

ORIGINAL RESEARCH ARTICLE

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

Hua Yao¹, Rendong Ji^{1,2}, Haiyi Bian^{1,2,*}

¹ Huaiyin Institute of Technology, Huai'an 223003, Jiangsu, China

² Faculty of Electronic Information Engineering, Huaiyin Institute of Technology, Huai'an 223003, Jiangsu, China

* Corresponding author: Haiyi Bian, bianhaiyi@163.com

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

ARTICLE INFO

Received: 6 July 2023

Accepted: 19 July 2023

Available online: 24 October 2023

COPYRIGHT

Copyright © 2023 by author(s).

Journal of Autonomous Intelligence is published by Frontier Scientific Publishing.

This work is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

<https://creativecommons.org/licenses/by-nc/4.0/>

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.

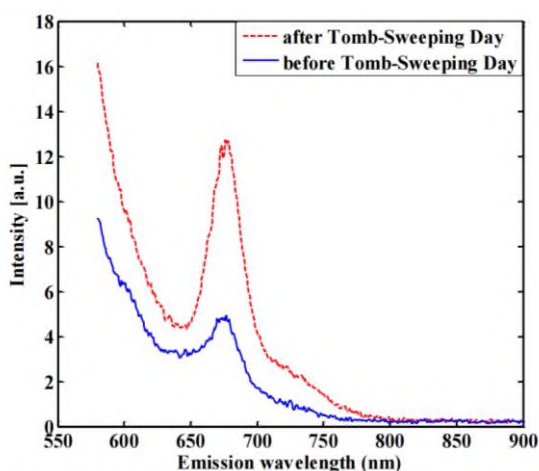


Figure 1. The fluorescence spectra of tea with excitation wavelength of 500 nm.

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.

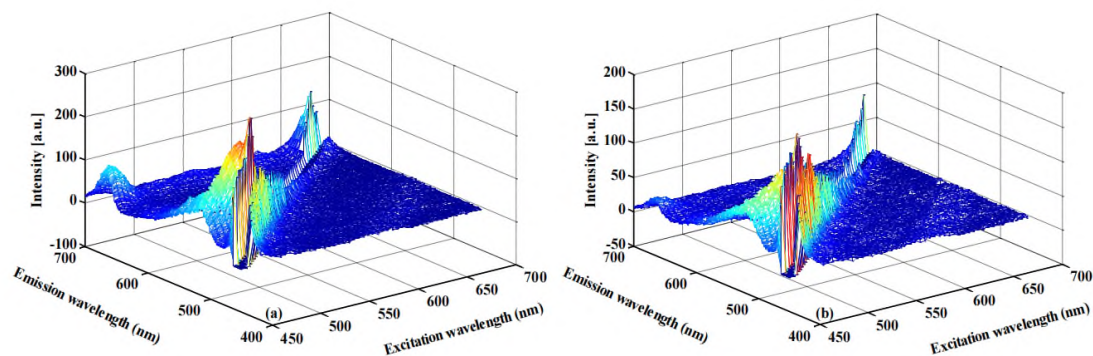


Figure 2. The 3D fluorescence spectra of Biluochun. (a) Biluochun picked after Tomb-Sweeping Day, (b) Biluochun picked before Tomb-Sweeping Day.

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.

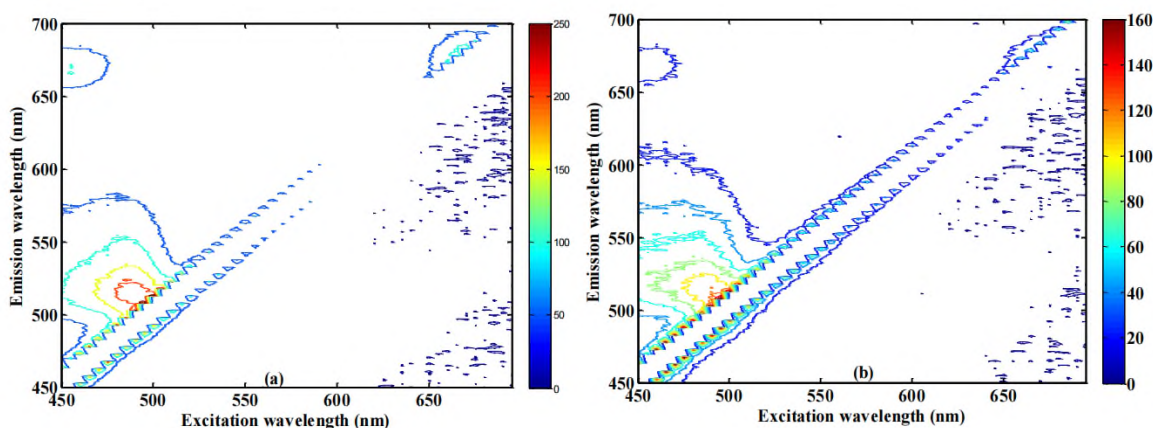


Figure 3. The contour maps of the fluorescent spectra of Biluochun. (a) Biluochun picked after Tomb-Sweeping Day, (b) Biluochun picked before Tomb-Sweeping Day.

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.

Acknowledgments

We would like to thank the Jiangsu Postdoctoral Foundation (Grant No. 1701045B); Natural Science Research of Jiangsu Higher Education Institutions of China (19KJD140001).

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.

References

1. Loudiyi M, Karoui R, Rutledge DN, et al. Fluorescence spectroscopy coupled with independent components analysis to monitor molecular changes during heating and cooling of Cantal-type cheeses with different NaCl and KCl contents. *Journal of the Science of Food and Agriculture* 2018; 98(3): 963–975. doi: 10.1002/jsfa.8544
2. Ji R, Ma S, Yao H, et al. Multiple kinds of pesticide residue detection using fluorescence spectroscopy combined with partial least-squares models. *Applied Optics* 2020; 59(6): 1524–1528. doi: 10.1364/AO.382311
3. Ozbekova Z, Kulmyrzaev A. Study of moisture content and water activity of rice using fluorescence spectroscopy and multivariate analysis. *Spectrochimica Acta Part A* 2019; 223: 117357. doi: 10.1016/j.saa.2019.117357
4. Nakaya Y, Nakashima S, Moriizumi M, et al. Three dimensional excitation-emission matrix fluorescence spectroscopy of typical Japanese soil powders. *Spectrochimica Acta Part A* 2020; 233: 118188. doi: 10.1016/j.saa.2020.118188
5. Yuan YY, Wang ST, Cheng Q, et al. Simultaneous determination of carbendazim and chlorothalonil pesticide residues in peanut oil using excitation-emission matrix fluorescence coupled with three-way calibration method. *Spectrochimica Acta Part A* 2019; 220: 117088. doi: 10.1016/j.saa.2019.04.080
6. Ju L, Lyu A, Hao H, et al. Deep learning-assisted three-dimensional fluorescence difference spectroscopy for identification and semiquantification of illicit drugs in biofluids. *Analytical Chemistry* 2019; 91(15): 9343–9347. doi: 10.1021/acs.analchem.9b01315
7. He Q, Zhang Z, Yi C. 3D fluorescence spectral data interpolation by using IDW. *Spectrochimica Acta Part A* 2008; 71(3): 743–745. doi: 10.1016/j.saa.2007.11.041
8. Soltzberg LJ, Lor S, Okey-Igwe N, Newman R. 3D fluorescence characterization of synthetic organic dyes. *American Journal of Analytical Chemistry* 2012; 3(9): 622. doi: 10.4236/ajac.2012.39081
9. Kamalraj D, Yuvaraj S, Yoganand CP, Jaffer SS. 3D contour fluorescence spectroscopy with Brus model: Determination of size and band gap of double stranded DNA templated silver nanoclusters. *Superlattices and Microstructures* 2018; 113: 283–290. doi: 10.1016/j.spmi.2017.11.008
10. Wu X, Zhao Z, Tian R, et al. Identification and quantification of counterfeit sesame oil by 3D fluorescence spectroscopy and convolutional neural network. *Food Chemistry* 2020; 311: 125882. doi: 10.1016/j.foodchem.2019.125882
11. Lin G, Ji R, Yao H, et al. Fluorescence detection of multiple kinds of pesticides with multi hidden layers neural network algorithm. *Optik* 2020; 211: 164632. doi: 10.1016/j.ijleo.2020.164632
12. Bian H, Yao H, Lin G, et al. Multiple kinds of pesticides detection based on back-propagation neural network analysis of fluorescence spectra. *IEEE Photonics Journal* 2020; 12(2): 1–9. doi: 10.1109/JPHOT.2020.2973653
13. Więckowski S. The influence of temperature and light intensity on the leaf growth and chlorophyll synthesis. *Acta Societatis Botanicorum Poloniae* 1963; 32(4): 719–730. doi: 10.5586/asbp.1963.043
14. Li Y, He N, Hou J, et al. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Frontiers In Ecology And Evolution* 2018; 6: 64. doi: 10.3389/fevo.2018.00064