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ORIGINAL RESEARCH ARTICLE

3D excitation-emission matrix fluorescence spectroscopy of Biluochun before and after Tomb-Sweeping Day

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ABSTRACT

Tea is an important public drink and its value is in accordance with the time of picking, for example, tea picked before Tomb-Sweeping Day is more expensive than that after Tomb-Sweeping Day. To avoid buying shoddy tea, it is necessary to identify the picking time of the tea. Considering the fact that the chemical components are related to the picking time, in this work, fluorescence spectroscopy is proposed to find the difference between these two kinds of Biluochun. The intensity of the excitation and emission wavelengths for the Biluochun was measured at the same to obtain 3D fluorescence spectra. The contour maps of Biluochun picked before and after Tomb-Sweeping Day were drawn and compared. The results shown that fluorescent intensity at the region from 660 nm to 680 nm, which is corresponding to chlorophyll, is obviously different for both kinds. The different fluorescent intensity demonstrated that the chlorophyll contained in Biluochun after Tomb-Sweeping Day was higher than that before Tomb-Sweeping Day, which can be used to classify these two kinds.

***Keywords*:** fluorescence spectroscopy; 3D fluorescence spectrum; Biluochun; chlorophyll

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1. Introduction

Tea is not only a public drink but also an important gift in China. Tea picked before Tomb-Sweeping Day is regarded as a valuable gift which induces higher price than tea after Tomb-Sweeping Day. To permit high profit, tea after Tomb-Sweeping Day is sold as tea before Tomb-Sweeping Day by some illegal businessmen. With the increasing complaint about the fake tea, it is necessary to develop a technology to classify tea before and after Tomb-Sweeping Day to standardize the market.

Considering the relationship between the picking time and the chemical components contained in tea, fluorescence spectroscopy is a suitable technology to realize the classification because of the advantages of non-contact, non-invasive and high speed[1–3]. 3D fluorescence spectral analysis is one of the key technologies of fluorescent analysis technology[4–6]. The intensity of the excitation and emission wavelengths (Emission-excitation matrix, EEM) for 3D fluorescence spectra can provide more fingerprint information which means that it can provide taxonomic differentiation on a finer scale[7].

Thus, 3D fluorescence spectral analysis has been widely used in chemical analysis field. For example, Soltzberg et al. reported the patterns for 65 dyes produced 3D fluorescence spectroscopy and realize the identification of dyes. The use of 3D fluorescence spectra can avoid the unambiguously distinguish dyes with similar hues with UV-visible absorption spectroscopy and the failure of distinguish isobaric dyes with mass spectroscopy[8]. Kamalraj et al. successfully determined the size and band gaps of the nanocluster in liquid form by combining 3D fluorescence spectra with Brus model. This new method avoided the requirement of polycrystalline aggregation[9]. Wu et al. proposed using 3D fluorescence spectroscopy combined with convolutional neural network to identify the counterfeit sesame oil. The good linear relationship between the predicted and actual values confirmed the validity of the method[10].

Herein, 3D fluorescence spectral analysis technology was proposed to identify Biluochun picked before Tomb-Sweeping Day. By comparing the contour maps of the fluorescent spectra of both Biluochun, we found that the fluorescent intensity value and number of contours for Biluochun picked before Tomb-Sweeping Day was lower which means the chlorophyll concentration was lower.

2. Materials and methods

2.1. Materials

Biluochun was bought from Dongshan tea factory. Biluochun before Tomb-Sweeping Day was picked one week before Tomb-Sweeping Day while Biluochun after Tomb-Sweeping Day was picked one week after Tomb-Sweeping. Each sample consisted of 2.7 g Biluochun and 100 mL water. When the tea sample cool down to the room temperature, 0.8 mL Biluochun was added into a 1.00 cm quartz cell and collected the fluorescence spectra with LS55.

2.2. Methods

LS 55 (PerkinElmer) was used to measure the fluorescence spectra of Biluochun. The light source is an ozone-free pulsed xenon lamp with wavelength range of 200–800 nm[11,12]. The excitation wavelength was scanned from 400 nm to 695 nm with a step of 5 nm. The emission wavelength was detected by a photomultiplier from 400 nm to 700 nm with a step of 0.5 nm. The width of the slit and scanning speed was set to 5 and 500 nm/min, respectively.

3. Results and discussion

Figure 1 was the average fluorescence spectra of Biluochun. The figure indicated that 676 nm, which is corresponding to chlorophyll, is the fluorescence characteristic peak of Biluochun when the excitation wavelength is 500 nm. The difference between Biluochun picked before Tomb-Sweeping Day and picked after Tomb-Sweeping Day seems to be the fluorescent intensity at 676 nm which relates to the concentration of chlorophyll.

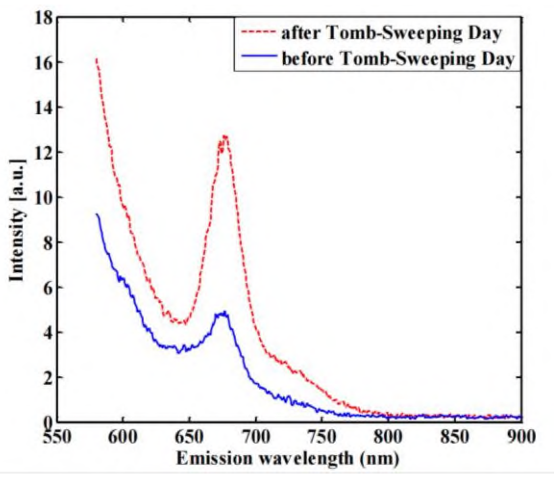

Figure 1. The fluorescence spectra of tea with excitation wavelength of 500 nm.

To demonstrate the difference, the EMM data was collected. The 3D fluorescence spectra were shown in **Figure 2**. The results shown in **Figure 2** also indicated that the fluorescent intensity at 676 nm for Biluochun after Tomb-Sweeping Day was obviously higher than that before Tomb-Sweeping Day. The difference of both Biluochun can be explained by the relationship between the growth of plants and temperature. The low temperature before Tomb-Sweeping Day inhibits enzyme activity which decrease the synthesis of chlorophyll. With the coming of Tomb-Sweeping Day, the temperature warm up slowly and the synthesis of chlorophyll will increase.

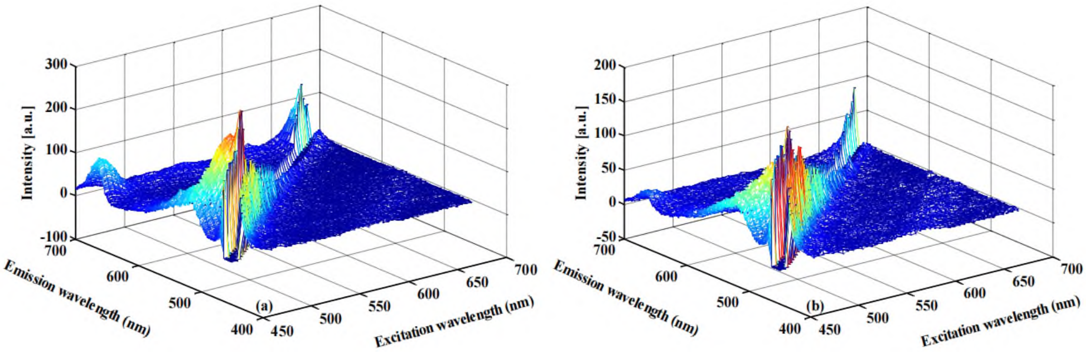

Figure 2. The 3D fluorescence spectra of Biluochun. **(a)** Biluochun picked after Tomb-Sweeping Day, **(b)** Biluochun picked before Tomb-Sweeping Day.

Figure 3 was the contour maps of the fluorescent spectra of Biluochun. The comparison between **Figure 3a** and **Figure 3b** shown that there was fluorescent package at the region from 660 nm to 680 nm for both Biluochun. However, the fluorescent intensity for Biluochun picked after Tomb-Sweeping Day has another contour whose fluorescent intensity is higher. It seems that when the excitation wavelength is larger than 480 nm, the fluorescent intensity corresponding to chlorophyll disappears in **Figure 3**. The reason for this phenomenon is the fluorescent package was overlapped with the scattering light and it was removed together with the scattering light.

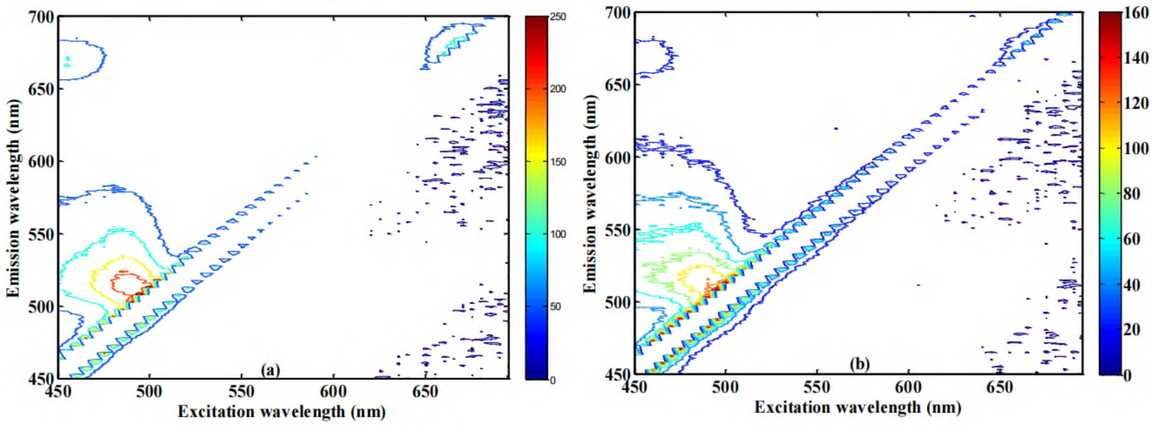

Figure 3. The contour maps of the fluorescent spectra of Biluochun. **(a)** Biluochun picked after Tomb-Sweeping Day, **(b)** Biluochun picked before Tomb-Sweeping Day.

4. Conclusion

In this work, Biluochun before and after Tomb-Sweeping Day was measured by fluorescence spectroscopy and EMM data was collected. By comparing the fluorescence spectra and the contour maps, we found that the fluorescent intensity of Biluochun picked after Tomb-Sweeping Day is higher than that picked before Tomb-Sweeping Day at the region from 660 nm to 680 nm. They can be obvious identified with contour maps according to the number and value of the contour. This difference is in accordance with the relationship between the growth of plants and temperature. The findings in this work can provide a technology to identify whether the Biluochun is picked before Tomb-Sweeping Day.

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Author contributions

Conceptualization, HY and HB; methodology, HY; software, HY; validation, RJ; formal analysis, HY; investigation, HY; resources, HB; data curation, HB; writing—original draft preparation, HY; writing—review and editing, HY; visualization, RJ; supervision, HB; project administration, HB; funding acquisition, HB. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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