Journal of Applied Science and Engineering Innovation, Vol.6, No.2, 2019, pp.93-98
ISSN (Print): 2331-9062
ISSN (Online): 2331-9070

Aromatic components analysis of Laoshan Green Tea by Enzyme Treatment

Lan Xin[1]

[1] *Institute of biochemistry, Qingdao Technical College, QingDao , 266555,China*

Abstract: In this study, Laoshan green tea before and after enzymatic hydrolysis was taken as theresearch object. Headspace-solid phase microextraction technology combined with GC-MS method wasused to analyze the changes of aroma components in Laoshan green tea. After enzymatic hydrolysis, the aroma components in the tea were rich, and these aroma components were converted from the original substances by the action of enzymes. Although the types of aroma components in Laoshan green tea before and after enzymolysis are the same, the specific components and relative contents are different. The research provides a reference for evaluating the processing methods of Laoshan green tea and improving its quality.

Keywords Laoshan green tea; Enzyme Treatment;Aromatic composition;HS-SPME,-GC-MS analysis

INTRODUCTION

Laoshan green tea is produced in Laoshan district of Qingdao, which is the highest latitude area for tea planting in China. Laoshan green tea production area is located near the mountain and the sea. its unique climate and geological conditions endow Laoshan green tea with excellent quality, and it is reputed as "the first tea in the north". Olfaction mainly refers to a sensation in the central nervous system caused by volatile substances in food stimulating olfactory nerve cells in nasal cavity. Aroma substances in tea are mainly volatile and semi-volatile compounds, with low content, complex composition and instability. Its analysis depends on appropriate extraction and adsorption methods and analysis methods. In recent years, the author has devoted to the analysis and research of flavor substances in Laoshan green tea. Solid Phase Microextraction (SPME) was proposed by Pawliszyn in 1989 and developed in the 1990s. Compared with traditional methods such as liquid-liquid extraction, solid-phase extraction (SPE) and supercritical extraction (SFE), it requires little or no organic solvent and complicated sample pretreatment. It is fast and simple, and can be directly analyzed by GC, GC-MS and HPLC. At present, it has become an internationally recognized suitable method for detecting tea aroma substances and has been widely applied in recent years [Zhang Ya, Gong Xue,TONG Lingju,CHEN Hui-min, etc.]. In this study, Laoshan green tea before and after enzymatic treatment was taken as the research object, and the changes of aroma components in Laoshan green tea were analyzed by headspace-solid phase microextraction combined with GC-MS method. It provides a reference for evaluating the processing methods of Laoshan green tea and improving its quality.

MATERIALS AND METHODS

Materials and Instrument

Materials： Laoshan green tea is the raw material of Laoshan district, which is made by Qingdao Xiaoyun industry and trade according to the conventional green tea processing technology.

Instrument ： Extraction bottle, manual SPME sampler and 75 μ m Carboxen / PDMS solid-phase microextraction head (U.S. Supelco company), thermostatic magnetic stirrer (Corning PC-420), Agilent GC 6890N-5973 mass selective detector(MSD).

Sample processing method

Weigh 6g of pulverized tea sample, add to the extraction bottle, build in a magnetic stirring rotor, brew with 60 ml 90℃ boiled water at 90℃ with sodium chloride concentration of 0.32 g/mL, seal the bottle mouth with tetrafluoro ethylene to prevent volatile components from overflowing, and immediately place in a constant temperature water bath at 80℃ for 5 min. Then insert the pre-aged solid phase microextraction head, turn on the thermostatic magnetic stirring device, take out the microextraction head after 60 min of headspace extraction, immediately insert it into the sample

Corresponding Author: Lan Xin, Institute of biochemistry, Qingdao Technical College, QingDao , 266555,China

inlet of chromatograph, analyze for 3 min, and perform data acquisition and analysis.

In this study, sodium chloride was added to reduce the solubility of volatile components and improve the sensitivity of headspace-solid phase microextraction by salting-out effect. Using magnetic stirring device can make the sample in a dynamic process, increase the precipitation of volatile and semi-volatile components, and improve the sensitivity and adsorption rate of SPME.

GC-MS and Analysis

GC conditions: HP-5MS Shi Ying capillary column (30 m× 0.25 mm× 0.25 μ m); Inlet temperature 250 ℃ , ECD detector temperature 250℃; The carrier gas is high purity helium with purity > 99.999% and flow rate of 1 ml/min. The initial column temperature was 50℃, kept for 5 min, raised to 160℃ at a rate of 6℃ per min, kept for 3 min, and then raised to 230℃ at a rate of 20 ℃ / min, regardless of inflow.

MS conditions: ion source ei; Ion source temperature 230℃; Electron energy 70 eV; The emission current is 100 μ A; Quadrupole temperature 150℃; Interface temperature: 280℃; Electron multiplier voltage 350 V; Quality scanning range: 50-550 ainu.

Data analysis: the data were retrieved by NIST 98. l standard spectral library, and the base peak, mass-core ratio and relative abundance were analyzed through comparison of mass spectrum materials to determine the components of each peak. the relative content was expressed as the percentage of peak area of each component to the total peak.

RESULTS AND DISCUSSION

Analysis of Flavor Compounds in Laoshan Green Tea Before and After Enzymatic Hydrolysis

Result shows in Table.1.Earlier studies compared three commercial solid-phase microextraction heads commonly used for determination of aroma substances. The results showed that 75 μ m CAR BOXEN/PDMS microextraction head suitable for extraction of gaseous sulfur compounds and organic volatile substances was most suitable for determination of aroma components in Laoshan green tea [Lan Xin,etc]. Laoshan green tea is a typical fried green tea with fresh chestnut flavor, which is contributed by aroma substances such as geraniol, pyrazine and pyrrole [Zhang Chao,etc.].

The general characteristics of odorous substances are: various types, extremely small content, poor stability, mostly non-nutritive substances; Volatile; It has both hydrophilic parts (only through mucous membrane of olfactory receptor) and lipophilic parts (only through lipid membrane of sensory cells); The molecular weight is between 26 and 300.

The aroma of any kind of food is not produced by a fragrant substance alone,but is a comprehensive reflection of various fragrant substances. Several major substances that contribute greatly to aroma are called "main aroma components".From the comparison of aroma components of Laoshan green tea before and after enzymatic hydrolysis, the aroma components of the tea after enzymatic hydrolysis are relatively rich. The aroma components of the two are similar, and there are many common aroma components. The test results are consistent with sensory evaluation. Common substances such as: Octadien ranked first in the aroma of green tea, with clear and sweet wood;Cyclohexene; with light and fragrant flowers; Among the high boiling point components of green tea, strong dispersive pear aroma hexanoic acid, 3-hexen yl ester; Sweet Naphthalene;Woody spicy earthy alpha.-Caryophyllene; Fresh citrus Limonene;Violet fragrance 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl); Toluene;o-Xylene;Heptanal; Pyrazine,2,5-dimethyl-;Benzaldehyde; Pyrazine;2-ethyl-5-methyl-;1H-Pyrrole-2-carboxaldehyde, 1-ethyl-; Pyrazine, 3-ethyl-2,5-dimethyl; Nonanal; Pyrazine, 3,5-diethyl-2-methyl-3,5-;Phenol,2-(1-methylpropyl); Tridecane; Naphthalene ;1,2-dihydro-1,1,6-trimethyl-; Butylated Hydroxytoluene; Tetradecane; Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl); Pentadecane; Naphthalene; Hexadecane; Octadecane; 1H-Pyrrole; 1-ethyl--N-; Pentadecane,2,6,10-trimethyl-, etc.

Rich flavor substances such as 3-Penten-2-one, 4-methyl- (honey flavor) were added after enzymatic treatment. 3-Hexen-1-ol, (Z)-;1,3,6-Octatriene,3,7-dimethyl-,(E);1-Octanol; Octadecane,1-(ethenyloxy)-; 2-Heptanone;;1-Octen-3-ol; D-Limonene (lemon flavor, citrus flavor); Octanoic acid, ethyl ester; Benzoic acid, 2-hydroxy-, 1-methylethyl ester; alpha.-Cubebene; Hexanoic acid, hexyl ester point 245 has the aroma of Qing Xiang, wax, herbs, tropical fruits and berries;3-Buten-2-One,4-(2,6,6-Trimethyl-2-Cyclohexen-1-YL)-(Terpenoids, Sweet Flowers); 5,9-undecadien-2-one,6,10-dimethyl-,(e)-fruity aroma; Nerolidol (rose and apple aroma; Oxirane, tridecyl-; Cyclotetradecane, etc.

These flavor substances are converted from the original substances by the action of enzymes [Wang, L. F.,etc.], such as: 3-cyclohexen-1-carboxalde, 3,4-dimethyl-, strong Qing Xiang and grass aroma, and orange aroma with boiling point of 75-78℃, high boiling point, low saturated vapor pressure, high viscosity, and relatively high molecular weight, with long durability; Phenylethyl Alcohol with rose fragrance；Hexanal; 1,3-Cyclopentadiene, 5-(1,1-dimethylethyl)- ; Allylphenyl sulfide; Furan, 2,5-dihydro-3-methyl- ； 1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-2,3-; 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)- 4- hexene -1- ol, 5- methyl-methyl vinyl; 4-Decenoic acid, methyl ester；2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester; alpha.-Farnesene; 3-(Prop-2-enoyloxy)dodecane; Tetradecane, 1-chloro-; Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-; Dodecane; Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-; Oleic Acid; Nonadecane; Naphthalene; 1,3a-Ethano-3aH-indene;1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-, [1R-(1.alpha.,3a.alpha.,7a.alpha.)]; Eicosane; 2-Dodecen-1-yl(-)succinic anhydride: 1,3,5-Cycloheptatriene; 2-Cyclopenten-1-one,3-methyl-2-(2-pentenyl)-, (Z)-, etc.

Tab.1 Comparison of kinds of aroma components identified in enzyme treatment LaoShan green Tea and Control

No	Library/ID	Area Pct of Control	Alrea Pct of enzyme treatment
1	Toluene	3.3187	0.9297
2	o-Xylene	0.4285	0.0449
3	Heptanal	1.914	2.1363
4	Pyrazine, 2,5-dimethyl-	1.2972	2.2932
5	Benzaldehyde	0.8403	0.6036
6	Pyrazine, 2-ethyl-5-methyl-	3.7983	2.8882
7	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)	1.8468	0.8652
8	1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	2.883	4.9268
9	Pyrazine, 3-ethyl-2,5-dimethyl	1.2502	3.008
10	1,6-Octadien-3-ol, 3,7-dimethyl-	2.1448	6.5907
11	Nonanal	2.9451	4.8125
12	Pyrazine, 3,5-diethyl-2-methyl	0.7277	0.6162
13	Phenol, 2-(1-methylpropyl)-	0.415	0.7107
14	Tridecane	0.9168	0.2679
15	Naphthalene, 1,2-dihydro-1,1,6-trimethyl	0.8496	0.4917
16	Hexanoic acid, 3-hexenyl ester, (Z)	0.581	1.053
17	.alpha.-Caryophyllene	0.8611	0.4148
18	Butylated Hydroxytoluene	0.2195	0.066
19	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	0.6753	0.3148
20	Hexanal	5.6783	—
21	1,3-Cyclopentadiene, 5-(1,1-dimethylethyl)-	0.0998	—
22	Allylphenyl sulfide	0.2961	—
23	Furan, 2,5-dihydro-3-methyl-	0.15	—
24	Limonene	0.7562	2.488
25	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-2,3	0.9954	—
26	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	1.677	—
27	4-Decenoic acid, methyl ester	0.7922	—
28	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	1.5581	—
29	Tetradecane	0.7058	0.4475
30	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	1.2903	0.0613
31	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-beta-	0.7791	0.7275
32	Pentadecane	0.4256	0.1947
33	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	0.9166	0.0613
34	.alpha.-Farnesene	0.3447	—
35	3-(Prop-2-enoyloxy)dodecane	0.6117	—
36	Tetradecane, 1-chloro-	0.8924	—
37	Oxirane, 2-decyl-3-(5-	0.5191	—

	methylhexyl)-,		
38	Hexadecane	0.5247	0.1818
39	Dodecane	0.6158	—
40	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	1.3132	—
41	Oleic Acid	0.3481	—
42	Nonadecane	0.3554	—
43	Octadecane	1.093	0.0291
44	1H-Pyrrole, 1-ethyl--N-	5.9546	0.2351
45	3-Penten-2-one, 4-methyl-	—	2.7746
46	3-Hexen-1-ol, (Z)-	—	0.1816
47	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	—	0.7586
48	1-Octanol	—	2.5064
49	Naphthalene	0.5477	—
50	1,3a-Ethano-3aH-indene, 1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-, [1R-(1.alpha.,3a.alpha.,7a.alpha.)]-	0.6714	—
51	Eicosane		
52	2-Dodecen-1-yl(-)succinic anhydride	0.9026	—
53	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	0.1043	—
54	Octadecane, 1-(ethenyloxy)	—	0.0281
55	1,3,5-Cycloheptatriene	0.4977	—
56	2-Heptanone	—	0.1826
57	1-Octen-3-ol	—	0.8522
58	D-Limonene	—	0.6153
59	Phenylethyl Alcohol	1.5934	—
60	Octanoic acid, ethyl ester	—	2.1211
61	Benzoic acid, 2-hydroxy-, 1-methylethyl ester	—	10.54
62	.alpha.-Cubebene	—	0.6483
63	Hexanoic acid, hexyl ester	—	0.0699
64	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	2.4341	—
65	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl	—	0.4538
66	5,9-Undecadien-2-one, 6,10-dimethyl-	—	0.1535
67	Nerolidol 1	—	0.1157
68	Oxirane, tridecyl-	—	0.2521
69	Cyclotetradecane	—	0.0138
70	Pentadecane, 2,6,10-trimethyl-	0.0842	1.0479

Effects of Enzymatic Hydrolysis on Types and Relative Contents of Flavor Components in Laoshan Green Tea

The flavor is determined by the type and content of aroma-producing substances.

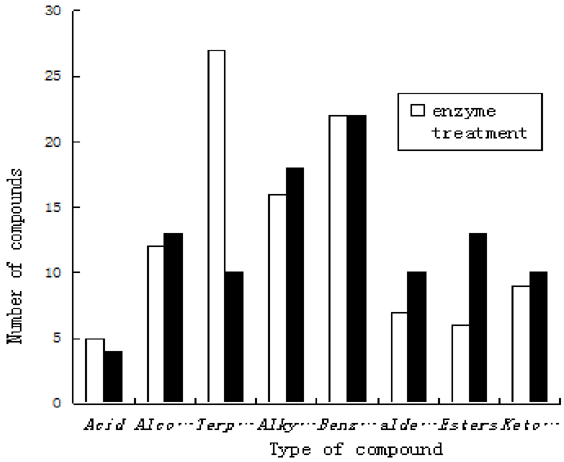

Fig.1 Comparison of number of aroma components between enzyme treatment LaoShan green Tea and Control

The analysis results showed that the flavor substances of Laoshan green tea before and after enzymolysis were mainly divided into 8 categories: terpenes, alcohols, ketones, aldehydes, acids, methoxybenzene and its derivatives (toluene in the figure for short) and alkanes. Alcohols from C1 to C3 have pleasant aroma, while alcohols above C7 have aromatic aroma. Ketones: acetone has a mint-like aroma; Heptyl ketone has a pear-like aroma; Butanedione with low concentration has cream aroma, but with slightly higher concentration, it has sour odor. Aldehydes: Lower aliphatic aldehydes have a strong pungent smell. With the increase of molecular weight, irritation decreases and pleasant aroma gradually appears. C8-C12 saturated aldehydes have good aroma, but α, β-unsaturated aldehydes have strong odor. Esters formed by lower saturated fatty acids and saturated fatty alcohols have various fruit aromas. Lactones, especially γ-lactones have special aroma. Aromatic compounds are mostly aromatic, such as benzaldehyde (almond aroma), cinnamaldehyde (cinnamon aroma), vanillin (vanilla aroma). Terpenoids such as ionone (violet aroma); Oenanthe javanica (spice aroma).

The results showed that the number of terpenes (from 10 to 27) and acids (from 4 to 5) increased, the number of Benzene remained unchanged, the number of Alcohol(from 13 to 12), and the number of alkyals (from 18 to 16). Aldehydes(from 10 to 7), Esters(from 13 to 6), Ketones(from 10 to 9) decreased.

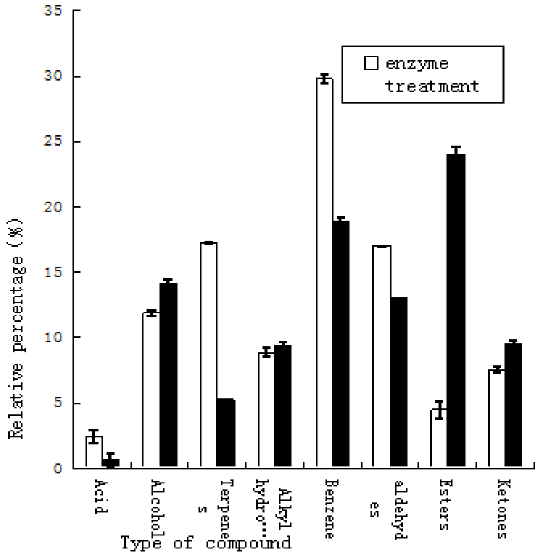

Fig.2 Comparison of relative percentage of aroma components between enzyme treatment LaoShan green Tea and Control

Whether it is fragrant or not is also related to the content of fragrant substances. The results showed that although the aroma components of Laoshan green tea were the same before and after enzymolysis, the specific components and relative contents were different. Changes in percentage content of enzymolyzed compounds: Terpenes increased significantly (from 5.2466 to 17.2432), Acid increased (from 0.6365 to 2.397) and aldehydes increased (from 13.013 to 17.014).

Although the number of Benzene compounds remained unchanged, its percentage content increased (from 18.8458 to 29.7944). Alcohol decreased (from 14.1456 to 11.8805), alkyl hydrocarbons decreased (from 9.3493 to 8.8548), Esters decreased (from 23.9612 to4.4714) and ketones decreased (from 9.5354 to 7.5191).

CONCLUSION

The aroma of tea is mainly formed through complex biochemical reactions in the process of making tea. Different tea species and processing technologies make the composition and relative content of aroma components very different. The tea aroma of tea is the comprehensive expression of various aromatic substances in different concentrations [Li YanQing,WANG Qiu-shuang, etc.]. The effect of enzymes on food aroma mainly refers to the process in which food raw materials form aroma substances under the catalysis of a series of enzymes during post-harvest processing or storage. Direct action of enzyme: enzyme catalyzes the precursor of a certain aroma substance to directly form aroma substance; Indirect action of enzyme: oxidation products formed by oxidase catalyze the oxidation of precursor of aroma substances to form aroma substances.

A kind of food has good flavor, not only can arouse people's appetite, but also can bring high economic benefits, so it is of great significance to the separation and identification of food flavor substances. However, the flavor components in food are very complex, because only a small amount of solute exists in highly complex mixtures, so how to effectively extract the target solute we need becomes the premise of current research[ZHANG Mingming,etc.]. At present, the evaluation of tea is mainly the sensory evaluation of tea soup. Due to the limitation of evaluation subject, the results are subjective, lack of stability and accuracy. Analysis of volatile components in tea soup can truly reflect the aroma of tea leaves after soaking. Solid phase microextraction combined with gas-mass spectrometry is a very suitable method for determination of aroma components in tea leaves. The principle of dynamic headspace technology (purge-cold trap trapping technology, Purg and Trap) is to enrich volatile substances by using intermediate trapping step before analyzing the sample, which can greatly improve the headspace sampling efficiency. this method is superior to static method, because it is not only suitable for components with higher volatility, but also for components with lower volatility and concentration, and is also very effective for samples with complex components and low content [Hai-Peng Lv,etc.]. In the solid phase microextraction operation process, a balance is established between the concentration of the analyte in the sample or the concentration of the analyte in the headspace and the concentration of the analyte adsorbed in the polymer coated on the molten silicon fiber, no organic solvent is required, the method is simple and convenient, the test is fast, the cost is low, the method integrates sampling, extraction, concentration and sample introduction into a whole, and can be combined with gas phase or liquid phase chromatography.

In this study, the aroma components of Laoshan green tea after enzymatic hydrolysis were analyzed in detail for the first time, and compared with the control aroma components, and the changes of aroma components after enzymatic hydrolysis were measured. This study establishes the basis for the further application of HS-SPME combined with GC-MS in the optimization and evaluation of Laoshan green tea processing methods.

ACKNOWLEDGMENT

The authors wish to thank the helpful comments and suggestions from my teachers and colleagues in intelligent detection and control lab of Institute of biochemistry, QingDao Technical College. .This work is supported by the study fund of Qingdao public domain department Technical Support Program Project (No.:12-1-3-52-(2)-NSH) and technological development planning in Qingdao development zone technology division(2012-2-58)

REFERENCES

CHEN Hui-min, SHI Zhi-gang, DI Tai-mei, HU Jian-hui, ZHANG Zhong-qiang, ZHANG Xin-fu.The Scenting Technology and Aroma Analysis by HS-SPME/GC-O-MS for Osmanthus Black Tea[J]. Modern Food Science and Technology ,2018,34:243~254.

GONG Xue，LIU Zhong-ying，LI Yan，JIANG Yan-yan，YIN Rong-xiu，YANG Chun，CHEN Zheng-wu, Analysis of Volatile Flavour Constituents in New Guizhou Green Tea Varieties by HS-SPME-GC-MS[J]. HuNan Agriculture Sciences, 2017,11: 69~72.

Hai-Peng Lv,Qiu-Sheng Zhong,Zhi Lin,Li Wang,Jun-Feng Tan,Li Guo. Aroma characterisation of Pu-erh tea using headspace-solid phase microextraction combined with GC/MS and GC﹣olfactometry[J].Food Chemistry, 2012, 130: 1074~1081.

Lan Xin, Wang Dong-feng, Zhang Li, Zhao JiHe. Aromatic components analysis of green tea in Lao Mountain by HS-SPME and GC-MS[J].Food and Machinary,2012, 28,96~101.

Li YanQing, Fu DaYou, Wang Rong. Research Progress in Determination Methods of Tea Aroma Components[J].Tea Science and Technology,2009, 1: 8~10.

TONG Lingjun, CHI Xuelu, ZHANG Xiaomei, AI Nasi, WANG Jing, SUN Baoguo. Analysis of Volatile Compounds of Black Buckwheat Milk Tea by HS-SPME-GC-MS[J]. Food Science,2017,38:134~139.

Wang, L. F., Lee, J. Y., Chung, J. O., Baik, J. H., So, S., & Park, S. K.. Discrimination of teas with different degrees of fermentation by SPME﹣GC analysis of the characteristic volatile flavour compounds[J].Food Chemistry,2008, 109:196~206.

WANG Qiu-shuang，QIAO Xiao-yan，WU Hua-ling，CAO Jun-xi ， CHEN Dong.Study on Aroma Components in Five Region of Sri Lanka Black Tea By HS-SPME/GC-MS. Food Research And Development, 2016,37:128~133.

Wondwosen Abebe Matebie , Wanchang Zhang and Guangbo Xie. Chemical Composition and Antimicrobial Activity of Essential Oil from Phytolacca dodecandra Collected in Ethiopia[J]. Molecules , 2019, 24, 342.

Zhang Chao,Lu Yan, Li YiXin, Huang WeiNing, Chen ZhengXing. Research Progress on Aroma Components of Tea and Mechanism of Aroma Formation[J].FuJian Tea,2005, 3: 17~19.

ZHANG Mingming, YIN Hongxu, DENG Yuliang, YAO Yuefeng, JIANG Yongwen, HUA Jinjie, YUAN Haibo,YANG Yanqin.Study on the Key Odorants for Chestnut-Like Aroma Green Teas using HS-SPME/GC×GC-TOFMS/OAV [J].Food Science ,2019,05,21.

Zhang Ya, Li Weifang,Xiao Bing.Analysis of volatile components of twenty-five Hunan and Shanxi Fuzhuan tea samples by HS-SPME-GC-MS[J]. Journal of Northwest A&F University, 2017,2:151~160.