing different components of a multi component system can be observed. In order to find the wavelength interval ($\delta\lambda$) between the excitation and emission wavelengths, which would result in distinct peaks corresponding to NADH, and flavins, spectra with different values of $\delta\lambda$ ($\delta\lambda = 10$ nm to 60 nm in steps of 10 nm) were recorded from pure honey and cane sugar syrup. The peak normalized SL spectra from honey and cane sugar syrup with three different offset values ($\delta\lambda = 20$ nm, 40 nm and 60 nm) are shown in Figure 3. Clear differences in synchronous fluorescence spectra of honey and cane sugar syrup can be seen at all the $\delta\lambda$ values investigated. However, there is an optimum value for $\delta\lambda$. As the value of $\delta\lambda$ is made substantially smaller than the ∼100 nm shift in the respective excitation and emission peaks of the two major bands of the samples, the synchronous luminescence signal decreases, and for larger $\delta\lambda$ the overlap between the spectra of honey and cane sugar increases. At a $\delta\lambda$ value of 40 nm , while the spectra for cane sugar syrup were characterized by a shoulder around 305 nm and a prominent band around 365 nm, the spectra from honey samples had a strong peak around 460 nm and a much weaker peak at 365 nm. SL spectra recorded from authentic NADH and flavins with the same $\delta\lambda$ ($\delta\lambda = 40$ nm)
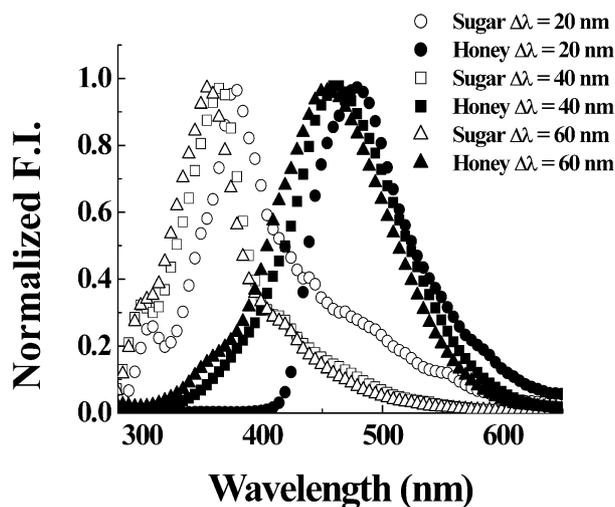
Fig. 3. The synchronous luminescence (SL) spectra with different offset values ($\delta\lambda = 20$ nm, 40 nm and 60 nm) between excitation and emission monochromators recorded from pure honey and cane sugar syrup. Fluorescence spectra have been normalized with respect to maximum fluorescence intensity for individual samples.
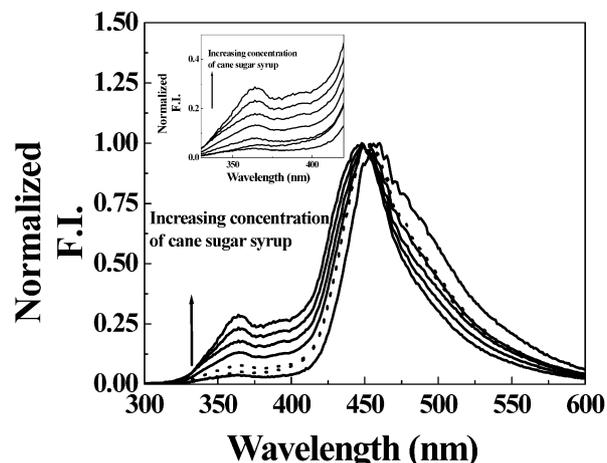

Fig. 4. The synchronous luminescence spectra ($\delta\lambda = 40$ nm) recorded from different mixtures of honey and cane sugar syrup. The arrow shows direction of increasing concentration of cane sugar syrup. The concentrations of cane sugar syrup shown here are 8.6%, 16%, 22%, 32%, 36%, 40% and 43% by weight. SL spectra have been normalized with respect to the peak fluorescence intensity for individual samples.
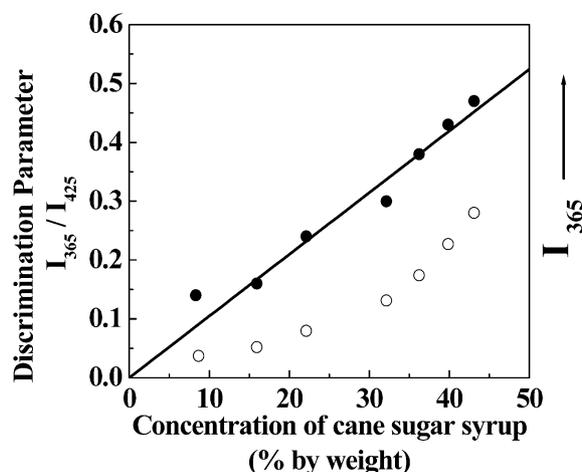

Fig. 5. The variation of intensity at 365 nm (I_{365}) of peak normalized SL spectra (open circles) and the ratio of intensity at 365 nm to that of 425 nm (I_{365}/I_{425}) (solid circles) derived from the peak normalized SL spectra as a function of percentage by weight of cane sugar syrup in honey.

confirmed that the peaks observed around 365 nm and 460 nm were primarily due to NADH and flavins respectively. The hump around 305 nm seen in the SL spectra of cane sugar syrup possibly arises due to fluorescence contributions from amino acids present in the sample (Majumder and Gupta, 2000). In Figure 4, we show SL spectra ($\delta\lambda = 40$ nm) recorded from mixtures of honey and cane sugar syrup where the concentration of cane sugar was varied from 8.6% to 43.0% by weight. It is apparent from the figure that, with increasing concentrations of cane sugar syrup in the mixture, the synchronous fluorescence peak at 365 nm increases. The synchronous fluorescence peak at 460 nm shows a slight blue shift with increasing concentration of cane sugar syrup in the mixture. This results in a decrease of intensity at 460 nm with increasing concentrations of cane sugar syrup. Since the value for the intensity at 365 nm increases with increasing concentrations of cane sugar syrup, the ratio of intensity at 365 nm to that of 460 nm (I_{365}/I_{460}) was found to increase non-linearly with increasing concentration of adulterant. In contrast, the intensity at 425 nm increases with increasing concentrations of cane sugar syrup and therefore the ratio (I_{365}/I_{425}) is expected to be more linear with respect to the concentration of cane sugar syrup. Figure 5 shows the variation of intensity at 365 nm (I_{365}) of the peak normalized SL spectra (open symbols) and the ratio of intensity at 365 nm to that of 425 nm (I_{365}/I_{425}) (solid symbols) derived from the peak normalized SL spectra. As expected, although the variation of I_{365} with increasing cane sugar syrup concentration is nonlinear, the ratio of intensity at 365 nm to that of 425 nm (I_{365}/I_{425}) is found to increase linearly with increasing concentration of cane sugar syrup beyond a concentration of 15%. This linearity of the ratiometric parameter (I_{365}/I_{425}) suggests that this can be used to quantitatively detect cane sugar

syrup adulteration of honey to as low as 15% by weight. It should be mentioned here that the 340 nm excited fluorescence emission spectra recorded from mixtures of honey and cane sugar syrup also showed increasing contribution of the 440 nm fluorescence band as a signature of increased concentration of cane sugar syrup in the mixture. However, since the difference in fluorescence spectral features between honey and cane sugar syrup was much enhanced in the SL spectra as compared to the 340 nm excited fluorescence spectra, use of SL spectra resulted in a more sensitive detection of cane sugar syrup concentration in its mixture with honey.

It is important to note that although some variation in

concentration and nature of flavonoids is to be expected in honey samples depending upon their floral origin, since the origin of fluorescence from cane sugar and honey is different, it should not affect the applicability of the method for monitoring cane sugar adulteration in honey. It may only affect the choice of the best ratiometric parameter for monitoring the adulteration of honey.

Conclusions

Fluorescence spectroscopic study of pure honey and cane sugar syrup revealed significant spectral differences between honey and cane sugar syrup. While the major contributor to the fluorescence of cane sugar syrup is NADH, the fluorescence of honey is dominated by flavonoids. The ratio of the intensity of synchronous luminescence spectra at 365 nm to that of 425 nm could be used to monitor adulteration of honey with cane sugar syrup.

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