Analysis of Volatile Components of Pu-Erh Ripe Tea Fermented with Exocarpium Citri Grandis by Hs-Spme-Gc-Ms

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Abstract: Aroma is an important reference index of tea quality. In this paper, we analyzed the volatile components extracted by Pu-erh ripe tea fermented with Exocarpium Citri Grandis (ECG). The HS-SPME-GC-MS analysis results showed that most of the volatile components of Pu-erh ripe tea increased significantly after co-fermented with ECG, especially D-Limonene. At the same time, 17 new volatile components were found in co-fermented tea, of which the predominant volatile compounds were (+)-delta-cadinene, (0.52%), and followed by 11-tetradecyne-1-ylacetate (0.51%), γ-Muurolene (0.41%), (1alpha,2alpha,5alpha)-2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hexan-2-ol (0.39%) and (+)-γ-Cadinene (0.34%). Our results demonstrated that co-fermentation with ECG was beneficial to the formation of the aroma of Pu-erh ripe tea, which provided an important reference for the innovative development and research of mixed fermented tea.

1. Introduction

With the deepening of research, more and more people are aware of the health benefits of tea, and the market demand for tea is also gradually expanding. In the tea market, the aroma of tea is an important factor affecting the quality and price of tea. Tea fragrance is produced by volatile aromatic substances in tea, its content in tea, although small, but can bring pleasant aroma. There are many factors affecting the aroma of tea, such as place of production, variety, planting conditions, picking time, tea making technology, storage method and time, etc. Good tea aroma can not only provide sensory and psychological pleasure, but also bring better economic value.

Exocarpium Citri Grandis (ECG), a well-known traditional Chinese medicine, is made from the dried unripe or ripe fruit peel of Citrus grandis Osbeck or Citrus grandis Osbeck var [1,2]. ECG produced in Huazhou City, Guangdong Province is very popular in the market because of its unique efficacy. Thanks to those compounds such as monoazole and pyrithylene, ECG has a good aroma. At the same time, relevant studies show that ECG has good medicinal value, such as quickly decanting alcohol and relieving the body discomfort when drunk [3], with good sputum effect [4] and antioxidant health function [5].

In this study, we explored the changes of volatile components of Pu-erh ripe tea after co-fermentation with ECG by HS-SPME-GC-MS, aiming to lay the foundation for the future development of a new type of mixed fermented tea with good aroma.

2. Materials and Methods

2.1 Materials and Reagents.

GCMS-QP2010 SE (Shimadzu, Japan). Manual solid phase microextraction device (Supelco, USA). Divinylbenzene / carboxen / polydimethylsiloxane (DVB / CAR / PDMS, 50/30 μm), 10mL headspace bottle (Supelco, USA). ECG was purchased from Huazhou City, Guangdong, and the tea was provided by Guangzhou Wenshan Tea Co., Ltd.

2.2 Gc-Ms Analysis and Identification for Volatile Components

Chromatography conditions. Rxi-5Sil MS Quartz capillary column (30m×0.25 mm, 0.25 μm, Restek, USA); High pure helium (99.999%) was the carrier gas set at a constant flow rate of 1mL· min^{-1}. The injection was performed by split mode with a split ratio of 20:1. The injection port temperature was 250 °C, and the interface temperature was set at 280 °C. The oven temperature program was as follows: column temperature was set at 50 °C initially for 5 minute, increased to 120 °C at the heating rate of 6 °C/min and was maintained for 5.0 min, increased to 160 °C at the heating rate of 3 °C/min and was maintained for 3.0 min, then increased to 220 °C at the heating rate of 10 °C/min and was maintained for 10.0 min [6,7].

Mass spectrometry conditions. Ion source temperatures was set at 230 °C. 70 eV of EI was adopted, and the mass scanning range was set from 45 to 450 atomic mass units in full scan.

Sample Preparation. The collected mixed fermented teas were ground into fine powder and flitted with 24-mesh filter. Put 0.1 g of ground tea sample into a sealed headspace bottle of 10 mL and analyzed using pretreated GC–MS system. The sample was balanced at 80 ℃ for 60 min, then the manual sampler with a 50/30 um DVB/CAR/PDMS extraction fiber head was inserted into the headspace bottle, and the extraction head was extracted for 50 min. Took out the manual sampler and inserted it into the injection port of the chromatograph immediately, desorption for 5 min [8].

Compound identification. All of the samples were represented by a GC–MS total ion chromatogram (TIC), and the peak areas of compounds in the chromatogram were integrated after GC–MS analysis. The identification of Volatile Components was achieved by comparing the mass spectrum with an authentic standard mass spectrum in National Institute of Standards and Technology (NIST) reference library. Those peaks with a similarity index more than 80% were the assigned compound names.

3. Results and Analysis

The separated volatile components of ECG co-fermented Pu-erh ripe tea were identified by HS-SPME-GC-MS, and determined by peak area normalization method. In the TIC of the tested tea sample, the peak was identified by NIST searching. 59 common compounds were identified from Pu-erh ripe tea and co-fermented tea samples of Pu-erh ripe tea and ECG. The information of these compounds is listed in Table 1, and the total ion current diagram is shown in Figure 1.

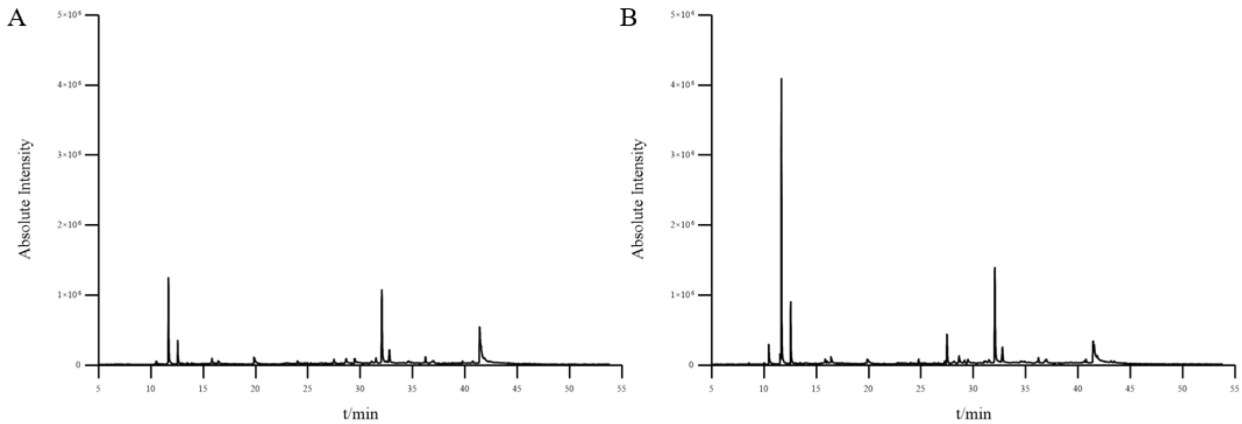

Fig.1 Total Ion Chromatograms of Different Teas by Hs-Spme/Gc–Ms

(A. Pu-erh ripe tea; B. Pu-erh ripe tea fermented with ECG)

In total, forty-one volatile compounds were identified and the predominant volatile compounds were 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (24.01%), followed by D-Limonene (16.68%), Caffeine (13.84%), 2-Pentadecanone, 6,10,14-trimethyl- (9.35%), Cedrol (5.29%) and γ-Terpinene (5.08%).

46 kinds of volatile substances were detected in ECG co-fermented Pu-erh ripe tea. After co-fermentation, there was almost no change in the types of higher volatile substances, namely

D-Limonene (30.54%), 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (18.10%), γ-Terpinene (12.563%), 2-Pentadecanone, 6,10,14-trimethyl- (6.46%), Germacrene D (5.44%) and Caffeine (4.55%).

Table 1 Volatile Compounds Commonly Detected in Ecg Co-Fermented Pu-Erh Ripe Tea by Hs-Spme/Gc-Ms

No.	t_R/min	CAS	Relative content /%		No.	t_R/min	CAS	Relative content /%	
			A	B				A	B
1	8.579	7785-70-8	0.10	-	31	31.066	40716-66-3	0.34	0.38
2	10.033	18172-67-3	0.14	-	32	31.230	629-94-7	-	0.45
3	10.469	123-35-3	2.92	0.97	33	31.501	2882-96-4	0.77	1.36
4	11.556	527-84-4	1.29	0.24	34	31.750	1139-30-6	0.15	-
5	11.676	5989-27-5	30.54	16.68	35	32.062	6846-50-0	18.10	24.01
6	12.563	99-85-4	7.33	5.08	36	32.437	19780-33-7	-	0.16
7	13.399	29050-33-7	0.21	-	37	32.610	1560-89-0	-	0.15
8	13.472	5989-33-3	0.15	-	38	32.788	77-53-2	3.44	5.29
9	13.904	78-70-6	0.30	-	39	34.609	3892-00-0	0.83	0.66
10	13.923	78-70-6	-	0.10	40	34.880	6785-23-5	-	0.43
11	15.744	39028-58-5	0.13	0.25	41	34.893	2883-05-8	0.15	-
12	15.850	14049-11-7	0.56	1.23	42	35.025	1560-92-5	-	0.15
13	16.040	17699-16-0	0.39	-	43	35.278	630-02-4	0.19	-
14	16.419	98-55-5	1.36	0.86	44	35.285	13475-75-7	-	0.17
15	19.710	540-97-6	-	0.12	45	36.845	629-78-7	0.33	0.44
16	19.888	634-36-6	1.48	2.41	46	36.993	1921-70-6	1.14	1.20
17	22.809	3856-25-5	0.17	-	47	39.775	6418-44-6	0.15	0.55
18	24.020	629-59-4	0.31	0.83	48	40.611	593-45-3	0.29	0.25
19	24.786	87-44-5	0.82	0.13	49	40.767	638-36-8	0.71	0.64
20	25.253	18252-44-3	0.33	-	50	41.453	58-08-2	4.55	13.84
21	27.274	30021-74-0	0.41	-	51	41.540	502-69-2	6.46	9.35
22	27.280	107-50-6	-	0.15	52	41.853	84-69-5	1.35	1.87
23	27.497	23986-74-5	5.44	1.26	53	41.960	33925-72-3	0.51	-
24	28.151	24703-35-3	0.33	-	54	42.319	102608-53-7	0.12	0.06
25	28.535	629-62-9	0.25	0.20	55	43.154	112-39-0	0.23	-
26	28.643	502-61-4	1.79	1.75	56	43.469	505-32-8	-	0.06
27	28.906	39029-41-9	0.34	-	57	43.475	142-50-7	0.10	-
28	29.154	483-76-1	0.52	-	58	43.580	85-69-8	-	0.20
29	29.479	17092-92-1	0.74	1.90	59	44.304	7098-22-8	-	0.07
30	30.730	25117-31-1	-	0.23					

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4. Conclusions

In this study, HP-SPME-GC-MS was used to analyze the volatile components of Pu-erh ripe tea, further clarifying the characteristic aroma of Pu-erh ripe tea, mainly 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (24.01%), D-Limonene (16.68%), Caffeine (13.84%), 2-Pentadecanone, 6,10,14-trimethyl- (9.35%), Cedrol (5.29%) and γ-Terpinene (5.08%).

The characteristic aroma components of ECG were terpenes, including monoterpenes and sesquiterpenes vinyl and D-Limonene was of the highest content in monoterpenes [9]. This article proves that ECG can significantly increase the content of terpene compounds such as limonene in

Pu-erh ripe tea. Moreover, seventeen new volatile components were found in co-fermented tea, which may be the reason why co-fermented tea has a more charming aroma.

Co-fermentation with ECG is beneficial to the formation of the aroma of Pu-erh ripe tea, which provides an important reference for the innovative development and research of mixed fermented tea. In the future, it is necessary to study the mixing ratio and fermentation conditions of ECG and Pu-erh ripe tea, and improve the flavor quality of mixed fermented tea by controlling the key conditions for the formation of volatile components.

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