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Research Article

Rapid Determination of Catechin Content in Black Tea by Fluorescence Spectroscopy

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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 R 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.

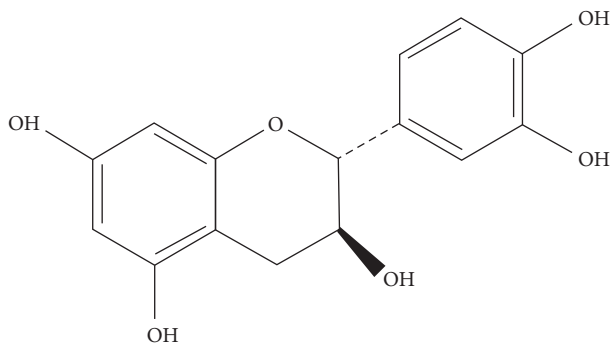

Figure 1: Molecular structure of catechin.

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, Guangxi Jinjunmei, Anhui Qimen Gongfu, Jiangxi Wuyuan, Hubei Jiyelichuan, Jiangsu Qianhong, Zhejiang Jiuquhongmei, 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_x is the concentration added, C_0 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:

$$C_0 = \frac{B - b_2}{k} - \frac{b_1}{k} \approx \frac{B - b_2}{k} \approx C_x, \quad (3)$$

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 10/90/0.05; 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 concentrates 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

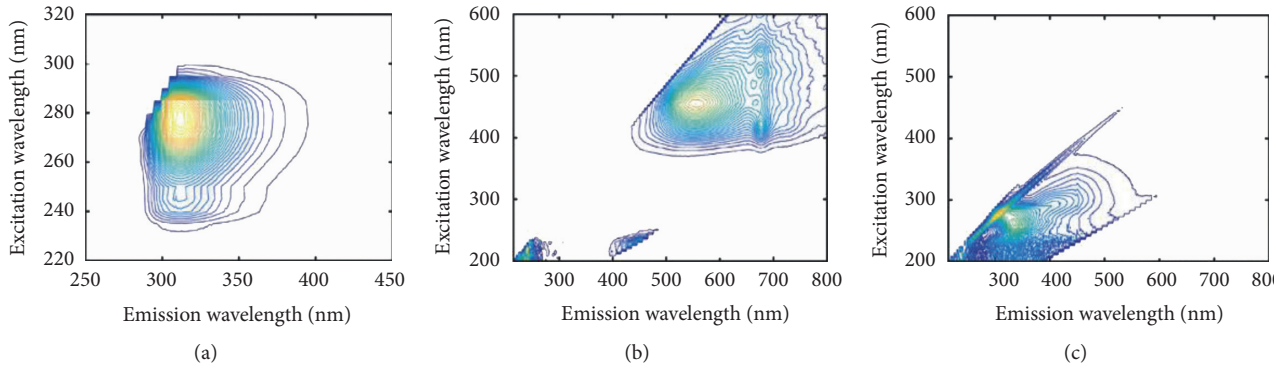

Figure 2: Three-dimensional fluorescence spectra of catechin monomer and different concentrations of tea solution. (a) Three-dimensional fluorescence spectrum of catechin monomer presents one characteristic excitation/emission ($\lambda_{ex} = 280$ nm/$\lambda_{em} = 310$ nm) maximum. (b) 0.02 g/mL and (c) 0.2 mg/mL of black tea clear liquid. Comparing (b) with (c), it was found that the fluorescence peak of catechin was obvious at low concentration.

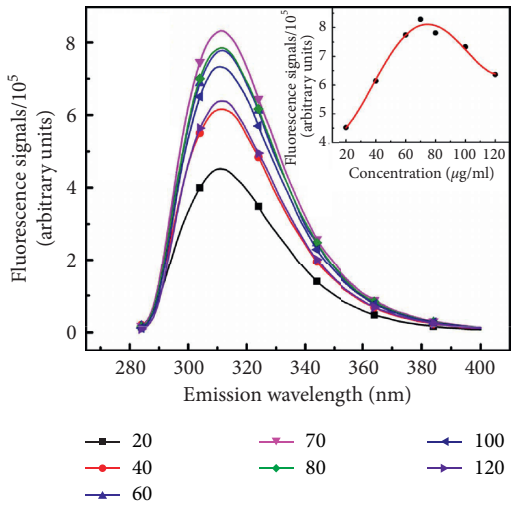

Figure 3: Fluorescence emission spectra of aqueous solution of different concentrations of catechin. The concentration of catechin is from 20 to 120 μg/mL, and the corresponding inset is the fluorescence peak of catechin aqueous solution as a function of concentration.

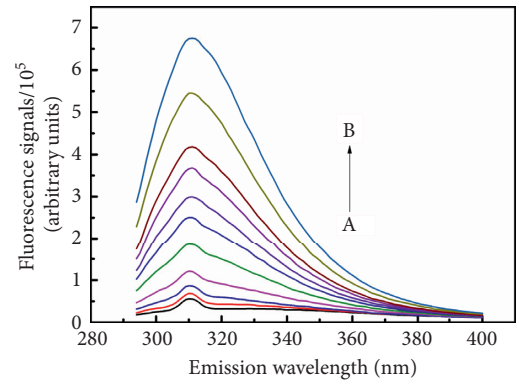

Figure 4: Fluorescence emission of black tea solution (tea = 0.2 mg/mL) with an increase in catechin concentration. Catechin varied from 0 μg/mL to 10 μg/mL. A to B indicates increased concentrations of catechin. The fluorescence intensity of the mixed solution gradually increased with the increase of catechin concentration.

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,

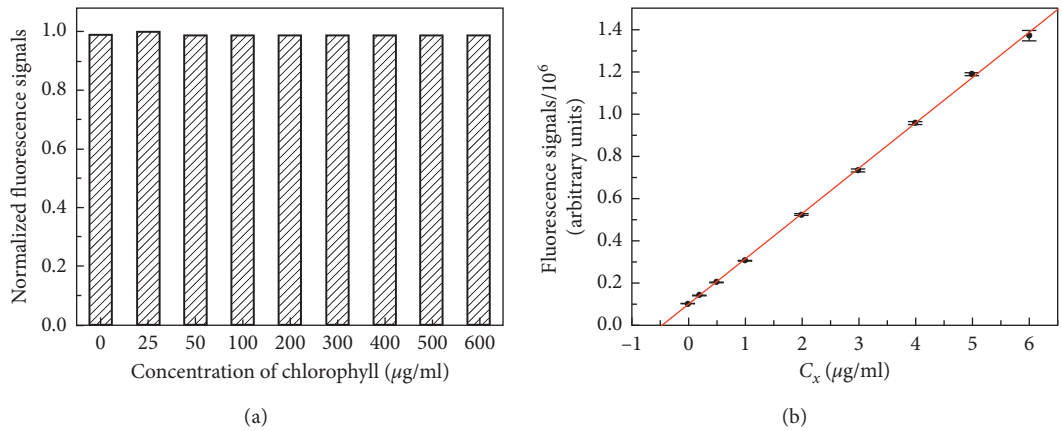

Figure 5: Fluorescence experiment of catechin in chlorophyll environment. (a) Comparison of emission peaks of chlorophyll aqueous solution at different concentrations. (b) Chlorophyll fluorescence area fitting, with increasing catechin concentration. The Pearson coefficient and the coefficient of determination of the fitted straight line in the figure are all greater than 0.99. All experimental data are averaged after three experiments.

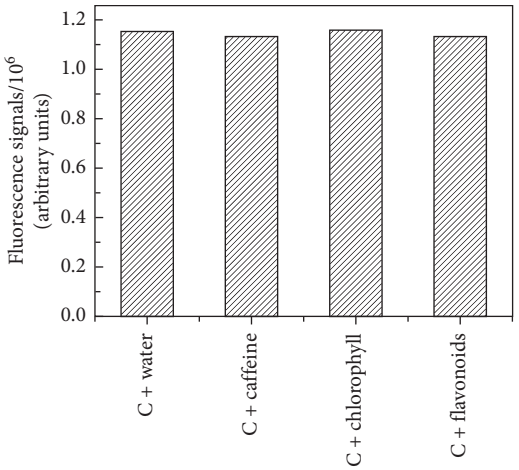

Figure 6: Fluorescence response of catechin (C) mixed with other interfering substances in tea.

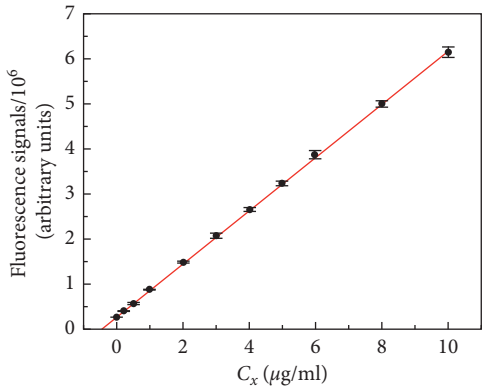

Figure 7: The concentration of catechin in Jiangxi black tea infusion. After removing the Raman effect, the fluorescence area fit image of the tea clear liquid increases with the concentration of catechin. The Pearson coefficient and the coefficient of determination of the fitted straight line in the figure are all greater than 0.999. All experimental data are averaged after three experiments.

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.

Table 1: Different methods for the determination of caffeine.

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

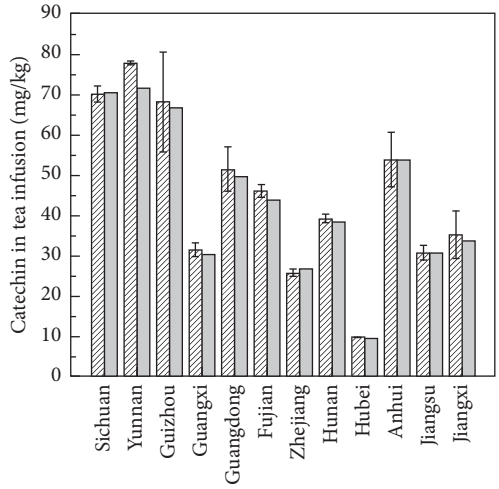

Figure 8: Catechin content of 12 tea samples as determined by high-performance liquid chromatography (shadow) or by fluorescence spectroscopy (gray). The results indicated that the catechin density deduced from fluorescence measurement are strongly linked to the value obtained by high-performance liquid chromatography.

Table 2: Catechin contents in tea infusion from different regions by fluorescence method and HPLC.

Name	Province	Tea region	Catechin (mg/kg)	
			Fluorescence	HPLC
Gongfu	Sichuan	Southwest	70.62	70.28 ± 1.96
Fengpai	Yunnan	Southwest	71.88	77.98 ± 0.50
Zunyihong	Guizhou	Southwest	67.00	68.38 ± 12.32
Jinjunmei	Guangxi	South China	30.54	31.60 ± 1.68
Yingde	Guangdong	South China	49.84	51.62 ± 5.49
Bama	Fujian	South China	44.06	46.22 ± 1.49
Jiuquhongmei	Zhejiang	Southern Yangtze	26.98	25.84 ± 0.88
Manxianghong	Hunan	Southern Yangtze	38.56	39.34 ± 1.01
Jiyelichuan	Hubei	Southern Yangtze	9.60	9.87 ± 0.11
Qimen Gongfu	Anhui	Southern Yangtze	54.06	53.96 ± 6.65
Qianhong	Jiangsu	Southern Yangtze	30.80	30.87 ± 1.75
Wuyuan	Jiangxi	Southern Yangtze	33.96	35.38 ± 5.97

Name is the brand of black tea, province is the area of black tea, and tea region is the area where tea leaves come from. Data were calculated based on three-replicate experiments and shown by mean value ± standard deviation.

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

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