Study on the Aromatic Components of Green Plum Wine by HS-SPME-GC-MS

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Abstract In order to better blend green plum wine and study aromatic components of green plum wine, a qualitative analysis on aromatic components of soaked base liquor, green plum soaked wine, green plum juice, and fermented wine of green plum juice by Head Space Solid-phase Microextraction (HS-SPME) and Gas Chromatograph Mass Spectrometer (GC-MS) was studied in this paper. Experiment results indicated that 14,32,17,and 46 kinds of aromatic components were identified respectively from four samples. Different aromatic components determined the special flavor and taste of green plum wine. Unique aromatic components generated in soaking process include benzaldehyde, 1-butanol, 2-methyl-, S-(-), benzoic acid ethyl ester, and 5-(hydroxymethyl). Special aromatic components in green plum juice were furfural, phenylethyl alcohol, and benzyl alcohol. The aromatic components in fermented wine of green plum juice mainly included phenylethyl alcohol (6.941%, relative content of peak area, same below), 1-butanol, 3-methyl- (6.940%), octanoic acid, ethyl ester (3.734%), decanoic acid, ethyl ester (2.590%), hexanoic acid, ethyl ester (2.479%), ethyl 9-decenoate (2.080%), and 5-hydroxymethyl (1.756%). This study was expected to provide scientific basis and data reference for quality improvement of green plum wine.

Key words Green plum wine, Aromatic components, Head Space Solid-phase Microextraction (HS-SPME), Gas Chromatograph Mass Spectrometer (GC-MS)

1 Introduction

China is home of green plum. It is mainly distributed in Changjiang Delta Area, South China, and southwest of China, including Guangdong, Guangxi, Fujian, Zhejiang, and Yunnan[1]. According to reports[2-4], fruit of green plum is rich in citric acid which can improve appetite, restore physical strength, and remove fatigue; besides, green plum can improve constipation, soothe the nerves, alleviate vexation, and promote tricarboxylic acid cycle, rapidly remove fatigue elements out of body, and generate energy to the maximum extent, and make foods completely burn. This is of significant realistic value for athletes and hot workers to restore physical strength. These organic acids can not only remove lactic acid accumulated in blood, but also inhibit generation of new lactic acid, to reach the function of cleaning blood. Also, the rich Vitamin B2 has function of anti-cancer. In addition, green plum is favorable for absorption of calcium, so it is an optimum fruit helping children and elderly people enriching the calcium. Processed products of green plum mainly include salted green plum, candied green plum, green plum drink, and green plum wine. In recent years, a lot of green plum wine products appear, but there are few products with special flavor and taste. Therefore, it is necessary to further study making of green plum wine.

Aroma is an essential indicator for wine products. Head Space Solid-phase Microextraction and Gas Chromatograph Mass Spectrometer (HS-SPME-GC-MS) combined method has been widely applied in analysis and detection of aromatic components of wine products[5]. Solid-phase microextraction (SPME) technology, developed in 1990, has been widely applied in study of aromatic substances of food[6]. HS-SPME method features short operation time, few samples, rapid and easy use, reagent not needed, and high repeatability[7-9], so we adopted this method to make preliminary treatment of samples. Besides, to better blend green plum wine, it becomes particularly important to know aromatic components of green plum soaked wine, green plum juice, and green plum juice fermented wine, to provide scientific basis and data reference for improving quality of green plum.

2 Materials and methods

2.1 Materials

2.1.1 Raw materials for experiment. Green plum wine, green plum soaked wine, and green plum juice were kept by laboratory. Soaked base liquor adopted Sichuan sorghum wine (suitable for medicated wine and soaking fruit wine), strong fragrant spirit, 60% Vol, produced by Luzhou Hong Man House Wine Co., Ltd.

2.1.2 Main instruments. Angilent 6890 GC gas chromatograph and 5975 MS mass spectrometer; chromatographic column DB-WAX (60 m × 250 μm × 0.25 μm); SPME manual sampling handle and extractor, produced by ANPEL Laboratory Technologies (Shanghai) Inc, and extractor was 50/30UM DVB/CAR on PDMS and made by American Supelco Company.

2.1.3 Main reagents. Sodium chloride (purity above 99.5%) was produced by Chengdu Kelong Chemical Co., Ltd; pure water was taken from Mount Emei drinking natural mineral water produced by Emeishan Emeisnow Mineral Water Co., Ltd.

2.2 Methods

2.2.1 Gas chromatograph and mass spectrometry conditions. Chromatographic column was DB-WAX (60 m × 250 μm ×

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0.25 μm), sample injection port temperature 250℃; column oven temperature rise process kept 35℃ for 5 minutes, then rose to 100 ℃ at 5 ℃ /min to hold 2 minutes, finally rose to 230℃ at 15 ℃ /min and held 10 minutes, the operation time was 38.667 minutes. Carrier gas: high purity nitrogen, flow rate of carrier gas: 1 ml /min. Mass spectrometry conditions: EI ion source, ion source temperature 230 ℃, interface temperature 250 ℃, ionization voltage 70 eV, quadruple pole temperature 150 ℃, and mass scan range 30 – 500 m/z. Qualitative determination of aromatic components: comparing with NIST05a. L spectrum library.

2.2.2 Operation method of Head Space Solid-phase Microextraction (HS-SPME). Put 9 ml samples and 1.8 g sodium chloride into 20 ml head space vial, and place it on 45℃ thermostatic water bath for 30 minutes, insert the aged extractor (aging temperature 230℃ and aging time 30 minutes) into the head space vial, extract 30 minutes, the extractor is 1 cm from the liquid level, and finally manually feed samples onto GC-MS to make chromatographic analysis.

3 Results and analyses

3.1 GC-MS result analysis Using Head Space Solid-phase Microextraction (HS-SPME), we extracted aromatic components of green plum soaked wine, green plum juice, and green plum juice fermented wine and made GC-MS analysis. Total ion of 4 samples was illustrated in Fig. 1. From Fig. 1, peaks of ions in green plum soaked wine (Fig. 1B) are more than in green plum soaked base liquor (Fig. 1A), showing that long time soaking of green plum in base liquor gives out new aromatic components. From Fig. 1C and Fig. 1D, we know that green plum gives out a lot of aromatic components through fermentation of yeast. Through NIST05a. L library search, 4 samples (A, B, C and D) had 14, 32, 17, and 46 aromatic components, we calculated peak area relative content of aromatic component using area normalization method and results were listed in Table 1, 2, 3, and 4.

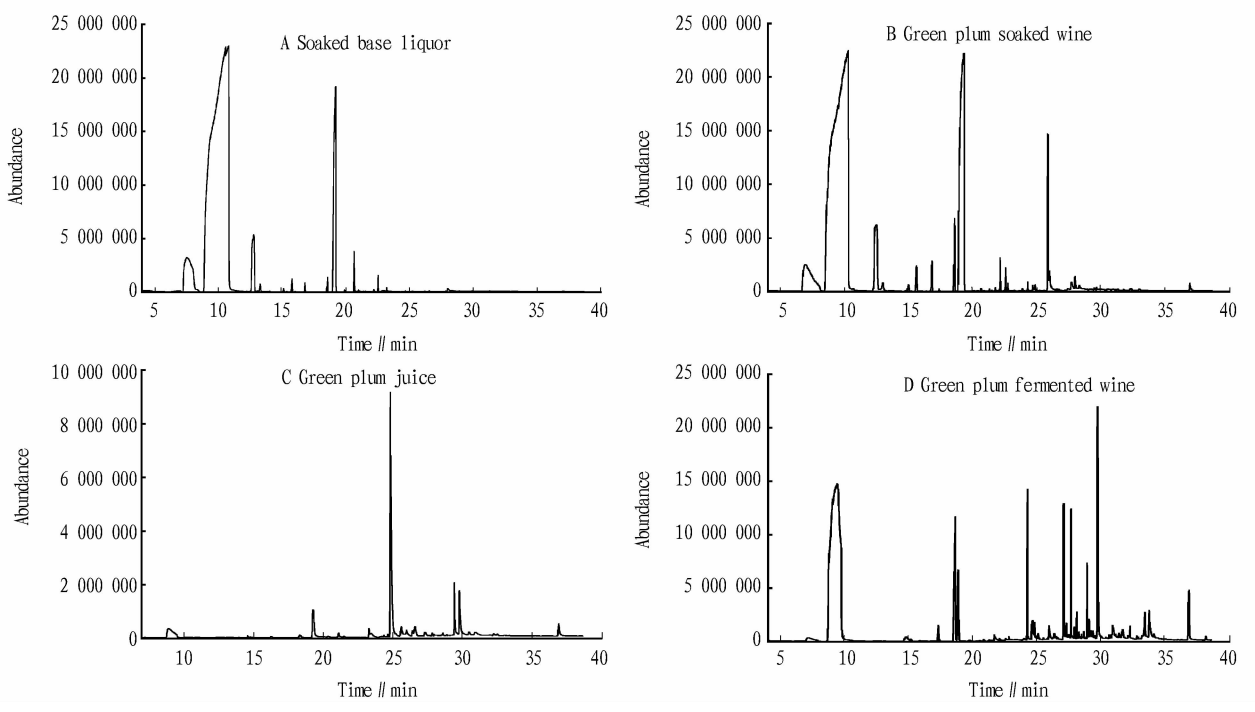

Fig. 1 Total ions of GC-MS

3.2 Aromatic components of soaked base liquor, green plum soaked wine, green plum juice, and green plum juice fermented wine From Table 1, we can see that soaked base liquor had 14 types of aromatic components, including 5 esters, 5 alcohols, and 4 others. These are mainly hexanoic acid, ethyl ester (relative content of peak area 8.796%, the same below), ethyl acetate (5.674%), butanoic acid, ethyl ester (2.883%), benzoyl bromide (0.414%), 1-Butanol, 3-methyl- (0.205%), 1-Propanol (0.183%), and propanoic acid, 2-hydroxy-, ethyl (0.169%), etc. This soaked based liquor has relative few aromatic components, so it is suitable for making fruit wine and medicated wine. From Table 2, we can see that green plum soaked wine had 32 types of aromatic components, including 16 esters, 9 alcohols, 3 aldehydes and 4 others. Aromatic components are mainly hexanoic acid, ethyl ester (relative content of peak area 19.283%, the same below), ethyl acetate (4.678%), butanoic acid, ethyl ester (3.513%), benzaldehyde (1.939%), 1-Butanol, 3-methyl- (0.841%), 1-Butanol (0.382%), heptanoic acid, ethyl ester (0.285%), 1-Propanol (0.280%), 1-Butanol, 2-methyl-, (S) - (0.245%), benzoic acid, ethyl ester (0.216%), Propanoic acid,2-hydroxy-, ethyl ester, (S) -L(-) (0.212%), Oxime-, methoxy-phenyl- (0.203%), 2-Furancar-

boxaldehyde, 5 -(hydroxymethyl) (0.165%), and 1-Propanol, 2-methyl- (0.163%).

Table 1 Aromatic components of soaked base liquor and the relative content of peak area

No.	Holding time min	Name of aromatic component	Relative content of peak area//%
1	7.536	Ethyl acetate	5.674
2	10.557	Ethyl alcohol	80.973
3	12.781	Butanoic acid, ethyl ester	2.883
4	13.283	1-Propanol	0.183
5	15.129	1-Propanol, 2-methyl	0.058
6	16.799	1-Butanol	0.095
7	18.569	1-Butanol, 3-methyl-	0.205
8	19.212	Hexanoic acid, ethyl ester	8.796
9	19.918	Styrene	0.026
10	20.665	Benzoyl bromide	0.414
11	20.998	Tridecane	0.015
12	22.211	Heptanoic acid, ethyl ester	0.030
13	22.544	Propanoic acid, 2-hydroxy-, ethyl ester	0.169
14	23.225	Oxime-, methoxy-phenyl-	0.100

Table 2 Aromatic components of green plum soaked wine and the relative content of peak area

No.	Holding time min	Name of aromatic component	Relative content of peak area//%
1	6.918	Ethyl Acetate	4.678
2	9.087	Ethyl alcohol	66.998
3	12.456	Butanoic acid, ethyl ester	3.513
4	12.981	1-Propanol	0.280
5	14.970	1-Propanol, 2-methyl-	0.163
6	16.809	1-Butanol	0.382
7	17.361	Hexanoic acid, methyl ester	0.033
8	18.509	1-Butanol, 2-methyl-, (S) -S-(-) -2-	0.245
9	18.574	1-Butanol, 3-methyl-	0.841
10	19.306	Hexanoic acid, ethyl ester	19.283
11	20.637	Benzoyl bromide	0.024
12	21.283	3-Hexenoic acid, ethyl ester, (Z) -2-	0.014
13	21.749	Hexanoic acid, propyl ester	0.024
14	22.130	Heptanoic acid, ethyl ester	0.285
15	22.471	Ethyl 2-hexenoate	0.005
16	22.536	Propanoic acid, 2-hydroxy-, ethyl ester, (S) -L(-)	0.212
17	22.726	1-Hexanol	0.069
18	23.212	2-Amino-5-methylbenzoic acid	0.007
19	23.868	Hexanoic acid, butyl ester	0.019
20	23.916	Butanoic acid, hexyl ester	0.009
21	24.259	Octanoic acid, ethyl ester	0.062
22	24.677	Isopentyl hexanoate	0.041
23	24.738	Propanoic acid, 2-oxo-, methyl ester	0.036
24	24.839	Furfural	0.043
25	25.840	Benzaldehyde	1.939
26	27.384	2-Furanmethanol	0.017
27	27.679	Benzoic acid, ethyl ester	0.216
28	27.969	Oxime-, methoxy-phenyl-	0.203
29	29.484	Benzyl alcohol	0.023
30	32.349	Oxirane, (ethoxymethyl) -	0.023
31	32.985	Octadecanoic acid, ethyl ester	0.026
32	36.932	2-Furancarboxaldehyde, 5 -(hydroxymethyl)	0.165

From Table 3, we can see that green plum juice had 17 types of aromatic components, including 2 esters, 8 alcohols, 4 aldehydes and ketones and 3 others. The aromatic components are mainly furfural (relative content of peak area, 52.07%, the same below), hexanoic acid, ethyl ester (9.67%), phenylethyl alcohol (9.17%), benzyl alcohol (7.36%), 2-Furancarboxaldehyde, 5-(hydroxymethyl) (3.19%), 2-Furancarboxaldehyde, 5 -methyl- (2.95%), Ethanone, 1-(2-furanyl) (2.63%), 2, 3-Butanediol (2.57%), ethyl alcohol (2.17%), 1-Propanol, 3-ethoxy- (2.66%), and 2-Butanone, 3-hydroxy- (1.01%).

Table 3 Aromatic components of green plum juice and the relative content of peak area

No.	Holding time min	Name of aromatic component	Relative content of peak area//%
1	8.900	Ethyl alcohol	2.17
2	18.337	1-Butanol, 3-methyl	0.45
3	19.298	Hexanoic acid, ethyl ester	9.67
4	21.131	2-Butanone, 3-hydroxy-	1.01
5	23.310	1-Propanol, 3-ethoxy-	2.66
6	24.670	3, 8-Dioxa-2, 9-disiladec-5-ene, 2, 2, 9, 9-tetramethyl-, (E) -	0.29
7	24.864	Furfural	52.07
8	25.638	Ethanone, 1 -(2-furanyl)	2.63
9	26.011	2, 3-Butanediol	2.57
10	26.614	2-Furancarboxaldehyde, 5 -methyl	2.95
11	27.320	Butyrolactone	0.52
12	27.381	2-Furanmethanol	0.80
13	29.451	Benzyl alcohol	7.36
14	29.810	Phenylethyl alcohol	9.17
15	30.508	2, 5-Furandicarboxaldehyde	0.77
16	30.957	Furyl hydroxymethyl ketone	0.50
17	36.944	2-Furancarboxaldehyde, 5 -(hydroxymethyl)	3.19

From Table 4, we can see that green plum juice fermented wine had 46 types of aromatic components, including 12 esters, 14 alcohols, 7 aldehydes and ketones and 13 others. Aromatic components are mainly ethyl alcohol (relative content of peak area, 60.480%, the same below), phenylethyl alcohol (6.941%), 1-Butanol, 3-methyl- (6.940%), octanoic acid, ethyl ester (3.734%), decanoic acid, ethyl ester (2.590%), hexanoic acid, ethyl ester (2.479%), Ethyl 9-decenoate (2.080%), 2-Furancarboxaldehyde, 5 -(hydroxymethyl) (1.756%), glycerin (1.173%), acetic acid (1.044%), 4-Fluorobenzoic acid, 2-phenylethyl ester (1.031%), etc.

3.3 Analysis on major aromatic components of green plum soaked wine and green plum fermented wine From Table 1 and Table 2, we can see that compared with soaked base liquor, there are 20 more aromatic components in green plum soaked wine. Major components are benzaldehyde (relative content of peak area, 1.939%, the same below), 1-butanol, 2-methyl-, (S) - (0.245%), benzoic acid, ethyl ester (0.216%), and 2-Furancarboxaldehyde, 5-(hydroxymethyl) (0.165%). Benzaldehyde has pleasant almond flavor and is unique aromatic component of green plum[10]. In the measurement of green plum soaked wine, Gao Min *et al*[11], Yang Hongya *et al*[12], and Zheng Xinhua *et al*[13] found there is benzaldehyde, the relative content of peak area is 4.740%, 10.870%, and 21.830% respectively, the volatile components in green plum soaked wine are high, which is consistent with results of our experiment. GC-MS experiment results of green plum soaked wine are consistent with sensory properties, proving that benzaldehyde is unique aromatic component of green

plum.

Table 4 Aromatic components of green plum juice fermented wine and the relative content of peak area

No.	Holding time min	Name of aromatic component	Relative content of peak area//%
1	7.177	Ethyl acetate	0.148
2	9.271	Ethyl alcohol	60.480
3	14.799	1-Propanol, 2-methyl-	0.515
4	15.170	1-Butanol, 3-methyl-, acetate	0.089
5	17.331	1-Butanol, 3-methyl-, propanoate	0.579
6	18.652	1-Butanol, 3-methyl-	6.940
7	18.872	Hexanoic acid, ethyl ester	2.479
8	19.757	Styrene	0.033
9	21.308	2-Butanone, 3-hydroxy-3-	0.030
10	21.704	2-Propanone, 1-hydroxy-	0.349
11	22.741	1-Propanol	0.020
12	22.852	4-Pyridinol4-	0.063
13	24.269	Octanoic acid, ethyl ester	3.734
14	24.624	Acetic acid	1.044
15	24.851	Furfural	0.430
16	25.104	7-Octenoic acid, ethyl ester	0.105
17	25.179	1-Hexanol, 2-ethyl-	0.045
18	25.53	Ethanone, 1-(2-furanyl) -	0.022
19	25.959	2, 3-butanediol	0.411
20	26.044	2, 3-Butanediol, [R-(R * , R *)]-(2R, 3R) -(-)	0.178
21	26.299	Ethanol, 2 -(1-methylethoxy) -	0.083
22	26.395	2, 3-butanediol, [R-(R * , R *)]-	0.165
23	26.503	2-Furancarboxaldehyde, 5 -methyl-	0.059
24	27.119	Decanoic acid, ethyl ester	2.590
25	27.262	Ethanone, 1 -(3-thienyl) -	0.210
26	27.331	2-Furanmethanol	0.333
27	27.502	Butanedioic acid, diethyl ester	0.137
28	27.663	Ethyl 9-decenoate	2.080
29	27.948	Oxime-, methoxy-phenyl-	0.219
30	28.133	3-Fluoro-4-methoxyphenylacetic acid	0.342
31	28.521	Cyclohexanepropanenitrile, 2-oxo-	0.114
32	28.935	4-Fluorobenzoic acid, 2-phenylethyl ester	1.031
33	29.061	Undecanoic acid, ethyl ester	0.487
34	29.406	Benzyl alcohol	0.155
35	29.759	Phenylethyl alcohol	6.941
36	30.644	1, 6, 10-Dodecatrien-3-ol, 3, 7, 11-trimethyl-, (E) -(6E) -	0.064
37	30.768	2, 5-dimethyl-4-hydroxy-3 (2H) -furanone	0.069
38	30.917	Octanoic acid	0.913
39	31.439	1, 3-dihydroxyacetone dimer	0.158
40	32.140	Eugenol	0.041
41	32.190	1, 3-Propanediol, diacetate	0.081
42	32.291	2-hydroxy-gamma-butyrolactone	0.254
43	33.446	4H-Pyran-4-one, 2 , 3-dihydro-3, 5-dihydroxy-6-methyl-	0.580
44	33.356	Phenol, 2, 4-bis (1, 1-dimethylethyl)	0.109
45	33.794	Glycerin	1.173
46	36.896	2-Furancarboxaldehyde, 5 -(hydroxymethyl)	1.756

From Table 3, we can see that relative content of furfural (relative content of peak area, 52.07%, the same below), hexanoic acid, ethyl ester (9.67%), phenylethyl alcohol (9.17%), and benzyl alcohol (7.36%) is high. Furfural is mainly generated through hydrolysis, dehydration, and distillation after reaction of pentose and vigabatrin. Pentose is isomerized to 1,2-alkene glycol, and further converted to deoxidized pentose, and finally forms furfural[14]. Since the formation process of furfural widely exists in plants and foods containing pentose type substances, it is reasonable that it also exists in green plum juice. Besides, extracts of green plum contain maleic acid and furfurtal substances, which have antibiotic and anti-inflammation effect[15]. Ethyl ester is aromatic component of strong fragrant spirit, like pineapple and banana flavor. The existence of ethyl ester in green plum can be understood that long time of soaking produces certain amount of ethyl ester which increases aroma of green plum. Benzyl alcohol has gentle sweet, fruit flavor, fragrance of flower, and aroma of oranges and tangerines. Benzyl alcohol is not fermentation product of common yeast. In the fermentation process, yeast has many reduction reactions, and many aldehydes and ketones can be reduced to alcohols[16]. Green plum contains benzaldehyde, and benzaldehyde can be reduced to benzyl alcohol through dehydrogenases. However, in the condition of oxygen, it firstly generates erythrose through pentose phosphate pathway, and then generates phenylethyl alcohol. The phenylethyl alcohol has sweet fragrance, rose and honey fragrance[12]. Therefore, furfural, benzyl alcohol and phenylethyl alcohol play important role in fragrance of green plum juice.

From Table 4, we can see that green plum juice produces a lot of aromatic components through fermentation of yeast. These aromatic components are mainly phenylethyl alcohol (6.941%), 1-Butanol, 3-methyl- (6.940%), octanoic acid, ethyl ester (3.734%), decanoic acid, ethyl ester (2.590%), hexanoic acid, ethyl ester (2.479%), Ethyl 9-decenoate (2.080%), 2-Furancarboxaldehyde, and 5 -(hydroxymethyl) (1.756%). 1-butanol, 3-methyl- and ethyl alcohol are generated in parallel and will bring fragrance of fruit. Ethyl ester, decanoic acid, ethyl ester, hexanoic acid, ethyl ester, and ethyl 9-decenoate separately have the aroma of pineapple, grape, banana, and pear. These aromatic components are closely related with fruit fragrance of green plum.

From Table 2 and Table 4, we can see that green plum fermented wine has more trace aromatic components than green plum soaked wine. The reason may lie in aromatic components of green plum are leached out and blended together with wine in the action of high concentration of spirit, forming unique aroma. High concentration of spirit also suppresses fermentation of natural yeast, leading to reduction of trace aromatic components in soaked wine. After dilution of green plum juice, sugar concentration drops to the level suitable for growth of yeast. Based on self substance of green plum, it generates important aromatic components, including 1-Butanol, 3-methyl- (6.940), octanoic acid, ethyl ester (3.734%), decanoic acid, ethyl ester (2.590%), hexanoic acid, ethyl ester (2.479%), ethyl 9-decenoate (2.080%). These contribute a lot to aromatic components of green plum wine.

4 Conclusions

Through HS-SPME-GC-MS sample analysis, there are 14, 32, 17, and 46 aromatic components in soaked base liquor, green plum soaked wine, green plum juice, and fermented wine of green plum juice . Unique aromatic components of green plum soaked wine include benzaldehyde (relative content of peak area, 1.939%, the same below), 1-Butanol, 2-methyl-, (S) - (0.245%), benzoic acid, ethyl ester (0.216%), and 2-Furancarboxaldehyde, 5-(hydroxymethyl) (0.165%). Aromatic components in green plum juice mainly include furfural (relative content of peak area, 52.07%, the same below), hexanoic acid, ethyl ester (9.67%), phenylethyl alcohol (9.17%), and benzyl alcohol (7.36%). The aromatic components in fermented wine of green plum juice mainly include phenylethyl alcohol (6.941%), 1-butanol, 3-methyl- (6.940%), octanoic acid, ethyl ester (3.734%), decanoic acid, ethyl ester (2.590%), hexanoic acid, ethyl ester (2.479%), ethyl 9-decenoate (2.080%), and 5-hydroxymethyl (1.756%). This experiment made a preliminary qualitative analysis on volatile substances in green plum fermented wine, green plum juice, green plum soaked wine, and base liquor. Analysis of special flavor components and principal components needs further study.

The experiment results indicate that green plum soaked wine, green plum juice, and green plum juice fermented wine have unique aroma of green plum, but there are essential differences in aromatic components, which also proves results of sensory analysis. Green plum soaked wine is high in concentration and the flavor is not good enough; green plum juice is too sweet and too thick, so a certain dilution is necessary; green plum fermented wine has low concentration, but the aroma is not sufficient, so the optimization of fermentation process needs further study. In addition, proper blending of these three types of green plum wine to make up the shortage of each other and enrich aroma and flavor is an effective approach to improve market prospect of green plum alcoholic drink at present.

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