Study on Extraction of Oolong Tea Assisted by Ultrasonic Wave and 4C Technique and Its Application

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Abstract [**Objectives**] The extraction conditions of formula oolong tea were investigated by an orthogonal experiment. [**Methods**] The technical conditions were optimized by the 4C method, and the application of formula oolong tea extract in cigarettes was studied. [**Results**] ① In the experimental range, the best sensory evaluation effect of formula oolong tea extract was obtained with extraction conditions of 70% ethanol as extraction solvent, extraction time h, extraction temperature 25℃, and ultrasonic frequency 80 kHz, and follow-up low-temperature concentration, low-temperature sedimentation and low-temperature centrifugation. ② The effects of different centrifugal speeds on the quality of formula oolong tea extract were explored. The formula oolong tea extract obtained under the conditions of 3 000 r/min and centrifugal time of 10 min showed the best evaluation effect with soft and delicate smoke, rich smoke fragrance, good comfort and refreshing mouthfeel. ③ The effective aroma components in the formula oolong tea extract were qualitatively analyzed by GC-MS. [**Conclusions**] This study provides high-quality raw materials and a theoretical basis for the research of independent flavor blending in cigarette industry enterprises.

Key words Combined application of ultrasonic wave and 4C technique; Orthogonal experiment; Formula extraction; Flavor

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Tea is an important kind of beverage in the world, which is widely concerned by people because of its high aroma and strong oxidation resistance[1]. At present, the classification of tea is mainly based on the varieties of raw materials and different processing techniques, and tea is divided into six categories: green tea, white tea, yellow tea, black tea, oolong tea and black tea[2]. Fragrance is one of the key factors that determine the quality of tea[3], and clarifying the characteristics of tea aroma is an important research content to improve the quality of tea products. At present, the methods of aroma extraction include supercritical fluid extraction, simultaneous distillation extraction (SDE), headspace adsorption, purge and capture, and solid-phase microextraction[4–15].

Materials and Methods

Raw materials and equipment

Table 1 and Table 2 show the uses and sources of raw materials and equipment, respectively.

Table 1 Raw materials

Name of material	Use	Producing area
95% ethanol	Extraction solvent	Weifang Ensign
Water	Extraction solvent	Yizhong Industrial Co., Ltd.
Fruit-fragrance Jinguanyin	Raw material	Fujian
Light-fragrance Tieguanyin	Raw material	Fujian
Fragrant Tieguanyin	Raw material	Fujian

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Table 2 Instruments

Name of material	Use	Producing area
Name of instrument	Use	Instrument location
Rotary evaporator	Concentration	Yizhong Industrial Co., Ltd.
Refrigerator	Settlement	Yizhong Industrial Co., Ltd.
Centrifuge	Centrifugation	Yizhong Industrial Co., Ltd.
50 L centrifuge	Extraction	Yizhong Industrial Co., Ltd.
Agilent GC	Analysis	Yizhong Industrial Co., Ltd.

Experimental method

The sensory evaluation of single oolong tea and formula oolong tea was carried out according to GB/T 23776-2018 Methodolody for sensory evaluation of tea.

Sensory evaluation of single oolong tea First, 5 g of three kinds of oolong tea were weighed, namely, light-fragrance Tieguanyin, fragrant Tieguanyin and fruit-fragrance Jinguanyin, and added into corresponding evaluation cups, into which boiling water was added with the ratio of tea to water (mass/volume ratio) at 1∶50, and they were then covered for 5 min. Next, the tea soup was filtered out, and evaluated according to the fragrance and taste. The results are shown in Table 3.

Table 3 Summary on sensory evaluation results of fragrance and taste of single oolong tea

No.	Name	Fragrance	Taste
1	Fruit-fragrance Jinguanyin	87.5	96
2	Light-fragrance Tieguanyin	94.5	94
3	Fragrant Tieguanyin	86.0	91

According to the results of sensory evaluation on fragrance and taste of five kinds of oolong tea at home and abroad, two kinds of oolong tea, Jinguanyin with fruit fragrance and fragrant Tieguanyin were finally selected. These two kinds of oolong tea had sufficient and elegant aroma and comfortable sweetness.

Sensory evaluation of formula oolong tea According to the sensory evaluation in "Sensory evaluation of single oolong tea", Jinguanyin with fruit fragrance and fragrant Tieguanyin were mixed into formula tea according to a certain ratio, and evaluated according to the evaluation method in "Experimental method". The sensory evaluation results of formula oolong tea are summarized in Table 4. The ratio in Table 4 is fruit-fragrance Jinguanyin: fragrant Tieguanyin (mass ratio).

Table 4 Summary on sensory evaluation results of fragrance and taste of formula oolong tea

No.	Ratio	Fragrance	Taste
1	1: 1	89.5	95.0
2	1: 2	94.0	95.5
3	1: 3	91.0	94.5
4	1: 4	92.0	94.0
5	1: 5	94.0	94.0
6	5: 1	87.5	95.0
7	4: 1	88.0	95.0
8	3: 1	89.5	94.0
9	2: 1	91.0	94.0

According to the summary results of sensory evaluation on the fragrance and taste of formula oolong tea in Table 4, the formula with a ratio of 1: 2 showed a sweet fragrance, which was a harmonious fragrance of paste and flowers and fruits. The formula tea emitted sufficient fragrance and showed good texture. The taste was sweet, mellow and fresh, and there was sweet aftertaste, so the formula tea with fruit-fragrance Jinguanyin: fragrant Tieguanyin = 1: 2 was selected as the raw material for this project.

Pretreatment of formula tea The fruit-fragrance Jinguanyin and fragrant Tieguanyin were accurately weighed in the mass ratio of 1: 2. The two kinds of tea was crushed and sieved with a 60 – 120 mesh sieve for later use.

Chemical composition detection methods

Chromatographic conditions Chromatographic column: RTX-5 ms quartz capillary column (30 m × 0.25 mm × 0.25 μm); injection port temperature: 210 ℃; carrier gas: helium gas; injection volume: 1.0 μl; split ratio: 5: 1; column flow rate: 0.7 ml/min, programmed heating: started with an initial column temperature of 50 ℃, which was maintained for 4 min and increased at a heating rate 2 ℃/min to 150 ℃, which was maintained for 1 min and increased at a heating rate of 5 ℃/min to 180 ℃, which was maintained for 5 min and increased at a heating rate of 10 ℃/min to 280 ℃, which was maintained for 30 min.

MS conditions MS interface temperature: 220 ℃; EI ionization energy: 70 eV; ion source temperature: 200℃; solvent delay: 2.8 min; mass range of scanning ions: 35 – 550 amu.

Data processing and mass spectrometry retrieval Search spectrum library: NIST02 library; quantitative analysis by area normalization method: the sum of all components showing peaks was calculated as 100%, and when the measurement parameter was peak area, the calculation formula was as follows:

$$Xi = Ai \times fi/fi(\sum Ai) \times 100\%$$

In the formula, Xi is the mass fraction of component i in the sample, %; fi is the correction factor of component i; and Ai is the peak area of component i.

In the case of ignoring the correction factor, the area could be normalized directly, that is, the calculation could be according to following formula: $Xi = Ai / \sum Ai$.

The separated components were searched, compared and analyzed by using NIST02 library.

Exploration and Optimization on Extraction Technique of Formula Oolong Tea

Exploration on extraction technique of formula oolong tea

Main factors affecting the product quality are extraction solvent, extraction time, extraction temperature and ultrasonic frequency. According to the experimental results of previous projects and literature reports, the extraction solvent can be water or ethanol, the extraction time, extraction temperature and ultrasonic frequency can be 2 h, 60 ℃, and 1: 10, respectively. Accordingly, an orthogonal experiment was designed by the project team with four factors, *i. e.*, extraction solvent (a), extraction time (b), extraction temperature (c) and ultrasonic frequency (d), each of which was set with three levels, as shown in Table 5.

Table 5 Factors and levels for orthogonal experiment of ultrasonic-assisted low-temperature extraction

	A (Extraction solvent)	B (Extraction time, h)	C (Extraction temperature, ℃)	D (Ultrasonic frequency)
Level 1	95% ethanol	2.0	65	100 kHz
Level 2	70% ethanol	1.5	45	80 kHz
Level 3	50% ethanol	2.5	25	60 kHz

The scheme of orthogonal experiment with four factors and three levels was designed based on the $L_9(3^4)$ orthogonal table, as shown in Table 5. Sample preparation was conducted on the basis of dried Taishan (Huagui) leaves, and the samples were numbered separately for sensory evaluation. Sensory evaluation was based on the evaluation of dried Taishan (Huagui) leaves as 8 points, and the added oolong tea extracts were evaluated respectively, and the application amount was 0.1%. The results are shown in Table 6.

According to the characteristics of orthogonal design, the range R could be used to judge the primary and secondary order of various factors. A greater R value meant that the effect of the level change of the factor on the test index was greater and the factor was more important. The results of sensory evaluation showed that the order of the factors was A > D > B > C, and the extraction solvent and solid-liquid ratio had great influence on the sensory evaluation of products, while extraction temperature and extraction time had little influence. The level of each factor with the largest k value was selected as the optimal level, and the optimal combination was determined as $A_2B_1C_3D_2$, that is, 70% ethanol as extraction solvent, extraction time 2 h, extraction temperature 25 ℃, and ultrasonic frequency 80 kHz. After the extraction was completed, the extracts were concentrated by a rotary evaporator (water bath temperature 50 ℃, reduced pressure 120 mbar, coolant temperature −5 ℃).

Table 6 Scheme for orthogonal experiment of ultrasonic-assisted low temperature extraction and its evaluation results

	A	B	C	D	Sensory quality evaluation
1	1	1	1	1	8.21
2	1	2	2	2	8.29
3	1	3	3	3	8.29
4	2	1	2	3	8.57
5	2	2	3	1	8.43
6	2	3	1	2	8.50
7	3	1	3	2	8.43
8	3	2	1	3	8.29
9	3	3	2	1	8.21
K_1	24.79	25.21	25.00	24.86	
K_2	25.50	25.00	25.07	25.21	
K_3	24.93	25.00	25.14	25.14	
k_1	8.26	8.40	8.33	8.29	
k_2	8.50	8.33	8.36	8.40	
k_3	8.31	8.33	8.38	8.38	
R	0.24	0.07	0.05	0.12	
Primary and secondary order	A > D > B > C				
Optimal level	A_2	B_1	C_3	D_2	
Optimal combination	$A_2B_1C_3D_2$				

Optimization by 4C extraction technique

According to the characteristics of oolong tea, optimization was performed according to the 4C method, which referred to cold extraction-cryoconcentration-cold settlement-cold centrifugation.

In order to further reduce substances detrimental to sensory effect in the extract, after consulting the chemical solvent manual and related research articles and patents, we found that two measures could reduce the content of substances detrimental to sensory effect. First, lowering the temperature can effectively reduce the solubility of pectin in solvents. Secondly, high-speed centrifugation can effectively remove macromolecular substances (large mass). Based on these two points, following optimization experiments were designed.

The oolong tea products concentrated at a low temperature were refrigerated at 4 ℃ for 72 h, and then quickly centrifuged at high speed (12 000 r/min, 6 min, 7 500 r/min, 8 min and 3 000 r/min, 10 min) with a high-speed centrifuge. The working temperature of the centrifuge was 4 ℃. The supernatants were taken out and evaluated for sensory quality after standing at 4 ℃ for 24 h.

The supernatant after high-speed centrifugation was sprayed into the Huagui leaf group for sensory quality evaluation according to the perfuming method, and the proportion of spraying was 0.1%. The results of sensory quality evaluation are summarized in Table 7.

Table 7 Evaluation results of products centrifuged at three different speeds

Rotation speed//r/min	Evaluation result
12 000	The smoke was slightly dry, slightly irritating and had a good smell.
7 500	The smoke was soft, sweet and comfortable.
3 000	The smoke was soft and delicate, rich in fragrance, and the product had good comfort and refreshing mouthfeel.

According to relevant information, these two results might be due to that the excessive centrifugal force of 12 000 r/min led to the excessive loss of beneficial molecules (monosaccharides and polysaccharides) in the extract, resulting in dry smoke and irritation.

Amplification experiment of formula oolong tea extraction assisted by ultrasonic wave and 4C technique

First, 6 000 g of pretreated oolong tea powder was accurately weighed into a 50 L barrel, and added with 18 kg of 70% ethanol solution, and the barrel was sealed with parafilm. The tea powder was extracted under the conditions of ultrasonic frequency of 80kHz and temperature of (25 ± 2) ℃ for 2 h, and the liquid was filtered to obtain a supernatant. The above experiment was repeated for three times, and the supernatants were combined. The obtained supernatant was concentrated under reduced pressure by a rotary evaporator, and the low-temperature concentrated oolong tea product was refrigerated at 4 ℃ for 72 h, and then quickly centrifuged at a high speed (3 000 r/min, 10 min) by a high-speed centrifuge at 4 ℃. The supernatant was taken out and evaluated for sensory quality after standing at 4 ℃ for 24 h.

Two batches were treated continuously, and the difference between batches was small. Follow-up evaluation of the extracts was carried out, and the interval of follow-up evaluation was one month. There was no significant difference in product quality.

Qualitative analysis of chemical components extracted from formula oolong tea by ultrasonic wave and 4C technique

The extract obtained from formula oolong tea with the assistance of ultrasonic wave and 4C technique was analyzed according to the method in "Chemical composition detection methods", and the total ion chromatogram data of oolong tea extract is shown in Fig. 1. Qualitative analysis was conducted through search in the NIST02 standard library, combined with some aroma internal standards. The peak area normalization method was adopted to calculate the relative content of each component. The obtained volatile components and their relative content data of oolong tea extract are shown in Table 8.

Through analysis, it was found that the main components in the oolong tea extract obtained by combining ultrasonic wave and 4C technique were furfural, phenol, linalool oxide, heptaldehyde, ethyl linolenate, N-ethyl succinimide, caffeine, guaiacol and so on. Its main chemical components are basically the same as those of tobacco leaves.

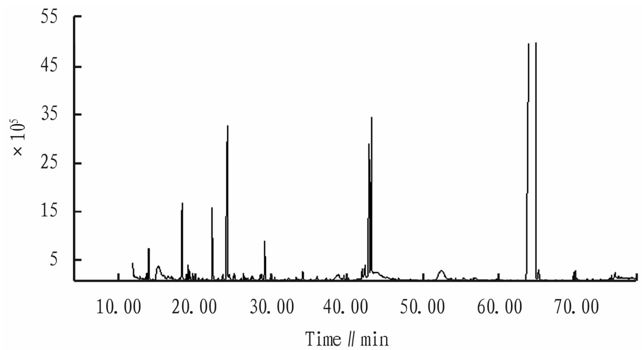

Fig. 1 Total ion chromatogram of oolong tea extract

Table 8 Volatile components and their relative contents in oolong tea extract

Retention time // min	Aroma components	Relative content // %
10.976	Heptanal	0.76
14.825	Furfural	5.98
16.906	Benzaldehyde	2.85
18.377	Linalool oxide	0.39
19.195	Hyacinthin	7.42
21.055	Phenylethyl alcohol	0.27
21.266	Butyrolactone	0.33
23.326	Furfuryl alcohol	2.91
23.406	Hotrienol	0.17
26.273	Epoxydihydrolinalool	0.69
26.812	α-Ionone	0.88
30.371	N-ethyl succinimide	1.70
30.939	β-Ionone	0.25
31.230	Heptanoic Acid	3.47
31.840	Acetyl pyrrole	0.43
32.026	Camphene	0.29
33.887	Phenol	2.22
33.906	Ethyl phenylacetate	0.30
35.480	Trans-nerolidol	0.45
35.670	Octanoic acid	0.57
36.390	6,10,14-Trimethyl-2-pentadecanone	0.43
37.358	α- Pentylcyclopentene	0.64
38.537	Nonanoic acid	0.41
39.571	Methyl palmitate	1.00
39.684	geraniol	0.53
41.412	Methyl 2-aminobenzoate	0.30
42.278	Trimethyl-tetrahydrobenzofuran	0.57
44.738	Guajacolum	1.47
47.846	Caffeine	1.58
57.978	P-phenol	0.52
62.875	Cedrol	0.10
66.203	Cadinol	0.35
69.621	Benzyl benzoate	0.10
72.451	Ethyl linoleate	3.50
73.144	Ethyl linolenate	1.76

Conclusions

In this study, an orthogonal experiment was carried out to explore the optimal extraction conditions of formula oolong tea, and the 4C method was adopted to optimize the technical conditions, and the application research of formula oolong tea extract in cigarettes was carried out. Finally, the obtained formula oolong tea extract showed soft and delicate smoke, rich smoke fragrance, good comfort and refreshing mouthfeel. Meanwhile, the effective aroma components in the formula oolong tea extract were qualitatively and quantitatively analyzed by GC-MS.

This study provides high-quality raw materials for the research of independent flavor blending in cigarette industrial enterprises, as well as a more reliable extraction method for independent flavor raw materials.

References

[1] LIU Y, ZHANG YJ, REN MX, *et al.* Application of bifenthrin and other pyrethroids in tea fields[J]. Journal of Tea, 2015, 41(4): 207–211, 217. (in Chinese).

[2] LUO ZM, LING TJ, LI LX, *et al.* A new norisoprenoid and other compounds from Fuzhuan brick tea[J]. Molecules, 2012, 17(3): 3539–3546.

[3] YIN DD, YUAN RY, WUQ, *et al.* Assessment of flavonoids and volatile compounds in tea infusions of water lily flowers and their antioxidant activities[J]. Food Chemistry, 2015(187): 20–28.

[4] YIN XZ, LI X, LI SH, *et al.* Study on extracting oil condition of Xinyi by steam distillation and its application in tobacco flavoring[J]. Journal of Zhengzhou University of Light Industry: Natural Science Edition, 2003, 18(4): 65–67. (in Chinese).

[5] SCALIA S, GIUFFREDA LP. Analytical and preparative supercritical fluid extraction of chamomile flowers and its comparison with conventional methods 1999, 21(3): 549–558

[6] NIAN XK, LI YY, LI XS, *et al.* Optimization of supercritical CO2 extraction conditions for Honghuadajinyuan tobacco essence and analysis of its volatile components[J]. Tobacco Science & Technology, 2011, 48(4): 46–52. (in Chinese).

[7] PAN NS, ZHANG XY, ZOU J, *et al.* Comparison of yield of volatile oil extracted from coarse powder of curcuma zedoary by steam distillation and petroleum ether extraction[J]. Chinese Traditional Patent Medicine, 2007, 29(10): 1519–1521. (in Chinese).

[8] YU ZH, ZHANG B, BAO CY, *et al.* Study on absoult oil extraction from safflower and its application in cigarette flavoring[J]. Journal of Henan Agricultural Sciences, 2010, 39(2): 44–48. (in Chinese).

[9] PANG QH, HE JH, ZENG RJ, *et al.* Comparison of volatile oil in *Alpinia officinarum* Hance extracted by different methods[J]. Chinese Journal of Pharmaceutical Biotechnology, 2008, 15(1): 54–58. (in Chinese).

[10] TIAN GH, LIU CF, LAI PH, *et al.* Study on volatile components and biology activities of the essential oil from the leaf of *Camellia sinensis* [J]. Food Science and Technology, 2007, (12): 78–82. (in Chinese).

[11] YANG J. Study on flavor components of tea by supercritical fluid extraction[J]. Food Science and Technology, 2008, 33(6): 83–85. (in Chinese).

[12] JOSHI R, SAINI R, GULERIA S, *et al.* Characterization of volatile components of tea flowers (*Camellia sinensis*) growing in Kangra by GC–MS[J]. Nat Prod Commun, 2011, 6(8): 1155–1158.

[13] ZOU YH. Green tea extract and preparation method thereof[P]. CN 105707973 A. 2016.10.16. (in Chinese).

[14] ZHANG Y, DING HY, YU Y, *et al.* Preparation method and application of oolong tea extract[P]. CN 104531359 A . 2014.12.15. (in Chinese).

[15] MA ST, FENG YJ, *et al.* A May tea extract, its preparation method and its application in cigarettes[P]. CN 107361398 A. 2017.06.26. (in Chinese).

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