Hindawi
Journal of Food Quality
Volume 2023, Article ID 3940081, 12 pages
https://doi.org/10.1155/2023/3940081

Research Article

Rapid Analysis of 18 Flavonoids in Tea by Ultrahigh-Performance Liquid Chromatography Coupled with Quadrupole-Time of Flight Mass Spectrometry

Jian Li,[1,2] **Junmei Ma**,[3] **Qiang Li**,[3] **Liangna He**,[3] **Mingxing Zhang**,[3] **Lei Zheng**,[1] **and Yan Zhang**[3]

[1]*School of Food and Biological Engineering, Hefei University of Technology, Hefei, China*
[2]*College of Applied Arts and Science, Beijing Union University, Beijing, China*
[3]*Hebei Food Safety Key Laboratory, Key Laboratory of Special Food Supervision Technology for State Market Regulation, Hebei Engineering Research Center for Special Food Safety and Health, Hebei Food Inspection and Research Institute, Shijiazhuang, China*

Correspondence should be addressed to Lei Zheng; lei.zheng@aliyun.com and Yan Zhang; snowwinglv@126.com

Received 15 February 2023; Revised 6 April 2023; Accepted 29 June 2023; Published 10 July 2023

Academic Editor: Narashans Alok Sagar

A method was established for the determination of 18 flavonoids in tea by ultra-performance liquid chromatography coupled with quadrupole/time-of-flight mass spectrometry (UPLC-Q-TOF/MS). The tea samples were extracted by 70% (V/V) methanol aqueous solution, and separation was achieved on a Kinetex F5 column (2.1 mm × 100 mm, 2.6 μm) with methanol and 0.1% formic acid in water as the mobile phase with a gradient elution. The samples were detected in TOF/MS and information-dependent acquisition (IDA)-MS/MS modes. The results showed that the relative standard deviations of 18 flavonoids were less than 5.0 ppm. The correlation coefficients (R^2) of the linear equation were greater than 0.998 in the range of 0.10–200 ng/mL. The limits of detection were 0.0010–0.040 ppm, and the limits of quantification were 0.0020–0.10 ppm. The recoveries ranged from 73.8% to 107% at spiked levels of 0.0020–1.0 ppm, with relative standard deviations (RSDs) being less than 10%. The method was simple, specific, and reliable. It could be used for the rapid screening and quantitative analysis of flavonoids in tea.

1. Introduction

Tea is a popular natural plant beverage [1, 2]. Due to the fact that tea contains tea polyphenols, caffeine, amino acids, tea polysaccharides, vitamins, and other nutritional functional components [3, 4], its good antiaging [5], anticancer [6], bactericidal [7], and lowering blood sugar [8] effects have always been valued by researchers. Tea polyphenols in tea mainly include catechins, flavonoids, phenolic acids, anthocyanins, and other substances, and their content accounts for about 20% of the dry weight of tea [9]. Our research team has established a detection method for catechins in tea in the early stage and analyzed the content of catechins in tea [10]. Flavonoids are another important tea polyphenol in tea. According to the different modifications of the central C ring, flavonoids can be divided into flavonols, flavanols, isoflavones, flavanones, and anthocyanins. Nearly one-third of the flavonoids are flavonols [11, 12]. Flavonols have a hydroxyl group in position 3 of the C ring, which is easy to be glycosylated (Figure 1) [13, 14]. The glycosylation makes flavonols commonly present in plants as glycosides and 3% to 4% of the dry matters of tea are flavonol glycosides. Flavonoids have the functions of strengthening the heart, lowering blood pressure, promoting cell proliferation, reducing the accumulation of sugar alcohols, and helping to prevent cataracts. They are also natural food antioxidants [15–19]. The main mechanism of healthcare effect of flavonoids comes from enzyme regulation, improved blood circulation, cell apoptosis regulation, and stem cell signal transduction and reduction [20, 21].

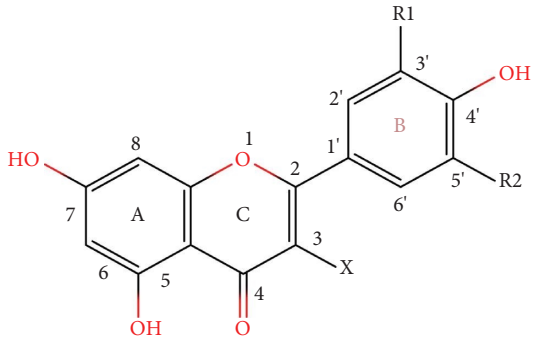

Figure 1: Structure of flavonoids.

Flavonoids play an important role in the organoleptic quality and physiological function of tea. Accurate determination of flavonoids in tea is of great significance for tea sensory taste research, raw material control, and tea production process improvement [22, 23]. How to accurately determine the content of flavonoids in tea is a key concern of tea researchers. Detection methods of flavonoids include ultraviolet spectrophotometry [24, 25], thin-layer chromatography (TLC) [26], high-performance liquid chromatography-diode-array detector (HPLC-DAD) [27, 28], and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [29–31]. Ultraviolet spectrophotometry is generally used to determine the content of total flavonoids. TLC suffers from a low accuracy and finds it difficult to distinguish similar compounds. It is difficult to separate various flavonoids using HPLC, and it has a long analysis time and poor specificity. The UPLC-MS/MS method has a high sensitivity and selectivity [32, 33], but the reference materials must constantly be purchased and renewed because of the limitation of their period of validity. These factors increase the detection time and costs [34, 35]. The development of the mass spectrometry technology makes Q-TOF/MS which has a high resolution and fast acquisition rate. UPLC-Q-TOF/MS has the characteristics of high resolution, high sensitivity, high accuracy, and wide scanning range. Moreover, it can also enable possession of the capability of nontarget matters screening and data tracing through the accurate mass, isotope distribution, and characteristic mass spectrum of MS/MS [36–39]. Researchers have made systematic studies and attempts on the IDA MS/MS mode using UPLC-Q-TOF/MS. IDA MS/MS is a mode in which the primary full scan is performed first, and then the highest ones are selected for the secondary mass spectrometry scan based on the abundance of ions in the primary full scan. Previously, we performed the targeted and nontargeted preliminary identification of flavonoids in tea using the Triple TOF™6600$^+$ MS system. The compounds were rapidly selected by the targeted and nontargeted peak finding approaches and then tentatively identified by comparing with the TCM MS/MS database, online ChemSpider database, or inferred through mass spectrometry fragment ion analysis and literature data. Second, based on the published literature [40, 41], we summarized and sorted out the contained and possibly contained flavonoids in tea. Finally, we identified the types of flavonoids to be studied, purchased standards of flavonoids, and quantified them accurately. In this paper, UPLC-Q-TOF/MS was first used for the fast and accurate determination of 18 flavonoids in tea. It can obtain accurate test results and provide reliable technical support for enterprises and regulatory authorities.

2. Materials and Methods

2.1. Reagents and Standards. Methanol was of HPLC grade and purchased from Fisher Scientific (Loughborough, UK). Formic acid was of HPLC grade supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Ultrapure water (resistivity 18.2 M·Ω·cm) was generated by a Milli-Q system (Millipore, USA). All tea samples were purchased from local tea shops in China.

Kaempferol, luteolin, quercetin, quercitrin, myricetin, isoliquiritigenin, silymarin, isovitexin, vitexin, afzelin, luteoloside, plantagoside, trilobatin, schaftoside, rutinum, baimaside, tiliroside, and camellianin A (purity were greater than 98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd.

2.2. Instruments and Equipment. The instruments used are high-speed refrigerator centrifuge (CR22N, Hitachi, Japan), ultrasonic cleaner (Elmasonic P300H, Elma Germany), water bath constant temperature shaker (SHA-B, Changzhou Runhua Electric Co., Ltd, P.R.China), and vortex mixer (Vortex Genius 3, IKA, Germany). Quantitative analysis of 18 flavonoids in tea was conducted on a Q-TOF/MS (UPLC-Triple TOF™6600$^+$, Sciex, USA). OS software (Version 1.6.0, Sciex, USA) was used for data processing, and Analyst® (Version 1.6, Sciex, USA) was used for data analysis.

2.3. Sample Preparation. 1.00 g of crushed tea (dry leaves) was weighed into a 50 mL centrifuge tube. After spiking of 20 mL of 70% methanol-water solution, it was vortexed for 1 min and then sonicated for 30 min at room temperature. The sample was centrifuged at 8000 ×g for 3 min at 4°C. 1 mL of supernatant was filtered through a 0.22 μm filter membrane and analyzed by UPLC-Q-TOF/MS.

2.4. Chromatographic Conditions. The chromatographic separation was performed on a Kinetex F5 column (2.1 mm × 100 mm, 2.6 μm). The column temperature was 35°C, and the injection volume was 5 μL. The chromatographic analysis was performed by a gradient elution with a mobile phase A (0.1% formic acid solution) and a mobile phase B (methanol). The gradient profile started from 10% B and linearly increased to 90% B within 11 mins and kept at 90% B for 5 mins before being returned to 10% B for the next injection. The postrun equilibrium time was 4 min, and the flow rate was 0.3 mL/min.

2.5. Mass Spectrometry Conditions. For the MS analysis, the Triple TOF™6600$^+$ equipped with a DuoSpray™ ion source was used. In this study, an electrospray ionization source mode was used for detection and an atmospheric pressure

chemical ionization (APCI) source was used for calibration. The spray voltage (ISVF) was set at 5500 V/4500 V and the source temperature at 400°C under the positive mode (ESI^+). The curtain gas pressure was 0.24 MPa, the nebulizer gas pressure was 0.34 MPa, and the auxiliary gas pressure was 0.38 MPa. The TOF MS data were collected with a duration time of 30 min (scan 100–100 Da), and the accumulation time was 0.15 s. The information-dependent acquisition (IDA)-MS/MS conditions were as follows: accumulation time was 0.05 s, high sensitivity mode, switch criteria 100 cps, isotopes within 4 Da were excluded, declustering potential was 80 V, and the collision energy was 40 ± 20 V. An automatic batch calibration was performed to ensure the accuracy and reproducibility.

2.6. Method Validation. In order to determine the specificity of the method, the chromatograms were compared between the 18 flavonoids and the tea samples were spiked with standards. 18 flavonoids mixed with standard intermediate solutions were accurately pipetted, and standard series solutions were prepared with methanol. The linear regression equation was established with the quantification of the peak area of the target primary excimer as the ordinate (Y) and the concentration of each component as the ordinate (X). A series of spiked samples were prepared to determine the limits of detection (LOD) and the limits of quantification (LOQ) when the signal-to-noise ratios of 18 flavonoids were about 3 and 10, respectively. The reproducibility and stability of the method were investigated by measuring the intraday and interday precision ($n = 3$) [42]. Two different concentrations were added to the standard samples at 0.2 and 2.0 ppm. Each additive concentration was measured 3 times in parallel, and the relative standard deviation of the results of the 3 replicates was evaluated for analysis.

The recovery and precision experiments were carried out by adding 18 flavonoids' standard solutions to tea samples at three concentration levels. The recovery and RSDs were calculated at six repetitions for each level. The recovery was calculated according to the following formula:

$$\text{recovery} = \frac{A \times 100\%}{B}, \quad (1)$$

where A is the measured sample concentration and B is the actual sample concentration.

3. Results and Discussion

3.1. Optimization of Extraction Solvents. The type of extraction solvent used directly affects the extraction effect of the target compound, so the choice of the extraction solvent is crucial. This experiment investigated the extraction effect of water with various concentrations of methanol (30%, 50%, 70%, and 90%). As shown in Figure 2, 70% of the methanol-water solution yielded the best recovery of the 18 flavonoids. Therefore, 70% methanol-water solution was selected as the extraction solvent.

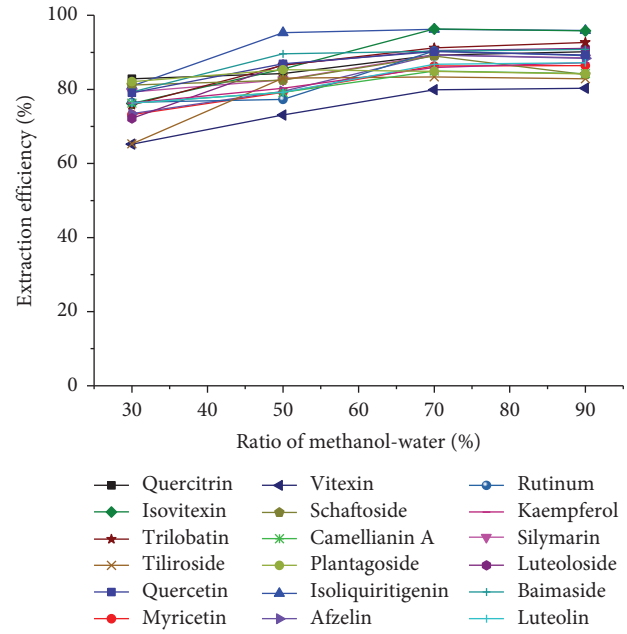

Figure 2: Comparison of extraction efficiency of methanol-water in different proportions.

3.2. Optimization of Liquid Chromatography Conditions. A series of preliminary experiments was carried out using different chromatographic columns including Waters Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 μm), Waters ACQUITY BEH C_{18} (2.1 × 100 mm, 2.5 μm), Waters ACQUITY UPLC BEH Shield RP18 (2.1 × 100 mm, 1.7 μm), and Phenomenex Kinetex F5 (2.1 × 100 mm, 2.6 μm). The results showed that Phenomenex Kinetex F5 was the best in reducing the analysis time and improving the separation degree. The peak shape of the obtained samples was the best, with a sharp symmetry, and could effectively separate the three groups of isomers: kaempferol and luteolin displayed parent ion at m/z 285.04046, quercitrin and luteoloside at m/z 449.10784, and isovitexin, vitexin, and afzelin at m/z 433.11292. The Phenomenex Kinetex F5 column (2.1 × 100 mm, 2.6 μm) was selected as the chromatographic column for this method. Figure 3 shows the extraction ion chromatograms of the 18 flavonoids.

3.3. Optimization of Mass Spectrometry Parameters. In order to obtain an accurate and reliable experimental data, the TOF-MS-IDA-MS/MS workflow was established by the Triple TOF™6600$^+$ instrument and the unique IDA function of the system was utilized to detect the target. The high-resolution mass spectrometry can obtain the primary parent ions and secondary fragment ions, thus obtaining the quantitative and qualitative analysis data. The dynamic background subtraction function of the instrument greatly reduces the background intensity of the secondary mass spectrum signal, and can improve the MS/MS signal of low-content targets in the sample. In this study, ionization voltage (ISVF), source temperature (TEM), pressure of the

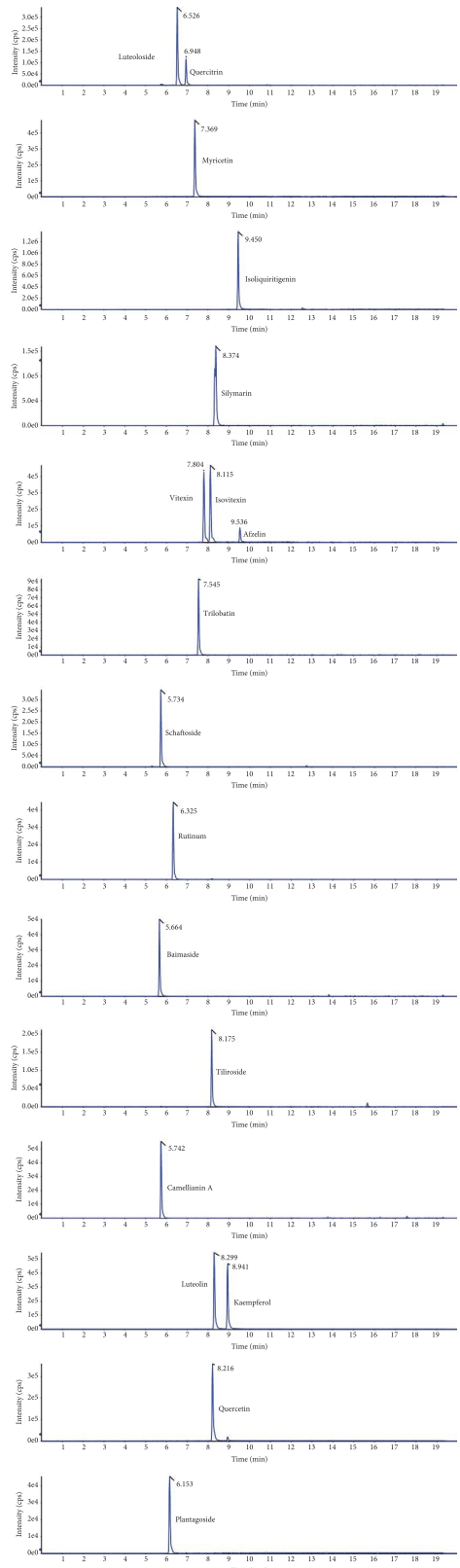

Figure 3: Extracted ion chromatograms of the 18 flavonoid precursor ions.

Table 1: Mass spectrometry information of the 18 flavonoids.

Compound name	Formula	Adduct/charge	Retention time (min)	Theoretical mass (m/z)	Experimental mass (m/z)	Mass error (×ppm)	Isotope ratio difference (%)
Quercitrin	$C_{21}H_{20}O_{11}$	$[M+H]^+$	6.948	449.10784	449.10720	−1.4	4.4
Myricetin	$C_{15}H_{10}O_8$	$[M+H]^+$	7.369	319.04484	319.04545	1.9	4.3
Isoliquiritigenin	$C_{15}H_{12}O_4$	$[M+H]^+$	9.450	257.08084	257.08150	2.6	4.2
Silymarin	$C_{25}H_{22}O_{10}$	$[M+H]^+$	8.374	483.12857	483.12780	−1.6	3.1
Isovitexin	$C_{21}H_{20}O_{10}$	$[M+H]^+$	8.115	433.11292	433.11323	0.72	4.8
Vitexin	$C_{21}H_{20}O_{10}$	$[M+H]^+$	7.804	433.11292	433.11303	0.25	3.7
Afzelin	$C_{21}H_{20}O_{10}$	$[M+H]^+$	9.536	433.11292	433.11231	−1.4	3.5
Luteoloside	$C_{21}H_{20}O_{11}$	$[M+H]^+$	6.526	449.10784	449.10827	1.0	3.5
Trilobatin	$C_{21}H_{24}O_{10}$	$[M+H]^+$	7.545	437.14422	437.14350	−1.6	4.3
Schaftoside	$C_{26}H_{28}O_{14}$	$[M+H]^+$	5.734	565.15518	565.15561	0.76	4.7
Rutinum	$C_{27}H_{30}O_{16}$	$[M+H]^+$	6.325	611.16066	611.16165	1.6	4.0
Baimaside	$C_{27}H_{30}O_{17}$	$[M+H]^+$	5.664	627.15558	627.15477	−1.3	3.2
Tiliroside	$C_{30}H_{26}O_{13}$	$[M+H]^+$	8.175	595.14462	595.14458	−0.067	3.5
Camellianin A	$C_{33}H_{40}O_{20}$	$[M+H]^+$	5.742	757.21857	757.21784	−1.0	3.3
Kaempferol	$C_{15}H_{10}O_6$	$[M-H]^-$	8.941	285.04046	285.04164	4.1	4.1
Luteolin	$C_{15}H_{10}O_6$	$[M-H]^-$	8.299	285.04046	285.04177	4.6	4.0
Quercetin	$C_{15}H_{10}O_7$	$[M-H]^-$	8.216	301.03538	301.03649	3.7	4.1
Plantagoside	$C_{21}H_{22}O_{12}$	$[M-H]^-$	6.153	465.10385	465.10391	0.13	2.9

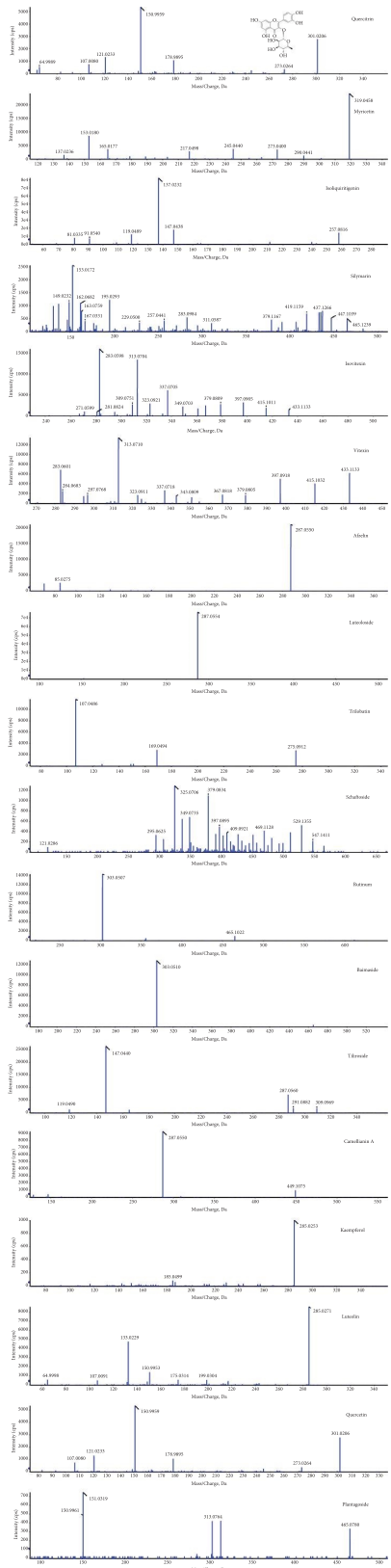

Figure 4: Secondary MS spectra of the 18 flavonoids.

Table 2: Linear relationships, LOD, LOQ, intraday, and interday precision of the 18 flavonoids.

Compound name	Linear equation	Linear range (ng/mL)	R^2	LOD (ppm)	LOQ (ppm)	Intraday precision (%)		Interday precision (%)	
						0.2 (ppm)	2.0 (ppm)	0.2 (ppm)	2.0 (ppm)
Quercitrin	$Y = 937.93572X - 60.94538$	2.0–200	0.999	0.020	0.040	6.33	2.35	5.98	4.33
Myricetin	$Y = 5573.98039X - 5141.38132$	0.50–200	0.999	0.0040	0.010	2.65	4.10	4.18	5.33
Isoliquiritigenin	$Y = 3.29233e4 - 684.58458$	0.10–200	0.999	0.0010	0.0020	2.11	4.16	4.21	3.99
Silymarin	$Y = 1303.67649X + 772.68571$	0.50–200	0.999	0.0040	0.010	1.75	5.29	1.32	6.22
Isovitexin	$Y = 7058.63154 - 6954.63317$	0.50–200	0.999	0.0040	0.010	2.03	3.18	3.56	4.00
Vitexin	$Y = 5907.43785 - 5540.04408$	0.50–200	0.999	0.0040	0.010	4.56	1.69	4.95	3.26
Afzelin	$Y = 933.85478X + 306.46076$	2.0–200	0.999	0.020	0.040	7.21	6.98	6.98	5.93
Luteoloside	$Y = 4256.71850X - 2641.93243$	0.50–200	0.999	0.0040	0.010	3.26	4.19	4.15	3.29
Trilobatin	$Y = 4758.23582X - 442.95230$	2.0–200	0.999	0.020	0.040	2.41	3.25	3.66	5.87
Schaftoside	$Y = 2857.85348X - 2820.37177$	0.50–200	0.999	0.0040	0.010	8.32	4.33	9.29	5.12
Rutinum	$Y = 614.44087X - 566.07374$	5.0–200	0.999	0.040	0.10	0.141	1.98	2.65	6.59
Baimaside	$Y = 337.15349 - 205.47768$	2.0–200	0.999	0.020	0.040	1.89	6.59	3.26	4.19
Tiliroside	$Y = 2058.79076X - 1139.28568$	2.0–200	0.999	0.020	0.040	6.12	7.11	4.21	8.77
Camellianin A	$Y = 328.76344X - 235.67547$	2.0–200	0.999	0.020	0.040	4.50	4.32	5.00	6.31
Kaempferol	$Y = 6203.83219X - 311.96940$	1.0–200	0.999	0.010	0.020	6.22	4.88	7.19	5.09
Luteolin	$Y = 6937.99180X - 180.55061$	1.0–200	0.999	0.010	0.020	1.25	3.21	5.36	4.08
Quercetin	$Y = 4111.83509X + 44.50690$	1.0–200	0.998	0.010	0.020	2.97	1.78	6.23	5.19
Plantagoside	$Y = 787.08819X + 1.18151$	1.0–200	0.999	0.010	0.020	7.11	5.82	7.08	8.17

Table 3: Linearity, LODs, and LOQs of the 18 flavonoids.

Compound name	Recovery (%)			RSD (%)		
	1LOQ	2LOQ	10LOQ	1LOQ	2LOQ	10LOQ
Quercitrin	86.7	88.1	88.5	1.35	2.11	1.24
Myricetin	81.4	90.2	81.4	1.45	1.02	1.65
Isoliquiritigenin	91.6	102	94.5	3.74	2.36	2.90
Silymarin	101	86.2	95.5	1.79	4.14	1.11
Isovitexin	84.7	80.9	88.4	5.37	2.48	3.45
Vitexin	77.6	79.5	73.8	2.40	2.15	2.40
Afzelin	80.2	88.0	88.0	2.13	3.49	0.340
Luteoloside	90.1	87.4	86.2	5.10	2.25	1.31
Trilobatin	89.7	84.2	91.2	1.69	4.59	1.00
Schaftoside	87.8	88.2	84.4	5.49	2.34	2.55
Rutinum	102	107	89.8	0.983	2.69	0.712
Baimaside	93.6	85.4	87.7	0.852	4.43	1.37
Tiliroside	85.3	83.7	85.7	3.51	3.60	8.05
Camellianin A	81.4	84.5	87.0	3.31	5.55	0.943
Kaempferol	84.8	91.0	91.2	3.08	3.48	1.62
Luteolin	82.2	86.5	88.1	1.68	1.03	0.544
Quercetin	86.4	93.3	90.3	3.18	1.19	0.135
Plantagoside	83.5	87.8	83.1	1.78	1.45	1.96

*1LOQ, 2LOQ, and 10LOQ represent the three different concentration levels: $1 \times$ LOQ, $2 \times$ LOQ, and $10 \times$ LOQ.

curtain gas (CUR), pressure of the nebulizer gas (GS1), pressure of the auxiliary gas (GS2), declustering potential (DP), collision energy (CE), and dynamic energy of collision (CES) parameters were optimized to achieve an effective isolation and response of all target compounds. CES is an important parameter to improve the sensitivity of the target compounds and to reduce the ion information loss of secondary fragments. CES was set at 20 eV which made the rich second-order fragment ion information of EPI scan maps at 20, 40, and 60 eV.

Different from the quantitative means of triple quadrupole, Triple TOF™6600$^+$high-resolution mass spectrometry carried out the first-order full scan and data-dependent second-order mass spectrometry simultaneously in the TOF-MS-IDA-MS/MS mode. The parent ion was used as the quantitative ion, and it simplified the optimization process of mass spectrum parameters and improved efficiency. It is more conducive to rapid screening and quantitative analysis. Electrospray ionization and positive/negative ion scanning mode were used in the experiment to obtain the first-order full-scan mass spectrograms. It is extracted from the theoretical mass of each compound $[M+H]^+$ and $[M-H]^-$. From Table 1, it can be seen that the relative deviations of the mass numbers of 18 flavonoids were all less than 5.0 ppm, which is in accordance with the European Union Decision 2002/657/EC about the LC/MS part [43]. Figure 4 shows the second-order characteristic maps of 18 flavonoids. The accuracy of the qualitative analysis can be improved through fragment ion information.

3.4. Specificity of the Method. Both standards and the spiked tea samples showed a sharp and symmetric peak in the chromatograms, and there were no peaks in the samples at the same retention time. Figure 2 shows that there are no interferences from the matrix components on the retention time of the 18 flavonoids.

3.5. Linearity, Sensitivity, Intraday, or Interday Precision. From the determination results in Table 2, it can be seen that the linearity of 18 flavonoids in the concentration range of 0.10–200 ng/mL was good, the $R^2 \geq 0.998$, LOD was 0.0010–0.040 ppm, and LOQ was 0.0020–0.10 ppm. The intraday precision ranges from 0.141% to 8.32%, and the interday precision ranges from 1.32% to 9.29%. Huang et al. established a method for the detection of nine flavonoids with LOD ranging from 10 to 66 μg/kg [19]. The LOD and LOQ of this method are lower than those of the published methods, indicating that the method has a high sensitivity.

3.6. Recovery and Precision. As shown in Table 3, when the supplemental level was 0.0020 ppm–1.00 ppm, the recoveries ranged from 73.8% to 107%, and RSD ranged from 0.135% to 8.05%. The results were able to meet the requirements of the "Criterion on quality control of laboratories-Chemical testing of food" [44], that is, when the content of the measured component is less than 0.1 mg/kg, the recovery is in the range of 60–120% with RSD $\leq$ 15%. The recoveries were considered acceptable to the method, and the results indicated that the precision was reasonable.

3.7. Application to Tea Samples. In order to investigate the content of the 18 flavonoids in tea, 10 tea samples from local tea shops were analyzed by using the established method in this research. Qualitative screening of the 18 flavonoids was conducted by using an accurate mass number and retention time. The results were confirmed by secondary characteristic fragment ions. The compositions and contents of flavonoid substances were different in ten tea samples. Isoliquiritigenin, silymarin, trilobatin, baimaside, and camellianin A were not detected. The determination results of tea samples are shown in Table 4. The content of plantagoside and luteoloside in green tea was higher than that in black tea,

Table 4: 18 Flavonoids content in 10 types of tea (ppm, $n = 3$).

Compound name	Black tea	Green tea								Others
	Congou black tea	Yellow mountain fuzz tip	Green tea 1	Green tea 2	Green tea 3	Sword-shaped green tea	Maojian tea	Yucca tea	Taiping kowkui	Dandelion
Quercitrin	0.5595 ± 4.7	1.135 ± 3.3	1.406 ± 0.93	1.095 ± 2.1	1.263 ± 7.3	1.461 ± 5.2	1.313 ± 3.4	0.4325 ± 5.5	1.677 ± 0.8	ND
Myricetin	5.480 ± 2.5	3.403 ± 3.2	3.904 ± 4.7	5.300 ± 2.8	11.15 ± 4.7	2.990 ± 1.9	3.003 ± 3.5	1.334 ± 4.8	3.511 ± 2.9	0.7766 ± 6.0
Isoliquiritigenin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Silymarin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isovitexin	13.02 ± 0.52	11.54 ± 5.2	13.74 ± 2.3	71.39 ± 3.9	11.90 ± 2.9	8.908 ± 4.4	64.90 ± 3.1	0.7285 ± 5.2	35.95 ± 7.4	0.7906 ± 5.2
Vitexin	7.952 ± 6.9	4.568 ± 4.0	4.870 ± 4.4	38.82 ± 4.4	4.530 ± 4.7	2.890 ± 2.9	29.88 ± 3.7	0.2906 ± 4.3	14.59 ± 2.0	ND
Afzelin	1.053 ± 7.7	3.783 ± 3.1	1.755 ± 2.7	4.131 ± 7.2	9.915 ± 2.1	2.125 ± 4.2	2.297 ± 0.93	0.2194 ± 3.5	5.844 ± 8.0	1.229 ± 4.7
Luteoloside	0.5801 ± 6.5	17.02 ± 2.2	14.69 ± 0.45	14.14 ± 5.8	2.621 ± 4.2	21.02 ± 0.91	7.701 ± 1.1	1.621 ± 4.5	26.77 ± 3.4	44.12 ± 2.8
Trilobatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Schaftoside	84.40 ± 0.86	62.12 ± 1.9	61.96 ± 2.7	214.4 ± 6.1	62.08 ± 1.6	50.98 ± 4.2	169.0 ± 3.2	6.912 ± 4.2	132.0 ± 1.7	0.8466 ± 6.2
Rutinum	471.8 ± 1.0	109.5 ± 3.0	280.6 ± 5.5	342.5 ± 3.8	1190 ± 6.5	179.2 ± 2.2	324.1 ± 4.7	218.5 ± 3.2	150.7 ± 0.83	9.693 ± 4.7
Baimaside	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tiliroside	31.44 ± 4.4	59.49 ± 2.6	26.30 ± 6.2	37.97 ± 1.8	39.87 ± 1.8	67.86 ± 2.8	39.11 ± 4.0	22.34 ± 3.7	83.06 ± 5.2	ND
CamellianinA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kaempferol	8.573 ± 3.2	2.308 ± 0.73	5.031 ± 0.91	8.291 ± 4.4	5.653 ± 0.52	6.883 ± 4.2	6.269 ± 2.7	3.427 ± 2.0	3.687 ± 4.2	0.3565 ± 6.0
Luteolin	0.8085 ± 2.1	0.3146 ± 4.5	0.1862 ± 6.3	0.5200 ± 3.6	0.3582 ± 1.9	0.1850 ± 2.0	0.5798 ± 2.9	0.1507 ± 3.7	0.5199 ± 6.4	26.41 ± 7.5
Quercetin	341.1 ± 4.6	97.43 ± 4.7	306.0 ± 5.2	293.9 ± 4.9	384.9 ± 6.2	159.6 ± 2.3	212.6 ± 2.1	141.2 ± 0.93	161.8 ± 1.9	1.076 ± 4.2
Plantagoside	6.632 ± 6.9	42.17 ± 7.2	41.10 ± 5.3	72.30 ± 4.2	9.552 ± 2.9	47.00 ± 4.1	76.94 ± 2.8	52.85 ± 4.3	67.97 ± 7.2	ND

*ND, not detected.

which may be due to the fermentation process. This may be due to the fact that flavonoids are unstable in the natural state and are transformed and degraded under the action of polyphenol oxidase, heat, microorganisms, and their secreted enzymes and oxidative substances during the fermentation process [45]. Correlation analysis was performed on the 18 flavonoids of 10 tea samples, and the results are shown in Supporting Information Table S1. As shown in the supplementary Table S1, there was an extremely significant correlation ($P < 0.01$) between the content of quercitrin and the content of tiliroside. There was an extremely significant correlation ($P < 0.01$) between the content of myricetin and the content of afzelin, rutinum, and quercetin. There was an extremely significant correlation ($P < 0.01$) between the content of isovitexin and the content of vitexin, schaftoside, and plantagoside. There was an extremely significant correlation ($P < 0.01$) between the content of vitexin and the content of schaftoside. There was an extremely significant correlation ($P < 0.01$) between the content of rutinum and the content of quercetin. There was an extremely significant correlation ($P < 0.01$) between the content of kaempferol and the content of quercetin.

4. Conclusions

At present, the imperfection of the relevant testing system and the lack of testing methods make tea quality supervision not in place, which restricts the improvement of tea product quality. In this study, a UPLC-Q-TOF/MS method was successfully established for the determination of 18 flavonoids in tea. The analytes were determined by Q-TOF/MS in the TOF MS and IDA-MS/MS modes. In the TOF MS mode, the target compounds are qualified by the retention time, accurate mass, isotope distribution, and isotope abundance ratio of the target, and quantified by the peak area of the excimer ion peak. In the IDA-MS/MS mode, the target compounds were further confirmed by the ion fragment information under the corresponding collision energy. In addition, the high-resolution mass spectrometry effectively reduced the matrix effect of tea. In this study, the determination conditions of flavonoids in tea were optimized by liquid chromatography and mass spectrometry (LC/MS), and the methodology was confirmed. The limits of detection were 0.0010–0.040 ppm, and the limits of quantification were 0.0020–0.10 ppm. The recoveries ranged from 73.8% to 107% at spiked levels of 0.0020–1.0 ppm, with RSDs less than 10%. The method was simple, sensitive, precise, and reproducible. It is suitable for the rapid determination of flavonoids in tea. This study provides a reference for quality evaluation of tea and its products, and promotes sustainable development of the industry. The quantitative detection of active ingredients in tea, the dose-effect relationship between active ingredients and body function, and the development of tea-functional products are expected to become the technology and market trend of industrial development.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

YZ and LZ conceived and designed the experiments. JL, JM, QL, and LH performed the experiments. MZ analyzed the data. JL, JM, and JZ wrote the original draft. All authors have read and approved the manuscript. Jian Li and Junmei Ma contributed equally to this work and share the first authorship.

Acknowledgments

This work was supported by the Major Science and Technology Projects in Anhui Province (Project no. 2020B06050001).

Supplementary Materials

Table S1: correlation of different flavonoids in tea samples. (*Supplementary Materials*)

References

[1] X. Y. He, J. K. Li, W. Zhao, R. Liu, L. Zhang, and X. Kong, "Chemical fingerprint analysis for quality control and identification of Ziyang green tea by HPLC," *Food Chemistry*, vol. 171, pp. 405–411, 2015.

[2] M. Sökmen, E. Demir, and S. Alomar, "Optimization of sequential supercritical fluid extraction (SFE) of caffeine and catechins from green tea," *The Journal of Supercritical Fluids*, vol. 133, no. 1, pp. 171–176, 2018.

[3] A. Di Lorenzo, S. F. Nabavi, A. Sureda et al., "Antidepressive-like effects and antioxidant activity of green tea and GABA green tea in a mouse model of post-stroke depression," *Molecular Nutrition & Food Research*, vol. 60, no. 3, pp. 566–579, 2016.

[4] L. Yan, C. H. Liu, H. Qu et al., "Discrimination and measurements of three flavonols with similar structure using terahertz spectroscopy and chemometrics," *Journal of Infrared, Millimeter and Terahertz Waves*, vol. 39, no. 5, pp. 492–504, 2018.

[5] P. Dubey, "Tea catechins as potent antioxidant and anti-inflammatory agents: possibilities of drug development to promote healthy aging," *Plant Bioactives as Natural Panacea Against Age-Induced Diseases*, vol. 56, pp. 253–269, 2023.

[6] K. W. Lange, "Tea in cardiovascular health and disease: a critical appraisal of the evidence," *Food Science and Human Wellness*, vol. 11, no. 3, pp. 445–454, 2022.

[7] A. I. Alghamdi, "Antibacterial activity of green tea leaves extracts against specific bacterial strains," *Journal of King Saud University Science*, vol. 33, no. 5, Article ID 102650, 2023.

[8] G. S. Zhao, J. Y. Teng, R. X. Dong et al., "Alleviating effects and mechanisms of action of large-leaf yellow tea drinking on diabetes and diabetic nephropathy in mice," *Food Science and Human Wellness*, vol. 12, no. 5, pp. 1660–1673, 2023.

[9] S. M. Li, L. Zhang, X. Wan, J. Zhan, and C. T. Ho, "Focusing on the recent progress of tea polyphenol chemistry and perspectives," *Food Science and Human Wellness*, vol. 11, no. 3, pp. 437–444, 2022.

[10] J. Li, J. M. Ma, Y. Zhang, and L. Zheng, "Determination of 19 polyphenolic compounds in tea by ultra-high performance liquid chromatography combined with quadrupole-time of flight mass spectrometry," *Food Science and Human Wellness*, vol. 11, no. 3, pp. 719–726, 2022.

[11] X. H. Jiang, Q. Meng, and L. L. Liu, "The extraction and purification and composition of celery seed medium yellow ketone of LC-MS detection," *China Health Industry*, vol. 10, no. 22, pp. 8-9, 2013.

[12] J. L. Lv, S. Rrbiguli, and A. Ajiaaikebaier, "Flavonoids from celerry seed," *Chinese Traditional Patent Medicine*, vol. 3, pp. 406–408, 2007.

[13] H. Wang, G. J. Provan, and K. Helliwell, "Tea flavonoids: their functions, utilisation and analysis," *Trends in Food Science & Technology*, vol. 11, no. 4-5, pp. 152–160, 2000.

[14] X. Lin and D. W. Sun, "Recent developments in vibrational spectroscopic techniques for tea quality and safety analyses," *Trends in Food Science & Technology*, vol. 104, pp. 163–176, 2020.

[15] C. J. Liu, F. Wang, and Y. F. Luan, "Optimization of ultrasound-assisted extraction of flavonoids from actinidia arguta fruits," *Food Science*, vol. 32, pp. 100–102, 2011.

[16] K. S. Ng, Z. Mohd Zin, N. MohdMaidin, H. Mamat, N. Juhari, and M. Zainol, "High-performance liquid chromatography (HPLC) analysis for flavonoids profiling of Napier grass herbal tea," *Food Research*, vol. 5, no. 1, pp. 65–71, 2020.

[17] J. Chen, Y. Liu, and Y. P. Shi, "Determination of flavonoids in the flowers of Paulownia tomentosa by high-performance liquid chromatography," *Journal of Analytical Chemistry*, vol. 64, no. 3, pp. 282–288, 2009.

[18] B. Dai, Z. Q. Hu, H. Y. Li, C. Yan, and L. Zhang, "Simultaneous determination of six flavonoids from Paulownia tomentosa flower extract in rat plasma by LC–MS/MS and its application to a pharmacokinetic study," *Journal of Chromatography B*, vol. 978-979, pp. 54–61, 2015.

[19] H. Huang, F. L. Wang, and W. Zhao, "Determination of nine flavonoids in celery seed by liquid chromatography-tandem mass spectrometry," *Food and Fermentation Industries*, vol. 47, pp. 259–265, 2021.

[20] L. Li, J. B. Teng, Y. L. Zhu et al., "Metabolomics study of flavonoids of taxillus chinensis on different hosts using UPLC-ESI-MS/MS," *Molecules*, vol. 26, no. 24, p. 7681, 2021.

[21] A. A. Shokry, R. A. El-Shiekh, G. Kamel, A. F. Bakr, D. Sabry, and A. Ramadan, "Anti-arthritic activity of the flavonoids fraction of ivy leaves (Hedera helix L.) standardized extract in adjuvant induced arthritis model in rats in relation to its metabolite profile using LC/MS," *Biomedicine & Pharmacotherapy*, vol. 145, Article ID 112456, 2022.

[22] L. L. Wang, Q. X. Lin, and Z. S. Song, "Spectrophotometric determination of total flavonoids in tea," *Acta Tea Sinica*, vol. 62, pp. 1–6, 2021.

[23] W. Z. Zhang, "Uncertainty evaluation of determination of total flavonoids in propolis by spectrophotometry," *Journal of Food Safety and Quality*, vol. 11, no. 13, pp. 4502–4506, 2020.

[24] X. Zhao, Y. J. Li, and W. Han, "Research on spectrophotometry for determination of total flavonoids content in peanut hulls," *Science and Technology of Food Industry*, vol. 40, no. 10, pp. 269–279, 2019.

[25] Y. C. Chen, J. Li, and H. Yao, "Comparative Identification and determination of total flavonoids in sandalwood," *Chinese Journal of Ethnomedicine and Ethnopharmacy*, vol. 29, no. 1, pp. 52–54, 2020.

[26] Y. Z. Li, C. Y. Zhao, C. Lu et al., "Simultaneous determination of 14 bioactive citrus flavonoids using thin-layer chromatography combined with surface enhanced Raman spectroscopy," *Food Chemistry*, vol. 338, Article ID 128115, 2021.

[27] X. Chen, X. Lei, and D. Wang, "Simultaneous determination of two flavonoids in rhizoma drynariae by HPLC," *Guangdong Chemical Industry*, vol. 49, no. 4, pp. 188–190, 2022.

[28] Y. J. Wang, W. G. Jie, and N. Guo, "Simultaneous determination of chlorogenic acid and total flavonoids in honeysuckle by HPLC and their extraction process," *Cereals & Oils*, vol. 35, no. 2, pp. 157–162, 2022.

[29] Z. L. Sheng, Y. M. Jiang, J. M. Liu, and B. Yang, "UHPLC–MS/MS analysis on flavonoids composition in astragalus membranaceus and their antioxidant activity," *Antioxidants*, vol. 10, no. 11, p. 1852, 2021.

[30] S. F. Su, X. Xue, and P. X. Gong, "Determination of endogenous phenolic acids and flavonoids in honey by UPLC/MS/MS," *Modern Food Science and Technology*, vol. 34, no. 8, pp. 260–266, 2018.

[31] J. B. Hou, W. Xie, Y. Qian et al., "Simultaneous determination of 16 flavonoids and ferulic acid in honey by solid phase extraction and high performance liquid chromatography-tandem mass spectrometry," *Chinese Journal of Chromatography*, vol. 38, no. 5, pp. 529–537, 2020.

[32] G. Wadhwa, K. V. Krishna, R. Taliyan et al., "A novel UPLC–MS/MS method for simultaneous quantification of trigonelline, 4-hydroxyisoleucine, and diosgenin from Trigonella foenum-graecum extract: application to pharmacokinetic study in healthy and type 2 diabetic rats," *Biomedical Chromatography: Biomedical Chromatography*, vol. 36, no. 2, Article ID e5275, 2022.

[33] G. Wadhwa, K. V. Krishna, and R. Taliyan, "Pre-clinical pharmacokinetic and pharmacodynamic modelling study of 4- hydroxyisoleucine using validated ultraperformance liquid chromatography-tandem mass spectrometry," *Royal Society of Chemistry*, vol. 10, pp. 5525–5532, 2020.

[34] Y. Zhao, P. Chen, L. Z. Lin, J. Harnly, L. L. Yu, and Z. Li, "Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS," *Food Chemistry*, vol. 126, no. 3, pp. 1269–1277, 2011.

[35] J. Li, J. M. Ma, Y. Zhang, and L. Zheng, "Determination of 4 psychoactive substances in tea using ultra high performance liquid chromatography combined with the quadrupole time-of-flight mass spectrometry," *Analytical Methods*, vol. 12, no. 40, pp. 4878–4884, 2020.

[36] J. Y. Zhong, N. Chen, S. C. Huang et al., "Chemical profiling and discrimination of green tea and Pu-erh raw tea based on UPLC-Q-Orbitrap-MS/MS and chemometrics," *Food Chemistry*, vol. 326, Article ID 126760, 2020.

[37] J. H. Huo, X. W. Du, G. D. Sun, W. T. Dong, and W. M. Wang, "Identification and characterization of major constituents in Juglans mandshurica using ultra performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-ESI-Q-TOF/MS)," *Chinese Journal of Natural Medicines*, vol. 16, no. 7, pp. 525–545, 2018.

[38] L. W. Chen, Q. Wang, K. M. Qin et al., "Chemical profiling of Qixue Shuangbu Tincture by ultra-performance liquid chromatography with electrospray ionization quadrupole-time-of flight high-definition mass spectrometry (UPLC-Q TOF/MS)," *Chinese Journal of Natural Medicines*, vol. 14, no. 2, pp. 141–146, 2016.

[39] Y. Wang, L. Liu, Y. Ma et al., "Chemical discrimination of Astragalus mongholicus and Astragalus membranaceus based

on metabolomics using UHPLC-ESI-Q-TOF-MS/MS approach," *Molecules*, vol. 24, no. 22, p. 4064, 2019.

[40] J. D. Freeman and E. D. Niemeyer, "Quantification of tea flavonoids by high performance liquid chromatography," *Journal of Chemical Education*, vol. 85, no. 7, pp. 951–953, 2008.

[41] N. Aziz, M. N. Khan, A. Ali et al., "Rapid analysis of flavonoids based on spectral library development in positive ionization mode using LC-HR-ESI-MS/MS," *Arabian Journal of Chemistry*, vol. 15, no. 4, Article ID 103734, 2022.

[42] J. Li, J. M. Ma, Q. Li et al., "Determination of 35 free amino acids in tea using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry," *Frontiers in Nutrition*, vol. 8, Article ID 767801, 2021.

[43] J. Wang, W. Chow, J. Chang, and J. W. Wong, "Ultrahigh-performance liquid chromatography electrospray ionization Q-Orbitrap mass spectrometry for the analysis of 451 pesticide residues in fruits and vegetables: method development and validation," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 42, pp. 10375–10391, 2014.

[44] FAO, *Criterion on Quality Control of Laboratories-Chemical Testing of Food*, FAO, Rome, Italy, 2008.

[45] J. H. Li, M. Zhao, and Y. P. Hu, "Changs of flavonol contents in Pu-erh tea during fermentation," *Journal of Southwest University*, vol. 34, no. 2, pp. 59–65, 2012.