



Characterization of volatile compounds of pu-erh tea using solid-phase microextraction and simultaneous distillation–extraction coupled with gas chromatography–mass spectrometry



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ABSTRACT

Two extraction methods, namely, solid-phase microextraction (SPME) and simultaneous distillation–extraction (SDE) both followed by gas chromatography–mass spectrometry were applied for the determination of a wide range of volatile compounds in pu-erh tea. The conditions of solid-phase microextraction including fiber selections and sampling condition optimization have been previously investigated. By the two methods, 79 volatile compounds belonging to different categories were identified. Among them, 61 volatile compounds were extracted by SDE and 67 by SPME. Qualitative and quantitative differences of pu-erh tea volatile profiles were observed by applying the two aforementioned extraction methods. SDE technique achieved higher percentages of high molecular weight alcohols, acids, and esters of low volatility, whereas SPME technique was found useful for analyzing low molecular weight alcohols, methoxy-phenolic compounds, aldehydes, ketones, and hydrocarbons of high volatility that were closely related to the characteristics of pu-erh tea aroma and its sensory perception. Therefore, SPME technique was a reliable extraction method for controlling pu-erh tea quality flavor.

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1. Introduction

Pu-erh tea, the most representative of dark teas, is made from leaves from mature wild “broad-leaf teas,” *Camellia sinensis* (L.) O. Kuntze var. *assamica* Kitamura, in southwestern China. Pu-erh tea is traditionally classified as post-fermented tea based on tea-making processing. The unique method of tea fermentation involves microorganisms that enter the fermentation process as a natural flora. Complex changes from the coordinated microbial metabolic action and natural oxidation occur during the post-fermentation and piling process to produce the special quality and flavor characteristics of the pu-erh tea. Pu-erh tea is a famous tea that wins recognition of tea lovers at home and abroad, which attributes the success to its multiple healthcare functions as well as its special aroma. The health benefits and biological activities of this tea are because of the presence of nonvolatile substances including polyphenols, polysaccharides, and tea pigments. Some studies have confirmed that pu-erh tea has various biological activities, including its antioxidant activity (Huang et al., 2013), inhibiting cholesterol biosynthesis (Lu & Hwang, 2008), preventing cancer (Lin, 2002), and having antimutagenic and antimicrobial effects (Wu et al., 2007). In contrast, volatile compounds in pu-erh tea contribute to its special aroma. The volatiles of pu-erh tea are similar to other teas, generally including alcohols, hydrocarbons, aldehydes, ketones, esters, nitrogenous

compounds, and phenolic compounds. Moreover, some special methoxy-phenolic compounds were also detected in pu-erh tea. The working together of these different categories of volatile compounds forms the unique, charming aroma, which makes pu-erh tea different from other teas.

The determination of volatile compounds usually includes an extraction step, which is followed by the analytical quantification of the individual compounds using gas chromatography–mass spectrometry (GC–MS). Over the past few decades, the conventional extraction methods applied by different researchers are vacuum hydrodistillation (Jumtee, Komura, Bamba, & Fukusaki, 2011), dispersive liquid–liquid microextraction (Sereshti, Samadi, & Jalali-Heravi, 2013), distillation–liquid/liquid extraction (Gu et al., 2010), and hydrodistillation (Tontul et al., 2012). Additionally, simultaneous distillation–extraction (SDE) is a technique applied with a Likens–Nickerson apparatus, which unites the advantages of liquid–liquid and steam distillation–extraction and has been widely used to the extraction of essential oils (Orav, Stulova, Kailas, & Müürisepp, 2004) and volatile compounds (Blanch, Reglero, & Herraiz, 1996) from numerous matrices. In terms of volatiles extraction, this technique shows the advantages over other traditional ones: adequate reproducibility and high efficiency (Gu et al., 2010). In recent years, a simple, rapid, solvent-free, and low cost technique named solid-phase microextraction (SPME) appeared. This technique is based on the distribution coefficient of analytes between the sample matrix, the gas phase, and the fiber coating with an adsorbent polar or apolar high polymer (Vichi, Guadayol, Caixach, López-Tamames, & Buxaderas,

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2007). Since the introduction of this technique, it has been widely used to analyze the volatile compounds of food, such as fruit (Cheong et al., 2010), bread (Poinot et al., 2007), sauce (Wei et al., 2013), and wine (Bonino et al., 2003).

Only one study was found in the literature about the volatile compounds of the entire set of pu-erh tea (Lv et al., 2012); that study coupled SPME technique using a 65 μm PDMS/DVB fiber with GC–MS for the identification of 66 volatile compounds in pu-erh tea. In our previous study, we found that the extraction efficiency of methoxyphenolic compounds from pu-erh tea was the best by using 75 μm CAR/PDMS fiber through comparing different SPME fibers (Du et al., 2013). Therefore, an appropriate fiber and optimized extraction conditions are essential for efficiently extracting and comprehensively analyzing the tea volatile components by using SPME method.

In the present work, four different fibers (PDMS, CAR/PDMS, PDMS/DVB, and DVB/CAR/PDMS) were compared in extracting the volatile compounds of pu-erh tea by using SPME method. The extraction conditions of the optimal fiber were then optimized. The optimal SPME procedure was finally compared with SDE method, and their advantages and limitations were analyzed. The final objective of this study is to identify and characterize new volatile compounds to obtain the widest volatile profile of pu-erh tea.

2. Materials and methods

2.1. Samples

Four pu-erh tea samples whose trademarks are 7572, 7262, 7562 and Jinzhenbailian (JZBL) obtained from Dayi Ltd., Inc. (Menghai, Yunnan, China) were used in this study. The tea samples were picked and processed in 2012. Before use, all of the tea samples were ground and processed in 2012. Before use, all of the tea samples were ground to pass through 30 mesh to 60 mesh and stored at room temperature in desiccators. The 7572, considered as the representative of pu-erh fermented tea, was selected for the optimization process.

2.2. Chemicals

The standards 1-octene-3-ol, benzyl alcohol, β -linalool, phenethyl alcohol, α -terpineol, isophytol, phytol, hexanal, (*E*)-2-hexenal, benzaldehyde, nonanal, β -cyclocitral, α -ionone, β -ionone, naphthalene, dimethyl phthalate, dihydroactinidiolide, methyl palmitate, 3,4-dimethoxytoluene, 1,2-dimethoxybenzene, 1,2,3-trimethoxy-5-methylbenzene, caffeine, linoleic acid, 1,2,3-trimethoxybenzene, 4-ethyl-1,2-dimethoxybenzene, indole, hexadecanoic acid, 1,2,4-trimethoxybenzene and 1,2,3,4-tetramethoxybenzene were purchased from Sigma-Aldrich, USA. A mixture of hydrocarbons ranging from heptane (C_7) to triacontane (C_{30}) was purchased from AccuStandard, Inc., USA. n-Pentane (analytical grade) was purchased from Sinopharm Chemical Reagent Co., China. Anhydrous sodium sulfate and sodium chloride (analytical grade) were purchased from Tianjin Chemical Reagents Company, China.

Four different coating fibers for SPME including polydimethylsiloxane (PDMS, 100 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) supplied by Supelco (Supelco, Inc., Bellefonte, PA, USA), which were preconditioned before the first extraction according to the manufacturer's recommendations.

2.3. Solid-phase microextraction

2.3.1. Extraction of volatile compounds

A total of 4 g pu-erh tea, 4.8 g NaCl, 16 mL of distilled water and a magnetic rotor were added into a 100 mL vial sealed with silicone septa (Sigma Chemical Co.), which was incubated at 60 $^{\circ}\text{C}$. After 15 min of sample conditioning, the SPME fiber was exposed in the headspace, placed for 60 min, and then immediately inserted into the

GC–MS injector port at 250 $^{\circ}\text{C}$ for 5 min. Each SPME process was performed in triplicate.

2.3.2. Evaluation of sensitivity and repeatability of the different fibers

Sensitivity for different categories of volatile compounds was evaluated on the basis of a parameter named “Cumulative Area Normalization Value (CANV)” (Tat, Comuzzo, Stolfo, & Battistutta, 2005), which was calculated by the absolute areas of different categories of the detected compounds. The calculation was as follows:

$$AV_K = \left[A_{K(100)} + A_{K(75)} + A_{K(65)} + A_{K(50/30)} \right] / 4$$

$$NA_{K(X)} = A_{K(X)} / AV_K$$

$$CA_{K(X)} = \sum_{n=1}^K NA_{n(X)}$$

K	Numbers of each category of volatile compounds
X	One of the four fibers: 100 μm PDMS, 75 μm CAR/PDMS, 65 μm PDMS/DVB, and 50/30 μm DVB/CAR/PDMS
AV_K	Average area of the compound “K” determined with the four fibers
$A_{K(X)}$	Absolute area of the compound “K” determined with the fiber “X”
$NA_{K(X)}$	Normalized area of the compound “K” determined with the fiber “X”
$CA_{K(X)}$	Cumulative area of one category of volatile compounds “from 1 to K” determined with the fiber “X”.

Not all the fibers were able to reveal the totality of volatile compounds. Therefore, we selected the analytes detectable (identified and unidentified) by all test fibers to evaluate the repeat performance of each fiber.

The relative standard deviations (RSD) of every selected compound adsorbed by each fiber were calculated, and the average value and standard deviation of RSD were also calculated from one fiber to another. The average value can be used as index of repeatability, and the standard deviation was an index of RSD variations.

GC area intensity of volatile compound is an important factor affecting the repeatability. Only the fiber showing an excellent repeatability for high area intensity of volatile compounds as well as low area intensity can be used for further test. Therefore, we grouped the detected compounds into two classes on the basis of the percentage of peak area: compounds with peak area higher than 1% were classified as class 1, whereas compounds with peak area lower than 1% were classified as class 2. Each fiber was replicated five times for the repeatability evaluations.

2.3.3. Optimization of the SPME procedure

To improve the extraction efficiency of volatile compounds, various experimental parameters including brewing proportion of tea and water (1:2, 1:3, 1:4, and 1:5), extraction temperature (50, 60, 70, and 80 $^{\circ}\text{C}$), extraction time (40, 50, 60, and 70 min) were optimized. By comparing the relative detector responses, the optimal extraction conditions were established as follows: extraction temperature, 70 $^{\circ}\text{C}$; extraction time, 60 min; brewing proportion of tea and water, 1:3.

2.4. Simultaneous distillation–extraction

A total of 20 g of grounded pu-erh tea was immersed in a flask with 250 mL of distilled water, and 40 mL of n-pentane was applied as organic solvent in another flask. Both flasks were placed in a Likens–Nickerson apparatus and heated up to their boiling points. Once the two flasks started to reflux, the distillation–extraction was continued for 4 h to allow the volatile components to collect in organic solvent. After the procedure, the extract was collected at room temperature

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