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# Authenticity determination of tea drinks in the Chinese market by liquid chromatography coupled to isotope ratio mass spectrometry

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ABSTRACT

Liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS) was developed for determinations of caffeine $\delta^{13}C$ from commercial tea in the Chinese market under lower temperature condition. On the basis of the carbon isotope analysis of 116 commercial tea samples including black tea, Oolong tea, green tea, dark tea, white tea, yellow tea and scented tea, it explained that the range of caffeine $\delta^{13}C$ for commercial tea were from −26.14 to −33.68‰. Partial least-squares discriminant analysis (PLS-DA) modeling of caffeine $\delta^{13}C$ data indicated that six main types of Chinese tea presented significant difference, and caffeine $\delta^{13}C$ in Chinese tea was related to the fermented process except yellow tea. The method was also used for measurement of caffeine $\delta^{13}C$ in the 19 tea drinks, and the results showed that caffeine in the seven tea drinks was not from the natural tea. Thus, LC-IRMS can be very promising tool for evaluation of natural caffeine from commercial tea and tea drinks authenticity.

## 1. Introduction

Tea is a popular beverage in the world, next only to water, which is derived from the leaves of *Camellia sinesis* and *Camellia assamica* [1]. The plant was originally discovered and grown in China 5000 years ago according to the Chinese medical book, the Pen T' Sao, the emperor Shen Nung discovered tea for the first time in 2737 BCE [2]. The production and consumption of tea has increased observably in recent years, and the literature reported that over two-thirds of the world's population have drunk tea [3]. Tea can generally be classified into six major categories based on their different processing techniques in China, which have named green tea (non-fermented), yellow and white teas (lightly fermented, 10–20%) Oolong tea (semi-fermented, 30–60%), black tea (fully fermented, 80–100%) and dark tea (post-fermented, ≤100%) [4]. Although there are detailed descriptions for discriminating the manufacturing suitability of tea cultivars according to size, color, constituents of tea leaves and chemical compositions in China [4,5], no determination of tea isotope ratio has been differentiated among the six categories.

LC-IRMS can be used to measure stable carbon isotope ratio of individual compounds. These measurements are based on the fact that chemical structure of every molecule can exhibit varying isotope contents depending on its origin. It has been used to measure precisely isotopic ratios $\delta^{13}C$ at low enrichment level [6]. LC-IRMS has found application in different fields including food authenticity, archaeology, physiology and geochemistry.

Many articles have been published concerning the possible application of liquid chromatography coupled to isotope ratio mass spectrometry, which has been applied to various different compounds, such as amino acids [6], volatile fatty acids [7], individual sugar (sucrose, glucose and fructose) [8], glutathione [9], carbohydrates [10], phenolic acids [11], dissolved inorganic carbon [12], glycerol and ethanol [13], dissolved inorganic carbon [14], galactose and glucose [15], phenols [16], caffeine [17], glucosamine [18], Sulfonamide [19], non-purgeable organic carbon [20], organic acids [21], DNA and RNA nucleotides [22], vanillin [23], glyphosate and AMPA [24], citric, malic, and tartaric acids [25].

The main components of tea are caffeine, theophylline, polyphenols, organic acids, polysaccharides and amino acids, which can be used to evaluate the quality and type of tea [26]. Especially, caffeine, an important chemical composition, is an influence on tea consumption [27]. LC-IMRS has been used as a screening method for identification of natural and synthetic caffeine [17]. Therefore, it is available for identification of six different categories of Chinese tea by $\delta^{13}C$ values of caffeine.

To evaluate these categories, we have developed a low temperature reversed-phase liquid chromatography coupled to isotope ratio mass spectrometry (LT-RPLC-IRMS) method to measure $\delta^{13}C$ values of

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caffeine in Chinese tea and other hydrophilic compounds including soluble sugars, theobromine, theophylline, catechin, epigallocatechin and epicatechin. We present the first characterization of $\delta^{13}C$ values for black tea, green tea, Oolong tea, yellow tea, white tea, dark tea and scented tea. The data of $\delta^{13}C$ values were analyzed by PLS-DA, to acquire the differences among these categories. Once we have established the variations seen in the six tea categories, we have analyzed 19 samples of tea drinks in the Chinese market, to identify the presence of significant difference between major tea categories and tea drinks.

## 2. Material and methods

### 2.1. Chemicals and instrumentation

Phosphoric acid (Sigma-Aldrich, Switzerland) and sodium persulfate (Sigma-Aldrich, Germany) were of analytical reagent and used without any purification. Carrier gas, helium (BIP grade), and $CO_2$ (high purity grade) reference gas were produced by Air Products (Dongguan, China). Solutions and dilutions were prepared with ultrapure MilliQ water (Merck Millipore, Germany) system. Caffeine standard IAEA-600 ($\delta^{13}C$, −27.771‰) (Max-Planck-Institute for Biogeochemistry, Germany) was used for accuracy of $\delta^{13}C$ measurement. The standard theobromine and theophylline were purchased by Sigma-Aldrich, USA.

The carbon isotope ratios of all samples were measured by a DELTA V Advantage (Thermo Fisher, Bremen, Germany) isotope ratio mass spectrometry coupled with a Dionex UltiMate 3000 HPLC system and the LC-IsoLink interface (Thermo Fisher, Bremen, Germany). The hydrophilic compounds of tea are identified by using LC-HRMS (Triple TOF 5600+, AB Sciex USA). All samples and standard materials were measured after the balance of reference gas, the standard deviations (SD) of reference gases, $CO_2$, was < 0.06‰ (n = 10). SW 30H ultrasonic cleaning bath (SONOSWISS, Ramsen, Switzerland) and Sigma 3-16P/3-16PK centrifuge (Sigma Laborzentrifugen GmbH, Niedersachsen Germany) were used in all experiments.

### 2.2. Samples and pretreatment

We prepared 116 samples of true commercial tea from Chinese market, including 34 black tea samples, 21 Oolong tea samples, 12 dark tea samples, 15 green tea samples, 10 white tea samples, 15 yellow tea samples and 9 flower tea samples. 15 yellow tea samples were directly purchased on the Chinese market by the Taobao Net and represent well-known yellow tea origin in China. And the other tea samples were provided by the Guangdong Inspection and Quarantine Technology Center, Guangzhou, China. Thus, tea samples were not Chinese products, but also other countries' products. Especially, black teas were collected from China, India and Sri Lanka. Tea samples (1.000 g) were accurately weighted in a 50 mL plastic centrifuge tube. 20 mL water was added and sonicated for 30 min at room temperatures. The resultant tea extracts were centrifuged at 10,000 rpm for 5 min at 25 °C. The tea preparation was diluted (1:10) and filtered (0.45 μm). Nineteen commercial tea drinks were also analyzed, representing the most popular tea drinks on the Chinese market. The drink samples were obtained by dilution (1:10) and filtration (0.45 μm).

### 2.3. The $\delta^{13}C$ analysis

All samples were subjected to reversed-phase chromatography using a HPLC system. Mobile phase was distilled water. Chromatographic separation was performed at an isocratic flow rate of 350 $\mu L \cdot min^{-1}$ using a HPLC column (Xbridge Shield RP C18, 5 μm, 2.1 mm × 50 mm; Waters Corp., Milford, MA) maintained at 50 °C. The injection volume was 5 μL for all samples. The oxidation reagent consisted of sodium persulfate in water (0.17 M, 20 $\mu L \cdot min^{-1}$), and the acid reagent was phosphoric acid (0.4 M, 35 $\mu L \cdot min^{-1}$) pumped by two heads. The mixture of these reagents, added by a T-piece to the mobile phase, flowed through a capillary oxidation reactor at 98.5 °C for the LC IsoLink. After the oxidation reactor, the mobile phase was cooled and the individual $CO_2$ peaks were transferred into a counter flow of helium. The liquid phase was completely degassed. The helium flow introduced the $CO_2$ samples to the isotope ratio mass spectrometry. The Isodat 3.0 software (Thermo Scientific Bremen, Germany) controlled the system. The analysis was repeated twice for each sample. In this paper, single-point anchoring method was used to measure the sample $\delta^{13}C$ values [28]. The stable carbon isotopic composition of a working gas is firstly recorded in the delta (δ) relative to the Vienna Pee Dee Belemnite (VPDB) standard, and the stable carbon isotopic composition of samples is measured relative to the isotopic composition of a working gas. In order to reduce normalization errors, the standard solutions of caffeine at concentrations between 10 and 100 $mg \cdot L^{-1}$ were injected the LC-IRMS. The $\delta^{13}C$ values of $CO_2$ reference gas and calibrated against the international standard of caffeine were performed at the beginning of the elution run, and then the working reference gas was calibration per 10 samples.

Caffeine, theobromine and theophylline were qualitative analysis by the standard substance. Catechin, epigallocatechin and epicatechin were identified on the basis of the accurate mass data of their protonated mass ion, isotope ion patterns, and fragment ions respectively by using the LC-Q-TOF-MS. For soluble sugars, the literature reported that the relative content of soluble sugars in tea was > 4% at the drying stage [29,30]. And usually soluble sugars are no retention when they were separated with the RP-C18 column. Thus, it is suggested that the first peak was soluble sugars with the isolation of tea samples by using the Xbridge Shield RP C18 column.

### 2.4. Statistical analysis

We performed a PLS-DA to determine the differences in the $\delta^{13}C$ values of caffeine profiles in the six tea categories using SIMCA-P software (version 14.1, Demo Umetrics, umea, Sweden). Cross-validation of the model was conducted by default leave-one-out procedure. The data set was mean-centered and pareto-scaled in a column-wise manner for the multivariate modeling [4].

## 3. Results and discussion

### 3.1. Separation of caffeine by LT-RPLC-IRMS

The caffeine in the standard substance and tea samples was clearly separated by LT-RPLC-IRMS using the defined conditions (Fig. 1). Early LC-IRMS had been successfully for the measurements of different highly soluble compounds, typically based on separation of ion exchange

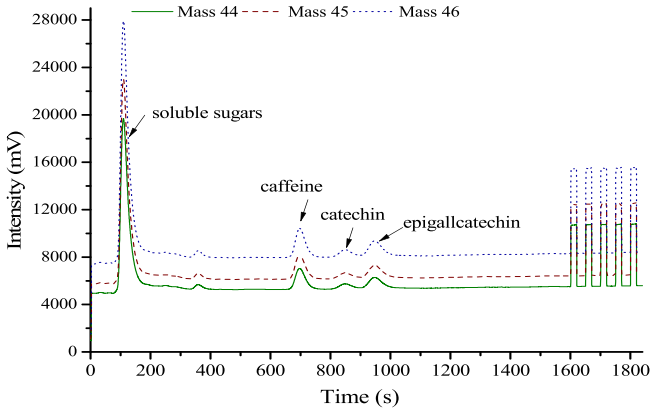


**Fig. 1.** LC-IRMS resolution of tea sample the special compounds: soluble sugars, caffeine, catechin and epigallocatechin.