The effects of Exocarpium Citri Grandis on the volatile components of Green tea based on HS-SPME-GC-MS

Xiaorong Wu1,3, Juxian Wu1,3,*, Baoling Huang2, Haibin Chen1, Haohao Ling1 and Baiqi Huang1

1Guangdong Polytechnic of Science and Trade, Guangzhou, China
2Guangzhou College of Technology and Business, Guangzhou, China
3Guangdong Modern Lingnan Food Heritage Innovation R&D Center, Guangzhou, China

*Corresponding author: chenhb@stu.scau.edu.cn

Abstract. Aroma is an important factor in the quality of green tea. In order to explore the effect of Exocarpium Citri Grandis (ECG) on the aroma formation of green tea, the aroma components of green tea were extracted and identified by HS-SPME-GC-MS. The results showed that the main aroma components of green tea were D-Limonene (58.69%), γ-Terpinene (14.51%), Myrcene (9.82%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (3.98%) and caffeine (2.67%). After co-fermentation with ECG, the main aroma components of green tea were changed to D-Limonene (33.64%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (11.7%), γ-Terpinene (9.38%), 2-pentadecanone (8.93%) and caffeine (6.04%). The content of terpenes such as D-Limonene decreased, while the content of aromatic components such as linalool increased, and the types of volatile components increased in green tea. Among the newly added volatile components, the higher ones were 2-pentadecanone (8.93%), β-ionone (2.76%) and dihydroactinidiolide (2.17%), which can give green tea fruity, violet and coumarin aroma. This study indicated that ECG contributes to the enrichment of greentea aroma, which provides theoretical support for the development of mixed tea with unique aroma.

1. Introduction
Green tea is popular among consumers because of its pleasant flavor and quality. The aroma of green tea is an important factor in evaluating the quality and influencing the price of tea [1]. The volatile components of green tea mainly include some aromatic substances with different chemical properties and content, including hydrocarbons, alcohols, ketones, acids, aldehydes, etc., which combined together to form different flavor types in different concentrations [2].

The typical green tea flavor is fragrant, floral, chestnut fragrance [3]. Previous research shown that the main aroma components and content of tea were significantly different due to cultivation conditions, processing technology, storage time [4].

Exocarpium Citri Grandis (ECG) is the immature or nearly mature dry outer pericarp of Citrrus Grandis Osbeck. In addition to bringing rich aroma, ECG also shows pharmacological effects such as moisturizing the liver, relieving cough, and anti-oxidation [5].
The co-fermentation of green tea and ECG is conducive to the formation of a new type of mixed tea that integrates the aroma of tea and ECG, thus improving the quality of green tea. However, there are few studies on the flavor changes of mixed tea, which need to be further studied. Based on HS-SPME-GC-MS, this study analyzed the volatile components of green tea and mixed tea, laying a foundation for the development of a mixed tea with more abundant and unique aroma components.

2. Materials and Methods

2.1. Instruments and materials
QP2010 gas-mass spectrometer (Shimadzu, Japan); Rxi-5Sil MS quartz capillary column (30 m×0.25 mm, 0.25 μm) (Restek, USA); Manual solid phase micro-extraction sampler with 50/30μm DVB/CAR/PDMS extraction fiber head (Supelco, USA); 10mL headspace bottle (Supelco, USA); ECG and the green tea were provided by Guangzhou Wenshan Tea Co., Ltd.

2.2. GC-MS analysis conditions

Chromatographic conditions: Helium at a flow rate of 1.0 mL min⁻¹ was used as the carrier gas. Splitting ratio was 20:1. The injector and interface were operated at 250 °C and 280 °C, respectively. The oven program was set at 50 °C for 5 min at first, increased to 120 °C at 6 °C min⁻¹ (held for 5 min), then further increased to 160 °C at 3 °C min⁻¹ (held for 3 min), finally increased to 220 °C at 10 °C min⁻¹ (held for 10 min)[6].

Mass spectrometry conditions: MS was operated in electron ionization mode (70 eV), with 230 °C ion source temperatures. Analysis was performed in full scan mode ranging from m/z 45 to 450.

2.3. Sample preparation
Grind the teas and filter them with a 24-mesh strainer. 0.1 g ground sample was put into headspace bottle and analyzed by "2.2" GC-MS system. After balanced at 80 °C for 60 min, manual sampler with extraction fiber head was inserted into the headspace bottle and extracted for 50 min. Then the extraction fiber head was quickly inserted into the injection port of the chromatograph and desorbed for 5 min.

2.4. Data analysis
Automatic mass spectrometry deconvolution and identification system was used to preprocess the data, including peak detection, deconvolution, chromatographic alignment and normalization. By comparing with the standard mass spectrometry in NIST17 database, volatile components were identified and their relative contents were calculated by area normalization method.

3. Results and analysis
The HS-SPME-GC-MS analysis and NIST17 mass spectrometry retrieval were performed to detect and identify the volatile components in green teas. The resulting total ionic current diagram and the relative contents of each volatile component were showed in Figure 1 and Table 1.
A total of 16 volatile components were identified in green tea, among which components with higher content were D-Limonene (58.69%), γ-Terpinene (14.51%), Myrcene (9.82%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (3.98%) and caffeine (2.67%), respectively, accounting for 89.67%.

30 volatile components were detected in ECG co-fermented green tea, among which D-Limonene (33.64%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (11.7%), γ-Terpinene (9.38%), 2-pentadecanone (8.93%) and caffeine (6.04%) were the components with higher content, accounting for 69.69%. Compared with the control group, the content of terpenes with floral and fruit aroma, such as D-Limonene and γ-Terpinene, decreased significantly, while the content of linalool with rose-scented

Figure 1. Total ion current diagram (A-control green tea; B-co-fermented green tea)
and woody fragrance increased from 0.57% to 2.01%, and the content of α-farnesene with floral fragrance increased from 0.93% to 1.31%.

Table 1. Volatile compounds of teas identify by HS-SPME-GC-MS

| No. | tR/min | CAS         | Relative content/% | Similarity (%) |
|-----|--------|-------------|--------------------|----------------|
|     |        |             | A                  | B             | A               | B               |
| 1   | 8.574  | 7785-70-8  | 0.36               | /             | 96             | /               |
| 2   | 10.033 | 127-91-3   | 0.38               | /             | 96             | /               |
| 3   | 10.488 | 123-35-3   | 9.82               | 3.07          | 96             | 96              |
| 4   | 11.587 | 527-84-4   | 1.53               | 0.62          | 95             | 94              |
| 5   | 11.684 | 5989-27-5  | 58.69              | 33.64         | 96             | 96              |
| 6   | 12.570 | 99-85-4    | 14.51              | 9.38          | 97             | 97              |
| 7   | 13.404 | 29050-33-7 | 0.56               | 0.29          | 96             | 93              |
| 8   | 13.855 | 78-70-6    | 0.57               | 2.01          | 97             | 96              |
| 9   | 14.084 | 5337-72-4  | /                  | 0.90          | /              | 84              |
| 10  | 15.865 | 39028-58-5 | 0.99               | 0.15          | 97             | 88              |
| 11  | 16.052 | 562-74-3   | /                  | 0.38          | /              | 85              |
| 12  | 16.437 | 98-55-5    | /                  | 1.70          | /              | 96              |
| 13  | 17.041 | 432-25-7   | /                  | 0.15          | /              | 85              |
| 14  | 24.029 | 629-59-4   | /                  | 0.30          | /              | 92              |
| 15  | 24.796 | 87-44-5    | 0.67               | /             | 96             | /               |
| 16  | 24.974 | 127-41-3   | /                  | 0.35          | /              | 91              |
| 17  | 26.753 | 3891-99-4  | /                  | 0.31          | /              | 92              |
| 18  | 27.500 | 79-77-6    | /                  | 2.76          | /              | 83              |
| 19  | 27.509 | 23986-74-5 | 2.92               | /             | 93             | /               |
| 20  | 28.168 | 473-13-2   | 0.26               | /             | 91             | /               |
| 21  | 28.676 | 502-61-4   | 0.93               | 1.31          | 96             | 96              |
| 22  | 29.495 | 17092-92-1 | /                  | 2.17          | /              | 97              |
| 23  | 29.921 | 31295-56-4 | /                  | 0.32          | /              | 83              |
| 24  | 31.113 | 2306-78-7  | /                  | 0.38          | /              | 85              |
| 25  | 31.247 | 638-36-8   | /                  | 0.38          | /              | 83              |
| 26  | 31.517 | 2882-96-4  | /                  | 0.74          | /              | 95              |
| 27  | 32.073 | 6846-50-0  | 3.98               | 11.70         | 96             | 96              |
| 28  | 32.808 | 19870-75-8 | /                  | 0.95          | /              | 82              |
| 29  | 39.775 | 6418-44-6  | /                  | 0.28          | /              | 94              |
| 30  | 41.461 | 58-08-2    | 2.67               | 6.04          | 92             | 95              |
| 31  | 41.543 | 502-69-2   | /                  | 8.93          | /              | 89              |
| 32  | 41.965 | 33925-72-3 | /                  | 1.03          | /              | 81              |
| 33  | 42.315 | 102608-53-7| /                  | 0.58          | /              | 85              |
| 34  | 42.731 | 630-01-3   | /                  | 0.28          | /              | 86              |
| 35  | 43.154 | 112-39-0   | /                  | 0.41          | /              | 94              |

ECG can not only increase the content of aroma components, but also increase their variety. 20 new volatile components were added into green tea by co-fermented with ECG, among which the higher contents were 2-pentadecanone (8.93%), β-ionone (2.76%) and dihydroactinidiolide (2.17%), 11-tetradecyne-1-ylocetate (1.03%) and 8-propanoyldecan (0.95%). Research have shown that 2-pentadecanone can show fruity aroma in tea, while β-ionone shows violet and woody aroma and dihydroactinidiolide shows coumarin aroma [7].
4. Conclusion
In this study, the volatile components of green tea were analyzed by HS-SPME-GC-MS, and the effect of ECG on the aroma of green tea was further illustrated by comparison. Terpenes are one of the main characteristic flavor components of green tea, and they are involved in forming the flowery and fruity aroma in chestnut fragrance [8]. After co-fermentation, although the content of terpenes such as D-Limonene and γ-Terpinene decreased significantly, the content of other aroma components such as linalool increased. At the same time, the aroma of green tea was enriched by the addition of new aroma components such as 2-pentadecanone. The result demonstrates that ECG can increase the content and variety of aroma components in green tea, resulting in attractive aroma of green tea.

With the improvement of people’s living standard, mixed tea with unique flavor has attracted more and more attention. In the future, the key to volatile component formation can be further investigated by determine the optimal fermentation conditions for ECG and green tea, providing technical and theoretical support for the industrial production of mixed green tea.

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On 1, 2, 3 and 4 are co-first authors.

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