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Abstract. Flavor, bioactivity, and commercial value are different in various kinds of tea. To improve the aroma and quality of Baihaoyinzhen white tea (BHYZ), different BHYZ was prepared by co-fermented with Vanilla (BHYZ-V) or Citri Grandis Exocarpium (BHYZ-C). The results showed that the volatile components of BHYZ were significantly changed after co-fermented, when HS-SPME-GC-MS was used for analysis. Thirty-nine, thirty-eight, and forty-four volatile compounds were identified by MS similarity in BHYZ, BHYZ co-fermented with Vanilla and BHYZ co-fermented with Citri Grandis Exocarpium (CGE), respectively. The volatile components in BHYZ-V with high content were similar to the BHYZ, but the content of most components increased. However, twenty-eight volatile components were only found in BHYZ-C, of which the predominant aroma compounds were D-limonene (39.28%), γ-terpinene (9.03%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (8.51%), linalool (7.25%), and germacrene D (4.61%). The results indicated that CGE enhanced aroma characteristic of BHYZ rather than Vanilla, which would be a feasible way to improve the quality of BHYZ.

Keywords: HS-SPME-GC-MS, baihaoyinzhen white tea, vanilla, citri grandis exocarpium, volatile component.

1. Introduction

Baihaoyinzhen white tea (BHYZ) is a kind of slightly fermented tea, which is processed only by sun-drying or heat-drying without killing or generally kneading. In China, BHYZ is one of the most popular tea for its unique flavor and potential health benefits [1].

Citri Grandis Exocarpium (CGE) is also one kind of famous traditional Chinese medicine with good therapeutic effect for relieving cough and reducing phlegm [2], which is made from the dried unripe or ripe fruit peel of Citrus grandis Osbeck. Previous research shown that the volatile components in CGE were mainly monoterpenes and their derivatives [3].

Vanilla, one of the world's most popular flavoring materials, has extensive applications in food, beverages, perfumery and pharmaceutical industry because of its unique fragrance [4]. Interestingly, Vanilla also has an appetite-enhancing effect [5].

In this study, we prepared two kinds of BHYZ co-fermented with CGE (BHYZ-C) or Vanilla (BHYZ-V), respectively, and measured the aroma component by HS-SPME-GC-MS in order to compare their difference, which would provide a good way to enhance the fragrance of BHYZ.

2. Materials and Methods

2.1. Materials and Reagents

QP2010 gas-mass spectrometer with NIST17 mass database (Shimadzu, Japan); Rxi-5Sil MS Quartz capillary column (30 m×0.25 mm, 0.25 μm) (Restek, USA); Manual solid phase microextraction device with extraction fibers (Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm)) (Supelco, USA); 10 mL headspace bottle (Supelco, USA); BHYZ, CGE and Vanilla were provided by Guangzhou Wenshan Tea Co., Ltd.

2.2. GC-MS Analysis

2.2.1. Sample Preparation. Sample was ground into fine powder and flitted with a 24-mesh filter. 0.1 g sample was put into a 10 mL headspace bottle and balanced at 80 ℃ for 60 min. Then a 50/30 um DVB/CAR/PDMS extraction fiber was inserted into the headspace bottle by anual solid phase microextraction device for extraction for 50 minutes. Finally, extraction fiber was took out and inserted into the injection port of the GC-MS immediately, and desorbed for 5 minutes [6].

2.2.2. Chromatography conditions. High purity helium (99.999%) was used as a carrier gas with a constant flow rate of 1mL/min. The sample was injected in the split mode with a split ratio of 20:1. The inlet temperature was 250 °C, and the interface temperature was 280°C. Briefly, the following oven temperature program was as follow: 50 °C for 5 min, 50 to 120 °C at 6 °C/min, 120 °C for 5 min, 120 to 160 °C at 3 °C/min, 160 °C for 3 min, 160 to 220 °C at 10 °C/min, and finally, 220 °C for 10 min [7].

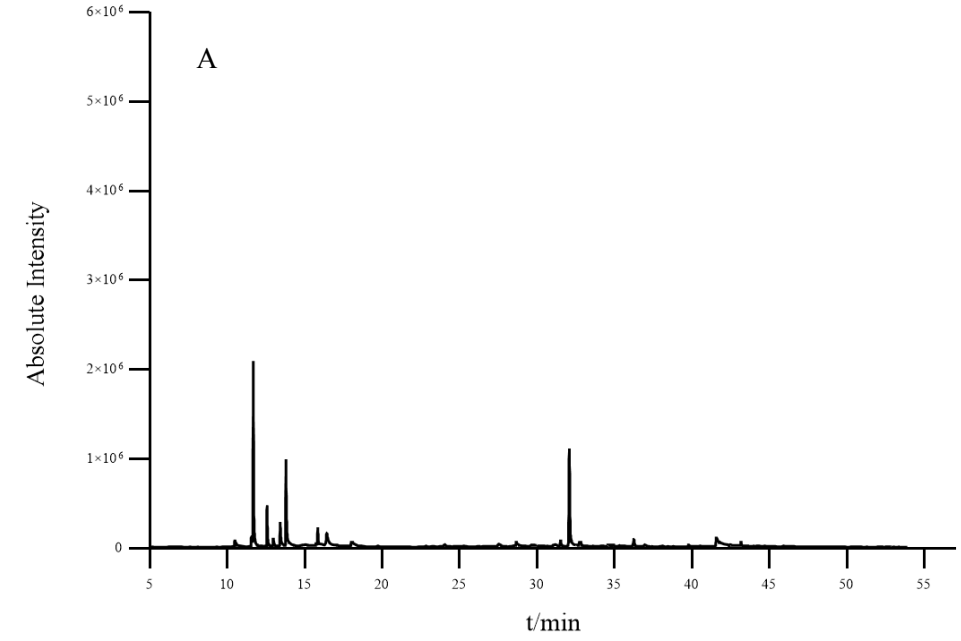
2.2.3. Mass spectrometry conditions. The ion source temperature was 230 °C, the ionizing voltage was 70 eV, and the analyses were performed in scan mode from 45 to 450 atomic mass units.

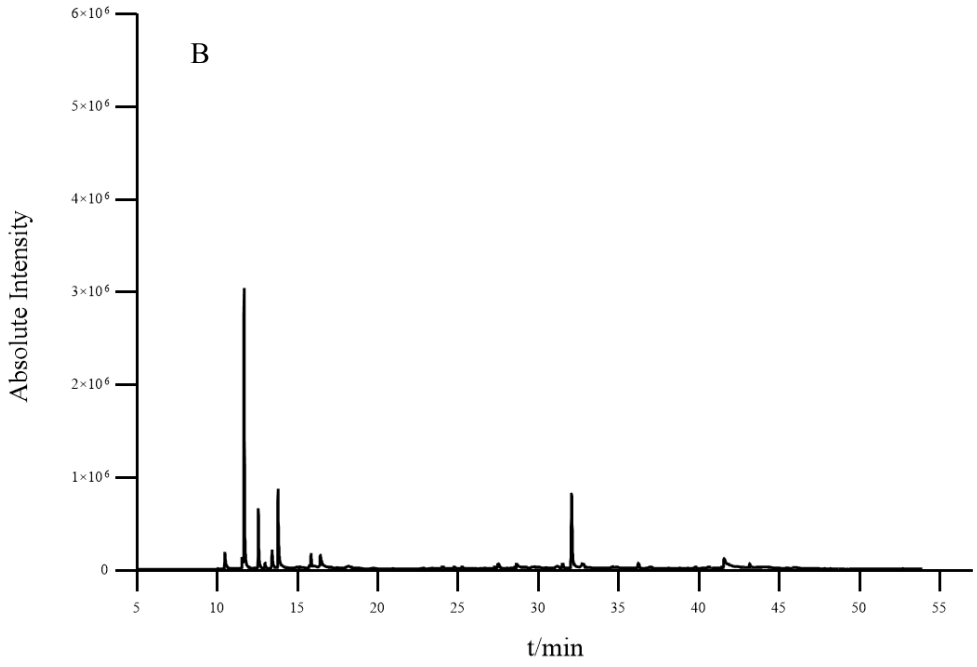
2.3. Analysis and Identification of Volatile Components

Volatile compound was identified by comparing the tested mass spectrum with the standard mass spectrum in NIST17 database, and its relative content was computed by area normalization method.

3. Results and analysis

In order to study the aroma characteristics, HS-SPME-GC-MS was used to detect and identify the volatile components in different BHYZ samples. Total ion chromatograms and the relative content of the identified compounds were showed in Fig. 1 and Tab. 1, respectively.


A
Absolute Intensity
6×10⁶
5×10⁶
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
5
10
15
20
25
30
35
40
45
50
55
t/min


B
Absolute Intensity
6×10⁶
5×10⁶
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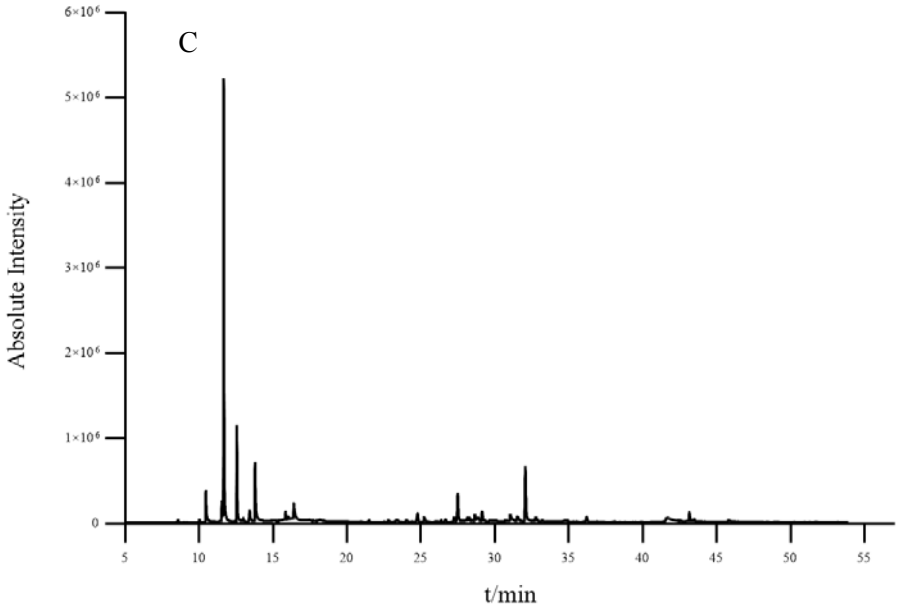

Figure 1. Total ion chromatograms (A: BHYZ; B: BHYZ-V; C: BHYZ-C)

Thirty-nine volatile compounds were identified in BHYZ, among which compounds with higher content were D-limonene (25.29%), 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (21.67%), linalool (14.33%), γ-terpinene (6.04%), 2-Furanmethanol, 5-ethenyltetrahydro-α, α,5-trimethyl-, cis- (4.04%). These five compounds accounted for 71.37% of total relative content of volatile components in BYHZ.

Totally, thirty-eight volatile substances were detected in BHYZ-V. The volatile components with higher content were similar to the BHYZ, such as D-limonene (32.44%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (14.14%), linalool (11.40%), γ-terpinene (7.36%), β-myrcene (3.55%). Inherently, the content of D-limonen, γ-terpinene, β-myrcene increased significantly, compared with BHYZ.

Notably, forty-four volatile compounds were identified in BHYZ-C, and the predominant volatile compounds were D-limonene (39.28%), γ-terpinene (9.03%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (8.51%), linalool (7.25%), and germacrene D (4.61%). Compared with BHYZ, germacrene D, α-Terpineol, caryophyllene and (+)-δ-cadinene (1.45%) were new fragrance substances added into BHYZ by co-fermented with CGE.

Table 1. Volatile compounds detected in three kinds of BHYZ by HS-SPME-GC-MS

No.	t_R/min	CAS	Relative content/%			No.	t_R/min	CAS	Relative content/%		
			A	B	C				A	B	C
1	8.572	7785-70-8	—	—	0.17	38	28.146	473-13-2	—	—	0.94
2	10.028	127-91-3	—	—	0.22	39	28.297	10208-80-7	—	—	0.49
3	10.460	123-35-3	1.71	3.55	3.89	40	28.540	629-62-9	0.07	—	—
4	11.335	586-62-9	—	—	0.08	41	28.634	502-61-4	1.08	1.13	1.43
5	11.409	18172-67-3	—	—	0.04	42	28.898	17334-55-3	—	—	0.57
6	11.539	527-84-4	1.75	1.48	2.36	43	29.140	483-76-1	—	0.34	1.45
7	11.671	5989-27-5	25.29	32.44	39.28	44	29.688	629-94-7	—	0.49	—
8	12.456	138-86-3	—	—	0.03	45	31.045	40716-66-3	0.80	0.85	1.37
9	12.554	99-85-4	6.04	7.36	9.03	46	31.235	112-95-8	—	0.50	—
10	12.979	34995-77-2	1.30	0.80	0.37	47	31.250	1560-92-5	0.36	—	—
11	13.420	5989-33-3	4.04	2.84	1.70	48	31.500	2882-96-4	1.10	0.74	—
12	13.787	78-70-6	14.33	11.40	7.25	49	31.731	1139-30-6	—	—	0.30
13	15.738	14049-11-7	—	0.32	—	50	32.055	6846-50-0	21.67	14.14	8.51
14	15.739	39028-58-5	0.40	1.96	0.17	51	32.606	3891-99-4	0.13	—	—
15	15.840	14049-11-7	2.84	—	0.94	52	32.765	544-76-3	0.55	0.70	—
16	16.021	562-74-3	0.46	0.54	0.76	53	32.784	19870-75-8	—	—	0.83
17	16.226	60-12-8	0.08	0.21	—	54	32.792	77-53-2	1.04	0.64	—
18	16.263	35907-10-9	—	—	0.32	55	34.632	3892-00-0	0.39	0.28	—
19	16.401	98-55-5	—	—	3.56	56	34.870	1795-21-7	—	0.23	—
20	16.422	119-36-8	3.90	—	—	57	36.992	1921-70-6	—	0.40	—
21	18.069	106-24-1	0.65	0.52	—	58	38.126	2425-77-6	0.20	—	—
22	19.715	540-97-6	0.07	—	—	59	39.760	6418-44-6	0.47	0.49	—
23	21.489	17699-14-8	—	—	0.24	60	40.608	593-45-3	—	0.16	—
24	22.807	3856-25-5	—	—	0.41	61	40.619	629-78-7	0.14	—	—
25	23.365	54324-03-7	—	—	0.23	62	40.769	638-36-8	—	0.23	—
26	23.433	33880-83-0	—	—	0.18	63	41.430	504-96-1	—	0.11	0.03
27	24.013	629-59-4	0.70	0.46	0.17	64	41.556	58-08-2	2.75	2.57	—
28	24.335	475-20-7	0.12	—	—	65	41.557	502-69-2	—	—	0.24
29	24.772	87-44-5	—	0.37	1.49	66	41.875	84-69-5	—	0.48	—
30	25.237	18252-44-3	—	0.24	0.76	67	41.968	6785-23-5	0.28	0.62	—
31	26.354	6753-98-6	—	—	0.15	68	43.145	112-39-0	0.65	0.62	1.18
32	26.661	157477-72-0	—	—	0.29	69	43.474	20016-73-3	—	—	0.16
33	27.249	30021-74-0	—	—	0.77	70	43.872	18435-22-8	0.05	—	—
34	27.489	23986-74-5	—	1.63	4.61	71	44.023	20016-72-2	—	—	0.04
35	27.506	14901-07-6	0.61	—	—	72	44.306	630-03-5	0.10	—	—
36	27.596	50464-95-4	0.42	—	—	73	45.804	2462-85-3	0.09	0.14	0.11
37	28.003	39029-41-9	—	0.25	0.38						

Note: A: BHYZ; B: BHYZ-V; C: BHYZ-C

4. Conclusions

After co-fermentation, volatile components of BHYZ verified significantly, especially in BHYZ-C, which indicated that CGE was much beneficial to the formation of aroma in BHYZ. The results showed that the main aroma substances added into BHYZ by co-fermented with CGE were germacrene D (4.61%) and α-terpineol (3.56%). Germacrene D is the characteristic aroma component of lychee, white flower salvia, and mint [8], and α-terpineol, naturally presenting in plant species, has a pleasant odor like lilacs [9].

Generally, results of this work indicated that Vanilla and CGE had a positive effect on increasing the number and content of aroma component in BHYZ, and CGE was much better. However, the best co-fermentation method for BHYZ and CGE is still not clear. Therefore, further studies are necessary to focus on the fermentation process and evaluation criteria of the fragrance.

Acknowledgments

This work was financially supported by 2020 Provincial Higher Vocational Education Teaching Reform Research and Practice Project Higher Vocational Enrollment Special Project (No. 200413114559687) fund.

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References

[1] Junjie Zh, Xuehong W, Weidong D, et al. Study of enrichment difference of 64 elements among white tea subtypes and tea leaves of different maturity using inductively coupled plasma mass spectrometry [J]. Food Res Int, 2019, 126:108655.
[2] Zhipeng S, Yaru Q, Kai Zh, et al. Inclusion Complex of Exocarpium Citri Grandis Essential Oil with beta-Cyclodextrin: Characterization, Stability, and Antioxidant Activity [J]. J Food Sci, 2019, 84 (6): 1592-1599.
[3] Zhiting Zh, Hao W, Weiwei S, et al. Effects of Total Flavonoids from Exocarpium Citri Grandis on Air Pollution Particle-Induced Pulmonary Inflammation and Oxidative Stress in Mice [J]. J Food Sci, 2019, 84 (12): 3843-3849.
[4] Arun K S, Upendra K S, Nandini S. A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents [J]. Int J Food Sci Nutr, 2008, 59(4):299–326.
[5] Kakuyou, Ogawa, Akira. Appetite-enhancing effects of vanilla flavours such as vanillin [J]. J Nat Med, 2018, 72 (3):798–802.
[6] Kazutoshi S, Kenichi T, Yoshihiro Y, et al. Characteristic Odor of the Japanese Liverwort (Leptolejeunea elliptica) [J]. J Oleo Sci, 2020, 69 (7): 767-770.
[7] Xiao X, Siduo Zh, David J M, et al. Multistarter fermentation of glutinous rice with Fu brick tea: Effects on microbial, chemical, and volatile compositions [J]. Food Chem, 2020, 309:125790.
[8] Nonghui J, Wei L, Peiyuan Y, et al. Analysis of Volatile Components of the'Yujinqiu' Litchi Pulp by Means of Solid-phase Microextraction Coupled with GC-MS [J]. Acta Agriculturae Universitatis Jiangxiensis, 2016, 38 (05): 829-835.
[9] Christina K, Nurhayat T, Gerhard B. α-terpineol, a natural monoterpene: A review of its biological properties [J]. Open Chem, 2018, 16(1): 349-361.