journal of food and drug analysis xxx (2017) 1–12

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Original Article

A comparative analysis for the volatile compounds of various Chinese dark teas using combinatory metabolomics and fungal solid-state fermentation

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ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form
14 November 2016
Accepted 21 November 2016
Available online xxx

Keywords:
Aspergillus niger
Eurotium cristatum
gas chromatography-mass spectrometry
methoxyphenolic compounds
Pu-erh tea

ABSTRACT

A total of 98 compounds including 20 aldehydes, eight arenes, six acids, 17 alcohols, 13 ketones, nine esters, nine methoxyphenolics, three alkenes, seven alkanes, and six other components were tentatively identified in six Chinese dark teas (CDTs) using gas chromatography–mass spectrometry. Multivariate statistical analysis revealed that dark teas from Yunnan and Guangxi provinces could be classified into one group, and other CDTs belonged to the other cluster. The diagnostic volatile compounds being responsible for CDTs' discrimination were observed as (*E*,*E*)-2,4-decadienal, methoxyphenolics, geraniol, *α*-terpineol, 2,4-heptadienal, *cis*-jasmone, linalool oxides, and 2-nonenal. Furthermore, mature tea leaves were separately fermented using *Eurotium cristatum* and *Aspergillus niger*. The results showed that *E. cristatum* increased the contents of *cis*-jasmone, *α*-terpineol, *ß*-ionone, nonanal, and 2-pentylfuran, whereas *A. niger* advanced the levels of geraniol, linalool oxides, 9,12-octadecadienoic acid, and *ß*-ionone after short-term fermentation. Fungus species may contribute to forming the flavor of Chinese dark teas by affecting the volatile compounds during postfermentation.

1. Introduction

Chinese dark tea (CDT) is a type of postfermented tea product from Southwestern China. CDTs usually possess significant geographical features. For example, ripened pu-erh tea is the typical CDT in Yunnan province. Furthermore, there are other famous CDTs in different regions, such as Ya'an Tibet tea (Sichuan dark tea; SCDT), Liubao tea (Guangxi dark tea; GXDT), Jingweifu tea (Shaanxi dark tea; SXDT), Fu-brick tea (Hunan dark tea; HNDT), and Qingzhuan tea (Hubei dark tea, HBDT)

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http://dx.doi.org/10.1016/j.jfda.2016.11.020

[1]. CDTs have been favored by the consumers because of their special flavors. They also contain some specific chemical constituents different from other unfermented or semi-fermented teas, such as green tea and oolong tea [2]. It has been reported that postfermentation produced the special secondary metabolites of dark teas [3]. Fungal fermentation could transfer the flavan-3-ols and L-theanine into 8-C-N-ethyl-2-pyrrolidinone substituted flavan-3-ols [4]. Also, the primary fungus is able to play an important role in the transformation of catechins during postfermentation [5]. Furthermore, some other B-ring oxidized flavan-3-ols have been identified in fu-brick tea [6]. These unique metabolites of catechins of CDTs are highly depended on the fermentation process and predominant fungi.

Traditionally, tea was classified according to the critical manufacture process, to be exact, the fermentation technology. For example, it could be classified as unfermented, semifermented, fully-fermented, and postfermented teas [7]. Fully-fermented tea (black tea) is oxidized by polyphenol oxidase(s) of tea leaves. The postfermentation process of CDTs could be depicted as a piling solid-state fermentation of deactivated tea leaves under natural or controlled conditions [8]. Some studies have demonstrated that there are multiple fungi species involved in postfermentation [9]. Different from other teas, the dark teas are more complicated in terms of flavor compounds because the tea ingredients can be highly affected by environmental microorganisms, temperature, and humidity.

To chemically distinguish CDTs, liquid chromatography coupled mass spectrometry combining metabolomics analysis has been applied in the reclassification of various CDTs [10]. The volatile compounds of some CDTs have been studied by comparing the teas' flavor compounds before and after postfermentation. It has been reported that methoxyphenolic compounds were the main volatile compounds of ripened pu-erh tea [11,12]. It was suggested that methoxyphenolic compounds were the metabolites of gallic acid and tannins after postfermentation. Furthermore, other types of CDTs, such as fu-brick tea, contained a high content of limonene. The unsaturated hydrocarbons in fu-brick tea usually gave a woody and fruity fragrance [13].

Although there are some studies regarding the volatile compounds of pu-erh tea and fu-brick tea, the systematic comparative study on various CDTs is still lacking. Furthermore, the effects of predominant microorganisms on the formation of the unique flavor of CDTs have not been studied in depth. Metabolomics has become a robust tool of non-targeted analysis of plant and bio-samples [14]. Integrative gas chromatography–mass spectrometry (GC-MS) and liquid chromatography coupled mass spectrometry analysis has been successfully applied in the research of primary and secondary metabolites in tea plants. Through the multivariate analysis, some marker volatile compounds were identified in different types of CDTs [10]. During the postfermentation, the fungi are very important for the transformation of compounds of CDTs. For example, *Aspergillus niger* is an important and main microorganism in the long-term fermentation of many CDTs rather fu-brick tea. Although the fungal fermentation caused a significant change of flavor in dark tea, very little is known about the effects of fungus on the transformation of raw volatile compounds of CDTs. In addition, the distinct aroma of CDT is a reflection of hundreds of chemicals, rather than a single flavor-active compound.

To explore the volatile compound profiling of different CDTs, a simultaneous distillation extraction (SDE) was used to extract the volatile compounds of tea samples, and subsequently subject to GC-MS analysis. The marker volatile compounds being responsible for the classification of various CDTs were characterized. Solid-state fermentation of deactivated tea leaves was also conducted by single fungus to study the trajectory of volatile compounds.

2. Methods

2.1. Tea samples

Yunnan dark tea (YNDT; pu-erh tea) was purchased from the Xishuangbanna, Yunnan province. HNDT (fu-brick tea) was purchased from the Anhua, Hunan province. HBDT (Qingzhuan tea) was produced in Chibi, Hubei province. GXDT (Liubao tea) was produced in Wuzhou, Guangxi province. SCDT (Tibet tea) was produced in Ya'an, Sichuan province. SXDT (Jingweifu tea) was produced in Xianyang, Shaanxi province. The green tea sample was used as control in the present study. Furthermore, the mature tea leaves of *Camellia sinensis* were used as the raw material for solid-state fermentation of single fungus.

2.2. Sample preparation

Six kinds of CDTs were extracted using SDE method. Briefly, for each kind of CDT, 15 g of tea leaves were placed in a 1-L flat-bottom flask containing 300 mL of boiling distilled water and immediately attached to the SDE apparatus. The flat-bottom flask containing tea was on one side of the Likens-Nickerson apparatus, and the flask containing solvents was on the other side. The solvent and tea infusions were heated via hot plates. Volatile compounds were extracted using diethyl ether for 90 minutes with three replications. After extraction, the extracts were dried using a small amount of anhydrous sodium sulfate and subsequently filtered. Then, the extract was concentrated to 1 mL. This concentrate was used for GC-MS.

During the SDE, 50 μL of ethyl decanoate was added into the tea sample as an internal standard, with the final concentration of 2.78 μg/g. The relative contents of volatile compounds were calculated with reference to the internal standard. For each tea sample, analysis was conducted in triplicate.

2.3. The solid-state fermentation by A. niger and E. cristatum

The solid-state fermentation of tea leaves single fungus referenced our previous study [5]. In brief, 5 mL of *A. niger* and *E. cristatum* spore suspension was incubated in potato dextrose agar plates with concentrations of 3.0–4.0 × 10^5/mL and 9.0–10.0 × 10^3/mL, respectively. The incubation condition was set at 30°C and 95% relative humidity. After 48 hours of

Please cite this article in press as: Cao L, et al., A comparative analysis for the volatile compounds of various Chinese dark teas using combinatory metabolomics and fungal solid-state fermentation, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2016.11.020