**Research Article**

**Rapid Determination of Catechin Content in Black Tea by Fluorescence Spectroscopy**

Chenxu Du,1 Chaoqun Ma,1,2 Jiao Gu,1,2 Lei Li,1 Chun Zhu,1,2 Lvming Chen,1 Tingyu Wang,1 and Guoqing Chen1,2

1School of Science, Jiangnan University, Wuxi, China
2Jiangsu Provincial Research Center of Light Industrial Optoelectronic Engineering and Technology, Wuxi, China

Correspondence should be addressed to Guoqing Chen; jncq@jiangnan.edu.cn

Received 15 October 2019; Revised 24 March 2020; Accepted 2 July 2020; Published 21 July 2020

Academic Editor: Alessandra Durazzo

Copyright © 2020 Chenxu Du et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Catechin can effectively prevent the occurrence of cancers due to its strong antioxidant capacity. In this study, the catechin contents of black teas from 12 different regions of south China were investigated using fluorescence spectroscopy. Herein, the catechin contents of various black teas with constant concentration were determined at the optimal excitation and emission wavelength combining the standard addition method and fluorescence spectroscopy. The results indicated that there was a linear relationship between the obtained concentration and fluorescence intensity, where the R² values were all greater than 0.99 and the limit of quantification (LOQ) was 0.02 μg/mL. Furthermore, the content of catechin monomer in the chlorophyll environment was measured under the same experimental conditions to demonstrate the correctness of the above experimental methods. It revealed that the experimental error was about 1.14% compared with the actual content. The current work was proved to be an efficient way to detect fluorescence spectrum through diluting the concentration of tea samples, thereby increasing the determination limit of catechin.

1. Introduction

Tea is one of the most popular beverages worldwide, which originates from China with a history of thousands of years [1]. There is increasing evidence that the tea is rich of flavonoids which offer a host of health benefits. Catechins are one of the most abundant flavonoids found in the tea, and the daily intake per person is about 120 mL [2–4]. Many reports have shown that the catechins in human diet can play an important role to prevent degenerative disease, cardiovascular disease, visceral disease, and some cancers [5–9]. In addition, catechin also has the health care functions of lowering blood fat and blood sugar and scavenging free radicals [10–12]. In addition, catechins have been widely used in medicine, chemistry, environment, and other fields. All the properties of catechin arises from its polyphenolic structure (as shown in Figure 1), which enables the catechin to exhibit a strong antioxidant activity. It is an important index for evaluating the tea quality [13, 14]. Therefore, it is great importance to determine the catechin content in different teas.

To date, the determination of catechin contents in tea was mostly carried out based on the high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) or ultraviolet/infrared/near-infrared spectroscopy, Fourier-transform infrared (FLD) spectroscopy [15–17], capillary electrophoresis techniques, and liquid chromatograph-mass spectrometer (LC-MS) [18–21]. However, HPLC-UV/FLD and GC-MS require complex separation and determination systems, which is inconvenient for rapid determination of catechin in tea. Because of the complex sample matrix and the low concentration levels of the compounds, the catechin analysis of teas generally relies on an extraction step that requires one or more purification or preconcentration procedures [22]. Compared with these methods, fluorescence technology is highly efficient and easy to operate and has become an effective method in the field of analysis.
Fluorescence spectroscopy is a commonly used determination technique. It can provide a relatively large number of physical parameters for qualitative and quantitative analysis. Furthermore, the fluorescence analysis possesses the characteristic advantage of high sensitivity, which is usually 2-3 orders of magnitude higher than that of a spectrophotometer [23–25]. It also allows nondestructive measurements for low concentration substances under various experimental conditions [26–32]. According to the molecular structure analysis of catechin, the fluorescent spectrometry is appropriate for its quantitative analysis [33,34]. However, the proper concentration range should be carefully selected for the quantitative analysis of catechin owing to the fluorescence quenching of catechin at high concentrations.

In this work, the three-dimensional fluorescence spectrum of catechin was measured. The fluorescence peak of catechin was around at 310 nm. However, the intrinsic fluorescence of catechin cannot be found in the spectrum of the original black tea infusion because of concentration quenching effect. The appropriate tea concentration for quantitative analysis was selected according to the linear relationship between its concentration and fluorescence intensity. The catechin qualities of black teas in twelve regions of China were measured by using the standard addition method, and the general rule was further analyzed. The catechin content in black tea infusions obtained by fluorescence analysis turned out to be in accordance with the results by HPLC. Above all, a rapid and sensitive fluorescence method for the determination of catechin content in black tea infusion was proposed in this work. It can help in the tea market supervision and quality evaluation.

2. Materials and Methods

2.1. Chemicals and Materials. Catechin, caffeine, and flavonol were purchased from Beijing Putian Tongchuang Biotechnology Co., Ltd. and chlorophyll was from Shanghai Ika Biotechnology Co., Ltd. The purity of catechin and chlorophyll is 92% or higher. Yunnan Fengpai, Sichuan Chuanhong Gongfu, Fujian Bama, Guizhou Zunyi, Guizhou Jinjunmei, Anhui Qimen Gongfu, Jiangxi Wuyuan, Hubei Jiayiliuchuan, Jiangsu Qianhong, Zhejiang Jiuhongmei, Guangdong Yingde, and Hunan Manxianghong black tea were bought from the market for analysis of catechin content. Ultrapure water was obtained from the Labonova purification system, and all the experiments were carried out with freshly prepared solution.

2.2. Apparatus. All fluorescence studies were performed using the FLS920-type steady-state and time-resolved fluorescence spectrometers. All HPLC tests are performed on the Agilent 1200.

2.3. Preparation of Sample Solution. A certain amount of catechin, caffeine, flavonol, and chlorophyll solids were weighed by a microelectronic balance, and different concentrations of catechin solution, caffeine solution, flavonol solution, and chlorophyll solution were prepared with ultrapure water for the fluorescence spectroscopy study.

2.4. Preparation of Tea Infusion. Quantitative determinations of catechin were carried out using commercially sold tea samples. Typically, 1 g of the tea leaf sample was added into 50 mL of boiling water in a beaker, and the mixture was allowed to stand after stirring for one minute. After cooling to room temperature, the supernatant was taken out and diluted to 0.2 mg/mL with ultrapure water. Then, 11 different concentrations of catechin aqueous solution (0, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 μg/mL) were added into 11 parts of 5 mL tea soaking solution samples, respectively. The fluorescence emission spectra of 11 samples were measured at an excitation wavelength of 280 nm.

2.5. Fluorescence Spectrum Acquisition. The emission spectrum was measured to be 294–400 nm at the excitation wavelength of 280 nm. The excitation and emission slit widths were set as 3 nm. All solutions were prepared in ultrapure water. All experiments were measured in time after configuration and performed in triplicate to analyze the average.

2.6. Standard Addition Method. The standard addition method is a routine chemical analysis method that is used for samples with complex matrices. It contains three main steps:

(1) The tea solution at a concentration of 0.2 mg/mL was divided into 11 portions, and then different concentrations of the catechin solution were added to the tea clear solution. For the prepared samples, the added concentration was graded.

(2) The emission spectrum of the sample to be tested at 280 nm was measured.

(3) The relationship between spectral intensity and elemental concentration is established by a least squares regression curve:

\[
I = kC_x + kC_0 + b_1 + b_2, \quad (1)
\]

\[
B = kC_0 + b_1 + b_2, \quad (2)
\]
where $I$ is the spectral intensity of the emission spectrum at 305–315 nm for area integral fitting and total fluorescence intensity, $C_0$ is the concentration added, $C_b$ is the original concentration of the element to be determined in the sample, $k$ is the slope of the fitted curve, $b_1$ is the noise background of the spectroscopic instrument, $b_2$ is the intensity of the emission spectrum Raman peak, and $B$ is the intercept value obtained by fitting the curve:

$$C_0 = \frac{B - b_2}{k} - \frac{b_1}{k} = \frac{B - b_2}{k} = C_x,$$

(3)

where the value of $C_0$ is more accurate when the value of $b_1/k$ is getting smaller. However, the background noise of the spectrometer is inevitable. If $C_0 \gg b_1/k$, the effect of parameter $b_1$ on the result is greatly reduced. If $C_0$ is not large enough, the spectral instrument background parameter $b_1$ will seriously affect $C_0$.

2.7. High-Performance Liquid Chromatography. Twelve black tea samples of 0.02 g/mL and 100 μg/mL aqueous catechin samples were taken and filtered through a 0.22 μm microporous membrane for HPLC analysis in a liquid phase bottle. HPLC conditions were as follows: injection volume, 5 μL; column, Zorbax SB-C18 (4.6 mm × 150 mm); mobile phase, acetonitrile/water/TFA 10/50/0.05; gradient elution, 50/50/0.05; determination wavelength, 280 nm; flow rate, 0.8 mL/min; column temperature, 30°C.

3. Results and Discussion

3.1. Fluorescence Quenching of Catechin. The three-dimensional fluorescence spectrum was ensured by the main fluorescent peak position of catechin. As shown in Figure 2(a), the main fluorescent peak position of catechin was located at 280/310 nm (excitation emission). In order to determine the presence of catechin in tea, three-dimensional fluorescence spectroscopy (Figure 2(b)) was performed based on black tea supernatant (0.02 g/mL). It can be seen that there was no fluorescence at the position of the catechin fluorescence peak, while catechin in the literature reports were indeed present in tea. Hence, the previous tea supernatant was diluted to 0.2 mg/mL for three-dimensional fluorescence spectroscopy (Figure 2(c)). By comparing the three-dimensional fluorescence spectra of tea supernatant at different concentrations, we found that the fluorescence peak position of catechin in tea supernatant at low concentrations were obvious but were absent at high concentrations. This phenomenon may be due to fluorescence quenching of catechin at high concentrations.

Therefore, 280 nm was selected as the excitation wavelength of the fluorescence emission spectrum to investigate the fluorescence changes of different concentrations of catechin monomers (Figure 3). It can be seen from Figure 3 that the peak of catechin emission barely changed with the concentration of catechin, but the fluorescence intensity of catechin has changed to some extent. Furthermore, fluorescence intensity values at the highest peak at each concentration were extracted and displayed in the inset of Figure 3. The results showed that the fluorescence intensity of catechin increased when the concentration of catechin was less than 70 μg/mL. When the concentration was more than 70 μg/mL, the fluorescence intensity was observed to decrease. Therefore, it is important to use a suitable tea concentration for quantitative determination of catechin.

By comparing the three-dimensional fluorescence spectra of (b) with (c) in Figure 2, the fluorescence intensity of the emission spectrum at 310 nm was significantly increased when the tea supernatant concentration was diluted to 0.2 mg/mL, implying the remarkable fluorescence signal. By comparing the emission spectra at 280 nm, the phenomenon of catechin fluorescence quenching in teas could be determined, and then the tea supernatant with a concentration of 0.2 mg/mL was selected for the next research.

Fluorescence emission spectroscopy was carried out by mixing the configured Guizhou black tea solution with different concentrations of catechin solution in equal volumes. Figure 4 exhibits that the fluorescence emission peaks of the mixed solution of all concentrations at the wavelength of 310 nm. The fluorescence intensity of the mixed solution gradually increased with the increase of catechin concentration. According to the above concentration quenching study, it is known that the concentration of catechin in the mixed solution is within the concentration range of quenching, which can be used as a quantitative analysis of catechin.

3.2. Interference Experiment. After calculations, it was found that there might be a Raman peak of water (310 nm) in an emission spectrum at an excitation wavelength of 280 nm. In order to eliminate the influence of the parameter $b$ on the result $C_0$ as much as possible, the intensity of the Raman peak must be subtracted. Since the most common component in tea is chlorophyll, the emission spectrum of chlorophyll aqueous solution at 280 nm at different concentrations was measured to exclude the influence of chlorophyll on catechin measurement (Figure 5(a)). In the figure, the fluorescence intensity of the emission peak of the chlorophyll aqueous solution at 310 nm was substantially unchanged as the concentration changes. By comparing the fluorescence emission peaks of ultrapure water and chlorophyll aqueous solution with different concentrations as well as calculating the Raman peak position of water, the emission peak at 310 nm was induced by the Raman scattering of water. Finally, the fluorescence intensity is to remove the intensity of the Raman peak to get a more accurate value of $C_0$.

We designed an auxiliary experiment to verify the correctness of the experiment through eliminating the interference effect of chlorophyll on catechin. The quality of catechin in the chlorophyll environment was measured using the principle of the above standard addition method by adding 50 μg of catechin solids into the aqueous solution of chlorophyll. As shown in Figure 5(b), the Pearson coefficient and the coefficient of determination of the fitted straight line in the figure are all greater than 0.99. In the experiment of chlorophyll as an interference condition, the
mass of the calculated catechin was about 49.43 μg, and the error with the actual mass was about 1.14%. The experiment demonstrated that chlorophyll had no effect on the fluorescence determination of catechin in the chlorophyll solution environment. It also excluded the interference of chlorophyll on catechin, which verified the accuracy of the experiment. In addition, we also studied the fluorescence effects of several other major substances in tea on catechin (Figure 6). The concentration of these interfering substances and catechin was 10 μg/mL, because the average amount of these interfering substances (after dilution) in teas ranges from 1 to 100 μg/mL. The cause of the error may be due to the noise influence of the instrument itself. Although the noise is very small for the actual fluorescence intensity, there may be a slight error in each fluorescence spectrum measurement since the noise cannot be reduced to zero. The total error of the proposed method may be attributed to the combined effects of these errors.

3.3. Catechin in Black Teas. The emission spectra of the equal mixture solution mixed by Jiangxi black tea solution and different concentrations of catechin solution (0, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 μg/mL) are measured at the excitation wavelength of 280 nm. After removing the influence of Raman scattering, the integral intensity of the fluorescence spectrum corresponding to different concentrations is linearly fitted (Figure 7). The calculated Pearson coefficient of the fitted line is 0.99, and the coefficient of determination is 0.99, which indicated that there is a good linear relationship of the line. In addition, by comparing the results obtained in this work with other methods in Table 1, it is clear that our work has a lower LOQ. Based on the derivation of formulas (1) and (2), the concentration of catechin in Jiangxi black tea was calculated about 0.3396 μg/mL, and the mass of catechin in 50 mL tea infusion of Jiangxi black tea was about 33.96 mg. The amount of catechin in black teas from different regions was tested in the same experimental method (70.62 mg/kg for Sichuan, 71.88 mg/kg for Yunnan, 67.00 mg/kg for Guizhou, 30.54 mg/kg for Guangxi, 49.84 mg/kg for Guangdong, 44.06 mg/kg for Fujian,
26.98 mg/kg for Zhejiang, 38.56 mg/kg for Hunan, 9.60 mg/kg for Hubei, 54.06 mg/kg for Anhui, and 30.80 mg/kg for Jiangsu).

3.4. High-Performance Liquid Chromatography Data. In order to demonstrate the reliability of the experimental method, the HPLC testing was employed to detect the tea catechin in the tea solution with same concentration. The results indicated that the catechin density deduced from fluorescence measurement are strongly linked to the value obtained by HPLC (Figure 8), which verified the accuracy of this paper. The error range was within the scope of 0.2~7.8%, which might be caused by the other polyphenols in tea or the unavoidable experimental error.

According to the test results of catechin contents (Table 2), the black teas in southwest possessed the higher catechin contents. Thereinto, the catechin content of the typical large leaf teas in Yunnan exhibited the highest amount of catechin, which might be attributed to the high surface area of teas and the high abundant organic compounds in soils. Furthermore, except for the catechin contents of black teas in Guangxi, the catechin contents in other two regions were relatively higher than those in Southern Yangtze, which might be caused by the differences of climates.

| Detection method          | LOQ (ng/mL) | Ref. |
|--------------------------|-------------|------|
| UHPLC-MS/MS              | 5           | [35] |
| HPLC                     | 720         | [36] |
| Micellar electrokinetic  | 500         | [37] |
| Fluorescence             | 20          | This work |

Table 1: Different methods for the determination of caffeine.
4. Conclusions

In this study, a novel sensitive, rapid, and low-cost fluorescence method was developed and applied to determine the catechin content in black teas from different areas. Due to the high concentration quenching of catechin, the fluorescence spectrum determination was carried out using the low-concentration tea clear liquid, so as to raise the determination limit. In addition, the method was verified by the designed experiments and the error was within the acceptable range, implying the practicability and feasibility of the method. And, the catechin content in actual tea samples was successfully detected by this method. Compared with the previously reported methods, the developed method in this work displayed a lower determination limit. This indicated that the enhanced sensitivity of the developed method made it suitable for routine analysis of foods containing catechin.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interests regarding the publication of the paper.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (grant number 2018YFC1604204), the National First-Class Discipline Program of Food Science and Technology (grant number JUFSTR20180302), the National Key Research and Development Program of China (grant number 2018YFC1604204), and the National Natural Science Foundation of China (grant number 31871821).
Development Program of China (grant number 2018YFD0400402), the Fundamental Research Funds for the Central Universities (grant number JUSRP11720), and the Jiangsu Province Post Doctoral Fund (grant number 2019K241).

References
[1] M. Z. Yao and L. Chen, “Tea germplasm and breeding in China,” Advanced Topics in Science and Technology in China, Springer, Berlin, Germany, 2012.
[2] Q. F. Collins, H.-Y. Liu, J. Pi, Z. Liu, M. J. Quon, and W. Cao, “Epigallocatechin-3-gallate (EGCG), A green tea polyphenol, suppresses hepatic gluconeogenesis through 5′-AMP-activated protein kinase,” Journal of Biological Chemistry, vol. 282, no. 41, pp. 30143–30149, 2007.
[3] N. Adnani, S. R. Rajski, and T. S. Bugni, “Symbiosis-inspired approaches to antibiotic discovery,” Natural Product Reports, vol. 34, no. 7, pp. 784–814, 2017.
[4] K. Wojciech, K. Wirginia, K. Łukasz, M. Zbigniew, S. Wojciech, and G. Kazimierz, “Green tea quality evaluation based on its catechins and metals composition in combination with chemometric analysis,” Molecules, vol. 23, no. 7, pp. 1689–1707, 2018.
[5] J. C. Vera, A. M. Reyes, F. V. Velásquez et al., “Direct inhibition of the hexose transporter GLUT1 by tyrosine kinase inhibitors,” Biochemistry, vol. 40, no. 3, pp. 777–790, 2001.
[6] J. P. E. Spencer, M. M. Abd El Mohsen, and C. Rice-Evans, “Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity,” Archives of Biochemistry and Biophysics, vol. 423, no. 1, pp. 148–161, 2004.
[7] Y.-S. Lin, Y.-J. Tsai, J.-S. Tsay, and J.-K. Lin, “Factors affecting the levels of tea polyphenols and caffeine in tea leaves,” Journal of Agricultural and Food Chemistry, vol. 51, no. 7, pp. 1864-1873, 2003.
[8] A. Dey and J. Lakshmanan, “The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver,” Food & Function, vol. 4, no. 8, pp. 1148–1184, 2013.
[9] T. Wollny, L. Aiello, D. D. Tommaso et al., “Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production,” British Journal of Pharmacology, vol. 127, no. 3, pp. 747–755, 2010.
[10] N. Oku, M. Matsukawa, S. Yamakawa et al., “Inhibitory effect of green tea polyphenols on membrane-type 1 matrix metalloproteinase, MT1-MMP,” Biomedical & Pharmaceutical Bulletin, vol. 26, no. 9, pp. 1235–1238, 2003.
[11] Q. V. Vuong, C. E. Stathopoulos, M. H. Nguyen, J. B. Golding, and P. D. Roach, “Isolation of green tea catechins and their utilization in the food industry,” Food Reviews International, vol. 27, no. 3, pp. 227–247, 2011.
[12] S. K. Connors, G. Chornokur, and N. B. Kumar, “New insights into the mechanisms of green tea catechins in the chemo-prevention of prostate cancer,” Nutrition and Cancer, vol. 64, no. 1, pp. 4–22, 2012.
[13] S. Oliver, A. Jofri, D. S. Thomas, O. Vittorio, M. Kavallaris, and C. Boyer, “Tunable catechin functionalisation of carbohydrate polymers,” Carbohydrate Polymers, vol. 169, pp. 480–494, 2017.
[14] X. Li and S. Wang, “Study on the interaction of (+)-catechin with human serum albumin using isothermal titration calorimetry and spectroscopic techniques,” New Journal of Chemistry, vol. 39, no. 1, pp. 386–395, 2015.
[15] P. Svoboda, H. Vlčková, and L. Nováková, “Development and validation of UHPLC-MS/MS method for determination of eight naturally occurring catechin derivatives in various tea samples and the role of matrix effects,” Journal of Pharmaceutical and Biomedical Analysis, vol. 114, pp. 62–70, 2015.
[16] G. A. C. Ribeiro, C. Q. D. Rocha, A. A. Tanaka, and I. S. D. Silva, “A fast, direct, and sensitive analysis method for catechin determination in green tea by batch injection analysis with multiple-pulse amperometry (BIA-MPA),” Analytical Methods, vol. 10, no. 17, pp. 2034–2040, 2018.
[17] D. A. El-Hady and H. M. Alshibi, “Alkyl imidazolium ionic liquid based sweeping-micellar electrokinetic chromatography for simultaneous determination of seven tea catechins in human plasma,” Journal of Chromatography B, vol. 969, pp. 224–229, 2014.
[18] M. Piovezan, D. Garcia-Seco, G. A. Micke, J. Gutiérrez-Mañero, and B. Ramos-Solano, “Method development for determination of (+)-catechin and (−)-epicatechin by micellar electrokinetic chromatography: annual characterization of field grown blackberries,” Electrophoresis, vol. 34, no. 15, pp. 225–2258, 2013.
[19] C. A. Ballus, A. D. Meinhart, R. G. de Oliveira, and H. T. Godoy, “Optimization of capillary zone electrophoresis separation and on-line preconcentration of 16 phenolic compounds from wines produced in South America,” Food Research International, vol. 45, no. 1, pp. 136–144, 2012.
[20] R. G. Peres, F. G. Tonin, M. F. M. Tavares, and D. B. Rodriguez-Amaya, “Determination of catechins in green tea infusions by reduced flow micellar electrokinetic chromatography,” Food Chemistry, vol. 127, no. 2, pp. 651–655, 2011.
[21] K. Wojciech, K. Wirginia, and K. Łukasz, “Black tea samples origin discrimination using analytical investigations of secondary metabolites, antiradical scavenging activity and chemometric approach,” Molecules, vol. 23, no. 3, pp. 513–524, 2018.
[22] A. Fromberg, A. Højgård, and L. Duedahl-Olesen, “Analysis of polycyclic aromatic hydrocarbons in vegetable oils combining gel permeation chromatography with solid-phase extraction clean-up,” Food Additives and Contaminants, vol. 24, no. 7, pp. 758–767, 2007.
[23] G. Brambilla, “Optical fibre nanotaper sensors,” Optical Fiber Technology, vol. 16, no. 6, pp. 331–342, 2010.
[24] S. K. Pandey, K.-H. Kim, and R. C. Brown, “Measurement techniques for mercury species in ambient air,” TrAC Trends in Analytical Chemistry, vol. 30, no. 6, pp. 899–917, 2011.
[25] O. L. Muskins, G. Bachelier, N. D. Fatti et al., “Quantitative absorption spectroscopy of a single gold nanorod,” The Journal of Physical Chemistry C, vol. 112, no. 24, pp. 8917–8921, 2008.
[26] T. K. Maiti, K. S. Ghosh, and S. Dasgupta, “Interaction of (−)-epigallocatechin-3-gallate with human serum albumin: fluorescence, fourier transform infrared, circular dichroism, and docking studies,” Proteins, vol. 64, no. 2, pp. 355–362, 2010.
[27] S. Bi, L. Ding, Y. Tian et al., “Investigation of the interaction between flavonoids and human serum albumin,” Journal of Molecular Structure, vol. 703, no. 1–3, pp. 37–45, 2004.
[28] W. He, Y. Li, C. Xue, Z. Hu, X. Chen, and F. Sheng, “Effect of Chinese medicine alpinetin on the structure of human serum albumin,” Bioorganic & Medicinal Chemistry, vol. 13, no. 5, pp. 1837–1845, 2005.
[29] J.-L. Yuan, Z. Iv, Z.-G. Liu, Z. Hu, and G.-L. Zou, “Study on interaction between apigenin and human serum albumin by
spectroscopy and molecular modeling,” *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 191, no. 2-3, pp. 104–113, 2007.

[30] J. Tian, J. Liu, Z. Hu, and X. Chen, “Interaction of wogonin with bovine serum albumin,” *Bioorganic & Medicinal Chemistry*, vol. 13, no. 12, pp. 4124–4129, 2005.

[31] J. Xiao, M. Suzuki, X. Jiang et al., “Influence of B-ring hydroxylation on interactions of flavonols with bovine serum albumin,” *Journal of Agricultural and Food Chemistry*, vol. 56, no. 7, pp. 2350–2356, 2008.

[32] C. Du, C. Ma, J. Gu, L. Li, and G. Chen, “Fluorescence sensing of caffeine in tea beverages with 3,5-diaminobenzoic acid,” *Sensors*, vol. 20, no. 3, pp. 819–827, 2020.

[33] D. E. Iglesias, S. S. Bombicino, A. Boveris, and L. B. Valdez, “(+)-Catechin inhibits heart mitochondrial complex I and nitric oxide synthase: functional consequences on membrane potential and hydrogen peroxide production,” *Food & Function*, vol. 10, no. 5, pp. 2528–2537, 2019.

[34] L. Trnková, I. Boušová, V. Staňková, and J. Dršata, “Study on the interaction of catechins with human serum albumin using spectroscopic and electrophoretic techniques,” *Journal of Molecular Structure*, vol. 985, no. 2-3, pp. 243–250, 2011.

[35] S. Pavel, V. Hana, and N. Lucie, “Development and validation of UHPLC–MS/MS method for determination of eight naturally occurring catechin derivatives in various tea samples and the role of matrix effects,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 114, pp. 62–70, 2015.

[36] H. Wang, G. J. Provan, and K. Helliwell, “HPLC determination of catechins in tea leaves and tea extracts using relative response factors,” *Food Chemistry*, vol. 81, no. 2, pp. 307–312, 2003.

[37] C.-M. Liu, C.-Y. Chen, and Y.-W. Lin, “Estimation of tea catechin levels using micellar electrokinetic chromatography: a quantitative approach,” *Food Chemistry*, vol. 150, pp. 145–150, 2014.