Comparative analysis of aroma substances of vanilla co-fermented black tea

Juxian Wu1,2, Xiaorong Wu1,2, Guoan Yuan1, Xuexun Chen1,2, Xiaoyan Shi3, Haibing Chen1 and Baiqi Huang1, *

1Guangdong Polytechnic of Science and Trade, Guangzhou, China
2Guangdong Modern Lingnan Food Heritage Innovation R&D Center, Guangzhou, China
3Agricultural Science and Technology Extension Center of Zhuhai Doumen Ecological Agriculture Park Management Committee, Zhuhai, China

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

Abstract. Different tea-making processes are different, and the aroma substances and main components of the tea-like products are different. The types of aromas and tastes of tea after processing are different. Vanilla, like tea, needs to be fermented and processed before it presents an attractive milk flavor. Based on the co-fermentation process of vanilla and tea, this study developed co-fermented black tea, and compared the aroma and content analysis of ordinary black tea and co-fermented black tea. The results showed that the special aroma substances of co-fermented vanilla black tea contained 2,3-dihydro-2,2,6-trimethylbenzaldehyde (0.42%); GermacreneD (1.54%); 3,7,11-Trimethyl-1,6,10-dodecantri-en-3-ol acetate (0.28%). It is a new type of mixed black tea product with richer ingredients and unique aroma.

Keywords: Vanilla, co-fermentation, black tea, aroma

1. Introduction
Aroma is a volatile aromatic substance in tea. Its content that in dry tea samples is small, usually 0.005% to 0.03%, but it is a mixture of many compounds with different properties and varying contents. The research on the aroma of tea can be traced back to the end of the 18th century and the beginning of the 19th century, but it was not until the development of micro-chemical analysis techniques in the 1950s that the industry began to conduct systematic research on the aroma of tea.

Different aroma substances, mixed in different proportions, reflect different aroma perceptions. According to the sensory aroma types of tea, it can be roughly divided into the following categories: millet aroma, tender aroma, floral, fruity, delicate, sweet, fire-flavored, mellow-scented, rosin-flavored [1].

The factors affecting the aroma of tea include varieties, planting areas, cultivation conditions, and picking conditions. The content of the contents in the fresh leaves of different tea species is different, such as aromatic substances, amino acids, proteins, sugars, etc. Takeo Tadaichi pointed out that the enolic alcohols that determine the aroma of tea are greatly affected by tea varieties, which are related to the different gene expression levels of different varieties [2].
Vanilla is a tropical vine native to Mexico [3]. My country is mainly grown in Hainan; fresh green pods do not have their characteristic aromas, and need to be de-greened, fermented, dried, and conditioned. These stages produce a series of enzymatic and non-enzymatic reactions, and finally produce a rich creamy aroma [4]; it is currently widely used in food, high-end tobacco and alcohol, perfume and pharmaceutical fields [3]. Studies have shown that vanilla has anti-oxidation, anti-cancer, blood-lipid lowering, antibacterial and other biological activities [5].

The traditional blended teas are jasmine tea, orange tea, etc. Some new varieties are rare in recent years. The market needs to innovate processing technology to achieve the purpose of improving quality and efficiency. Based on the co-fermentation process of vanilla and tea, this study analyzed co-fermented tea and analyzed its aroma substances, laying the foundation for further product development.

2. Instruments and materials
QP2010 gas-mass spectrometer (Shimadzu, Japan); manual solid phase micro-extraction device (Supelco, USA); 50/30μm DVB/CAR/PDMS extraction fiber head (Supelco, USA); 10mL headspace bottle (Supelco, USA). The vanilla for the experiment was commercially available, and the tea was provided from Guangzhou Wenshan Tea Co., Ltd.

3. Methods and results

3.1. GC-MS analysis conditions
Chromatographic conditions: Rxi-5Sil MS quartz capillary column (30m×0.25 mm, 0.25 μm, Restek, USA); carrier gas is high-purity helium (99.999%); volume flow rate 1.0 mL/min; Split ratio 20:1; inlet temperature 250°C; interface temperature 280°C; program temperature rise, 50.0°C hold for 5.0 min, rise to 120.0°C at a rate of 6.0°C/min, hold for 5.0 min. The rate of 3.0°C/min was increased to 160.0°C, and the temperature was maintained for 3.0 minutes, and the temperature was increased to 20.0°C at a rate of 10.0°C/min, and the temperature was maintained for 10.0 minutes. The total program time was 54.0 minutes.

Mass spectrometry conditions: ionization source EI; electron energy 70eV; ion source temperature 230°C; scanning range m/z 45~450.

Map search: The NIST11 mass spectrum library is used for data search and analysis.

Pretreatment of co-fermented tea: crush the raw materials to coarse powder, and pass through a No. 2 sieve for use.

3.2. HS-SPME method
Take 0.1g of co-fermented tea powder pretreated in "2.1", put it in a 10mL headspace bottle, equilibrate at 80°C for 60min, insert the manual sampler with 50/30μm DVB / CAR / PDMS extraction fiber head into the bottle In, the extraction head is pushed out and extracted for 50 minutes, taken out and immediately inserted into the injection port of the chromatograph, and desorbed for 5 minutes [6].

4. Results and analysis
Take the volatile components under "2.3", analyze and identify them with GC-MS under the conditions of "2.1", search through the NIST11 mass spectrum library, and determine the chemical components based on standard mass spectra and related literature reports. Then the peak area normalization method is used to analyze the relative content of each chemical component in the volatile component. The black tea of the control group is labeled A, and the co-fermented black tea is labeled A1; the results are shown in Table 1, and the total ion current diagram is shown in Figure 1-2.
Table 1. Types of volatile components of vanilla co-fermented black tea

| No. | ts/min | CAS        | A   | Similarity (%) | CAS        | A1  | Similarity (%) |
|-----|--------|------------|-----|----------------|------------|-----|----------------|
| 1   | 10.027 | 18172-67-3 | 0.09 | 92             | 127-91-3   | 0.08 | 90             |
| 2   | 10.457 | 123-35-3   | 4.73 | 96             | 123-35-3   | 4.16 | 96             |
| 3   | 11.341 | 527-84-4   | 0.89 | 96             | 527-84-4   | 0.75 | 95             |
| 4   | 11.555 | 5989-27-5  | 35.77| 96             | 5989-27-5  | 34.85| 96             |
| 5   | 11.670 | 99-85-4    | 9.03 | 97             | 99-85-4    | 8.12 | 97             |
| 6   | 12.309 | 34995-77-2 | 0.62 | 96             | 34995-77-2 | 0.49 | 95             |
| 7   | 12.556 | 586-62-9   | 0.13 | 87             | 589-33-3   | 1.81 | 93             |
| 8   | 12.963 | 34995-77-2 | 1.55 | 95             | 78-70-6    | 0.39 | 94             |
| 9   | 13.380 | 78-70-6    | 0.68 | 96             | /          | 0.06 | /              |
| 10  | 13.429 | /          | 0.06 | /              | /          | 0.08 | /              |
| 11  | 13.904 | 5337-72-4  | 0.17 | 80             | 39028-58-5 | 0.38 | 95             |
| 12  | 14.085 | 39028-58-5 | 0.43 | 95             | 14049-11-7 | 1.18 | 94             |
| 13  | 15.727 | 14049-11-7 | 1.11 | 94             | /          | 0.18 | /              |
| 14  | 15.785 | 562-74-3   | 0.22 | 85             | 98-55-5    | 0.21 | 88             |
| 15  | 15.851 | 98-55-5    | 0.31 | 94             | 116-26-7   | 0.42 | 81             |
| 16  | 16.046 | /          | 0.26 | /              | 629-59-4   | 0.45 | 96             |
| 17  | 16.426 | 629-59-4   | 0.44 | 97             | 87-44-5    | 0.13 | 93             |
| 18  | 24.021 | 87-44-5    | 0.11 | 84             | /          | 0.21 | /              |
| 19  | 24.090 | /          | 0.14 | /              | 23986-74-5 | 1.54 | 85             |
| 20  | 24.786 | 30021-74-0 | 1.28 | 83             | 629-62-9   | 0.19 | 93             |
| 21  | 25.256 | 502-61-4   | 0.36 | 94             | 502-61-4   | 0.53 | 94             |
| 22  | 26.747 | 17092-92-1 | 0.50 | 91             | /          | 0.14 | /              |
| 23  | 27.277 | /          | 0.81 | /              | /          | 0.11 | /              |
| 24  | 27.497 | 40716-66-3 | 0.58 | 94             | 16729-01-4 | 0.14 | 81             |
| 25  | 27.615 | 638-36-8   | 0.37 | 88             | 17092-92-1 | 1.39 | 97             |
| 26  | 28.642 | 2882-96-4  | 0.52 | 95             | /          | 0.07 | /              |
| 27  | 28.925 | 6846-50-0  | 12.29| 96             | 2306-78-7  | 0.28 | 83             |
| 28  | 29.164 | 77-53-2    | 2.49 | 91             | 638-68-6   | 0.38 | 88             |
| 29  | 29.529 | 3892-00-0  | 0.20 | 88             | 2882-96-4  | 0.67 | 96             |
| 30  | 29.565 | 6785-23-5  | 0.14 | 88             | 6846-50-0  | 11.91| 96             |
| 31  | 30.717 | /          | 1.61 | /              | 77-53-2    | 3.02 | 92             |
| 32  | 31.039 | /          | 0.37 | /              | 3892-00-0  | 0.43 | 90             |
| 33  | 31.185 | /          | 0.24 | /              | /          | 0.05 | /              |
| 34  | 31.265 | 6418-44-6  | 0.18 | 94             | 1795-21-7  | 0.11 | 84             |
| 35  | 31.501 | 593-45-3   | 0.24 | 95             | /          | 1.49 | /              |
| 36  | 32.064 | 544-76-3   | 0.30 | 90             | 6418-44-6  | 0.30 | 94             |
| 37  | 32.791 | 58-08-2    | 8.38 | 95             | 593-45-3   | 0.25 | 95             |
| 38  | 34.878 | 502-69-2   | 6.94 | 92             | 638-36-8   | 0.47 | 94             |
| 39  | 36.230 | /          | 1.14 | /              | 58-08-2    | 8.88 | 95             |
| 40  | 39.780 | 84-69-5    | 1.35 | 94             | 502-69-2   | 8.05 | 94             |
| 41  | 41.457 | 504-96-1   | 0.54 | 87             | /          | 1.19 | /              |
| 42  | 41.544 | /          | 0.56 | /              | 84-69-5    | 1.85 | 96             |
| 43  | 41.735 | /          | 0.25 | /              | 102608-53-7| 0.53 | 84             |
| 44  | 41.870 | /          | 0.15 | /              | /          | 0.18 | /              |
| 45  | 41.961 | 102608-53-7| 0.18 | 92             | 504-96-1   | 0.14 | 88             |
| 46  | 42.314 | 112-39-0   | 0.29 | 96             | 112-39-0   | 0.31 | 94             |
| 47  | 43.150 | /          | 0.26 | /              | /          | 0.07 | /              |
| 48  | 43.285 | 84-74-2    | 0.39 | 96             | 84-74-2    | 1.01 | 97             |
| 49  | 43.477 | 556-68-3   | 0.08 | 82             | 629-94-7   | 0.10 | 86             |
In the control group, 50 compounds were identified in the volatiles of black tea. Among them, the compounds with higher content are (+)-limonene (35.77%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (12.29%), γ-terpinene (9.03%), caffeine (8.38%), phyton B (6.94%), which together account for 72.41% of the volatile components.

Fifty compounds were identified in the volatiles of vanilla co-black tea. Among them, the compounds with higher content are the same as the control group, respectively (+)-limonene (34.85%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (11.91%), γ-terpinene (8.12%), caffeine (8.88%), phyton (8.05%), accounting for 71.81% of the volatile components.
Compared with the control group, the special scent is 2,3-dihydro-2,2,6-trimethylbenzaldehyde (0.42%), which has a sweet, grass-like fragrance, saffron-like fragrance, and a strong smoke fragrance.; Big root myrcene (1.54%), with mint, woody, medicinal, sweet, similar to hay and tea with the aroma of dried tobacco; 3,7,11-trimethyl-1,6,10-12 Alkaltrien-3-ol acetate (0.28%) has a faint floral fragrance similar to rose and apple, with a very sweet, fresh and long-lasting fragrance.

5. Conclusion

Usually the tea with floral and fruit aroma is mainly black tea, and its main aroma substances are alcohols, such as Geraniol, Benzyl alcohol, Phenethyl alcohol, Nerolidol, linalool, etc [7]. The above alcohol aroma substances are rich in types and relatively high in the type, which is inferred to form the basis of the floral and fruity camellia and fruit aroma [8].

In this experiment, headspace solid-phase micro-extraction technology was used to collect volatile components. The design of the GC oven temperature program was more reasonable during identification, and the compounds were fully collected, separated, and identified, so the identified volatile components were more abundant than traditional methods. The types and quantities of volatile components obtained from the control group and the co-fermentation group were basically similar, but the special aroma content was different. At the same time, the ingredients contain terpenes and their derivatives with strong antibacterial properties. On the whole, co-fermented vanilla black tea is a new mixed black tea product with richer ingredients and unique aroma.

Acknowledgments

On 1, 2, 3, 4, 5and 6 are co-first authors.

This work was financially supported by 2020 Provincial Higher Vocational Education Teaching Reform Research and Practice Project Higher Vocational Enrollment Special Project (No. 200413114559687), and Guangdong Province's 2020 key scientific research platform for ordinary universities (No.2020GCZX015) fund.

References

[1] Shi Zhaopeng. Tea review and inspection [M]. 2010: China Agriculture Press.
[2] Takeo Tadao. Relationship between tea plant aroma characteristics and tea plant system variation with monoterpene alcohols [J]. Foreign Agronomy. Tea, 1985, (1):13-15.
[3] Gallage N J, Miller B L. Vanillin-bioconversion and bioengineering of the most popular plant flavorand its de novo biosynthesis in the vanilla orchid [J]. Molecular Plant, 2015, 8 (1): 40-57.
[4] OdouxE, Grisoni M. Vanilla [M]. Boca Raton: CRC Press, 2011: 173-175.
[5] Baqueiro-Peña I, Guerrero-Beltrán J Á. Vanilla (Vanilla planifolia Andr.), its residues and other industrial by-products recovering for high value flavor molecules: A review [J]. Journal of Applied Researchon Medicinal& Aromatic Plants. 2017, 6: 1-9.
[6] Wang Ruyi, Zhou Weiming, Chen Liusheng, et al. Analysis of volatile components in different parts of five-color plum using HS-SPME-GC-MS [J]. Modern Food Science and Technology, 2016, 38 (08): 1-9.
[7] WANG Qiu shuang, CHEN Dong, XU Yong-quan, et al.Investigation and comparison of the aroma components in Guangdong black tea [J]. Journal of Tea Science, 2012, 32 (1): 9-16.
[8] HU Jie, LIU Tong-xun, Effect of different process conditionson microbes and enzyme activities during the pilefermentation of pu-erh tea [J]. Modern Food Science and Technology, 2013, 29 (3): 571-575.