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COMPARATIVE STUDY ON THE CHANGES OF AROMA COMPONENTS IN THE GRAPE AND DRY RED WINE OF CABERNET SAUVIGNON

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ABSTRACT

The aroma evolutions in the grape and dry red wine of Cabernet Sauvignon from geographic origin of Ningxia Helan Mountain Eastern Region were studied. The volatile organic compounds were extracted by solvent extraction and analyzed by GC-MS, and their relative contents determined by area normalization. 43 and 56 compounds were separated from the grape and wine respectively, in which 42 from grape and 55 from wine's volatile organic compounds were identified. Comparing with dry red wine of Cabernet Sauvignon growing different regions in the world, the detection rate was 98.95% and 99.71% of the total peak areas. There were mainly fatty acids, fatty acid esters, lactone, fatty alcohol, alkanols, lower fatty acid, fatty ketones, heterocyclic compounds and other chemical groups. Comparing with dry red wine of Cabernet Sauvignon growing different regions in the world, the higher relative contents and patterns of the volatile organic compounds were similar but characteristic aroma trace components were very different, which lead to a similar and unique aroma and style for the same variety wines. In order to release capability of free aroma substance and improve the quality of dry red wine, the results were significant for wine fermentation technology.

Keywords: Cabernet Sauvignon, dry red wine, aroma component, GC-MS
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INTRODUCTION

Cabernet Sauvignon, originating in France, is the traditional varieties to brew red wine in Bordeaux, it is one of the grape varieties that are the most widely planted in the world (Robinson *et al*., 2006). It has been introduced to Ningxia for the large scale of cultivation in the early 1980s, and has become the primary red wine grape varieties, for its unique aroma, high adaptability, are cultivated in many of our wine grapes areas, which is the main raw material to produce quality red wines. Cabernet Sauvignon grape wine produced with this unique breed grass aroma, deep red wine color, strong tannins, high polyphenols content, rich bouquet, full bodied, best suited age in oak barrels for several years, the wine is dominated by black fruit and plum incense, botanical incense (such as grass and green pepper) and roasted aroma-based. Dry red wine's aroma components are mainly made up of berry aroma and fermentation aroma. The volatile organic compounds are complex and significant in determining the characteristics of grape and wine flavor and typicality, their type, content, proportion, sensory threshold and their interaction play an important role in the style and quality of wine (Li *et al*., 2010; Ma, 2009). However, until now, only few researchers report about single variety dry red grape and wine aroma components in China (Hu Boran, 2004; Ferreira V *et al*., 2000). In this study, the test material was Cabernet Sauvignon from geographic origin of Ningxia Helan Mountain Eastern Region. The volatile organic compounds were extracted by solvent extraction and analyzed by GC-MS.

MATERIALS AND METHODS

Material and processing: Cabernet Sauvignon grapes were planted in 2000 at the vineyard of the vine experimental station in Yuquanying located into Geographic origin of Ningxia Helan Mountain Eastern Region, no grafted seedlings, spacing of 0.5m×3m, upright and single arm fence shelf, pruning with a multi-main vine. It began to bear fruit in 2002, grew well in the field, had pure varieties and was managed according to conventional. Hand harvesting in September 25~30, 2012, the grapes were ripe abundantly, which physicochemical index was: 21.9% total soluble solids, 198.3g/L reducing sugar, 7.2 g/L titratable acid, 0.59 g/L total phenol, 3.16pH value. Randomly Juice samples were obtained from fresh berries through de-stemming and crushing by mechanical press in 30t tanks after 48hours soaking with fruit and skin. The pretreatment of samples were taken it to centrifugal size 1000r/min, to remove the precipitate and to make the juice 200mL (Wang, 2001; Tu Zhengshun, 2001; Ferreira *et al*., 2000).

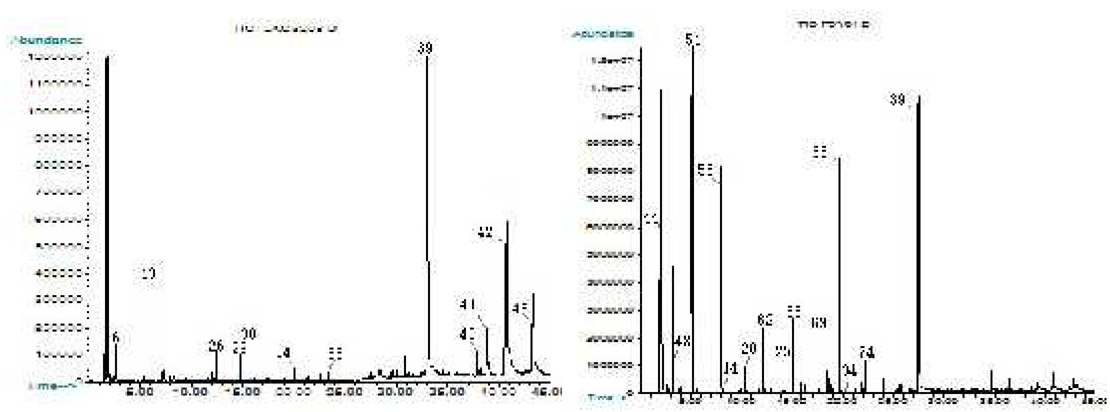
The wine were made by the standard wine processing in the 30t tanks using the following techniques: De-stemming and crushing, addition of 50μg/L SO2 and RC212 yeast cell/ml, fermentation at 25°C for 8~9 days followed by gentle pressing the pomace, the duration of fermentation was started in the end of September in 2012 without any fining treatments (such as no bentonite) in late maturation of wine. Malolactic fermentation not occurred and wines were stored in the steel tanks until analysis began in March 2013. Wine general components were: 12.8% (v/v)alcohol content, 2.14 g/L residual sugar, 6.3g/L titratable acid content, 3.39 pH value, 23.8 g/L extracts , 25.2 g/L free SO2 , 0.912g/L total phenol content.

Extraction and concentration: At the testing center analysis, Yangzhou University in China, the test was started on the end of March, 2013.The volatile compounds were extracted with continuous liquid-liquid method. The Samples of pretreatment juice and wine were taken respectively 200mL, 350mL, Each 200,350ml wine samples in a 500ml spherical flask with 100, 80, 60ml of freshly redistilled dichloromethane were extracted three times respectively. Every three organic fractions were combined and dried on sodium sulfate anhydrous and then concentrated to a final volume of 1mL by a rotary evaporator (0~5°C) for GC-MS (Ortega-heras *et al*., 2002; Li Jiming, 1997; Ferreira *et al*.,1996). In all cases, the samples were analyzed with a GC-MS system in duplicate. As used herein, Caldir and other studies (Caldeira *et al*., 2007; Ortega-heras *et al*., 2002) had shown that dichloromethane could extract most kinds of compounds, and could have greater yield.

GC-MS: Analysis was performed with a Hewlett-Packard 6890 gas chromatograph equipped with a detector model HP 5972.Samples were injected in split mode(50:1),and volatiles were separated by a column (HP-WAX:30m×0.25μm×0.25mm) with GC grade helium as carrier gas at a flow rate of 1ml/min. The working conditions were as follows: injector temperature 250°C; detector temperature 280°C; column temperature 60ºC; heated to 240°C at 5°C/min; (Held for 30min),and then heated to 270°C at 10°C/min (held for 30min); for mass spectrometry energy of 50eV was used in electric impact (EI) mode 1800V. In all cases, the samples were injected two times into GC. Sample compounds were identified by comparing their retention time and matching with a mass spectral library collection in NBS/WILEY spectrogram.

RESULTS AND DISCUSSION

The aroma compounds were analyzed by GC-MS systems and their relative contents determined by area normalization. GC-MS total ion chromatograms of aroma components in the samples of juice and dry red wine of Cabernet Sauvignon were plotted by HP MSD Chem Station as follows Fig.1: these were also some GC-MS analytical results of aroma (Table 1).The grape of 43 compounds were separated, and 42 of them were identified, one of them were unidentified. These identified constituents represent 98.95% of the total peak areas. 56 kinds of compounds were separated in the dry red wine, and 55 of them were identified. One of them was unidentified. These identified constituents represent 99.71% of the total peak areas.



a fruit of Cabernet Sauvignon b. dry red wine of Cabernet Sauvignon

Fig.1. GC-MS total ion chromatogram of aroma components in the grape and dry red wine of Cabernet Sauvignon

To retrieve NBS/WIL EY standard library by HP MSD Chem Station and combine related literature standard spectra to check (Li Hua *et al*., 2004; Hu Boran, 2004; Cong Puzhu *et al*., 2000), 43 compounds of grape were separated, and 42 of them were identified, one of them was unidentified. These identified constituents represent 98.95% of the total peak areas. 56 compounds of the dry red wine were separated, and 55 of them were identified, one of them was unidentified. These identified constituents represent 99.71% of the total peak areas.

Fruit aroma and fermented aroma of Cabernet Sauvignon composition were shown in Table 1. 42 kinds of volatile composition of fruits were identified that were all fruit aroma, and 55 kinds of aroma compositions were defined in the wine that had 7 kinds of aroma compositions that were same with fruit aroma, there were Hexanol, Hexanoic acid, Benzenemethanol, Bezeneethanol, octanicacid, 2-Cylohexen-1-one,3,5,5-trimethyl, 1-Butanol, 3-methyl-(impure). These seven types of ingredients can exist stably in the overall of the process; this indicated that they were derived from the fruit ingredients. In addition to these seven kinds of composition were the same, there were the same 3-Hexene-1-o1 is not within the species of the aroma, it may be converted or compounded to other aroma components in the finished wine and in the process of bottle in storage

Table 1. GC-MS analysis result of aroma components in the grape and dry red wine of Cabernet Sauvignon

No.	Volatile organic compounds	Molecular formula	Molecular mass	Rel. Content (%)	
				Fruit of Cabernet Sauvignon	Dry red wine
1	2-Butenal	C_4H_6O	70	0.066629796	
2	Butanal, 3-methyl-	$C_5H_{10}O$	86	0.043751062	
3	3-Buten-2-one,3-methyl-	C_5H_8O	84	0.118007219	
4	2-methyl-3-Buten-2-ol	$C_5H_{10}O$	86	0.047994561	
5	2-Butenal	C_4H_6O	70	0.322532382	
6	Hexanal	$C_6H_{12}O$	100	1.466692763	
7	3-Penten-2-ol-	$C_5H_{10}O$	86	0.050434773	
8	3-methyl-1-Butanol	$C_5H_{12}O$	88	0.088669183	
9	Furan,2-pentyl-	$C_9H_{14}O$	138	0.054289833	
10	1-Pentanol	$C_5H_{12}O$	88	0.064796147	
11	Cyclohexanone	$C_6H_{10}O$	98	0.072174717	0.029346193
12	2-(5H)-Furanone, 5-ethyl-	$C_6H_8O_2$	112	0.265467994	
13	2-Heptenal,(E)-	$C_7H_{12}O$	112	3.821351905	
14	1-Hexanol	$C_6H_{14}O$	102	0.154489646	1.153770259
15	3-Hexen-1-ol,(Z)-	$C_6H_{12}O$	110	0.054483529	0.043811
16	Nonanal	$C_9H_{18}O$	142	0.441849051	
17	2-Hexen-1-ol,(E)-	$C_6H_{12}O$	110	0.435060641	0.012632751
18	2-Octenal,(E)-	$C_8H_{14}O$	126	0.23069916	
19	1-Octen-3-ol	$C_8H_{16}O$	128	0.227259061	
20	Acetic acid	$C_2H_4O_2$	60	0.204037609	1.564331227
21	2,4-Heptadienal,(E,E)-	$C_7H_{10}O$	116	0.24762696	
22	1-Octanol	$C_8H_{18}O$	130	0.061872569	
23	2-Cyclohexen-1-one,3,5,5-trimethyl-	$C_9H_{14}O$	138	0.129931173	0.136586695
24	2-Octen-1-ol,(E)-	$C_8H_{16}O$	128	0.26549731	
25	Butyrolactone	$C_4H_6O_2$	86	0.401329734	1.233935623
26	2-Decenal,(E)-	$C_{10}H_{18}O$	154	1.15936998	
27	2,4-Nonadienal	$C_9H_{14}O$	138	0.07041366	
28	2-Decenal,(E)-	$C_{10}H_{18}O$	154	0.118058522	
29	2,4-Decadienal,(E,E)-	$C_{10}H_{16}O$	152	1.04649184	
30	2,4-Nonadienal	$C_9H_{14}O$	138	1.498778131	
31	Hexanoic acid	$C_6H_{12}O_2$	116	0.123072963	0.685878627
32	Benzenemethanol	C_7H_8O	108	0.114959048	0.313602011
33	Benzeneethanol	$C_8H_{10}O$	122	0.157120413	10.64407625
34	Octanoic acid	$C_8H_{16}O_2$	144	0.58260378	
35	Hexadecanoicacid,ethyl ester	$C_{18}H_{36}O_2$	284	0.487969479	0.38998759
36	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.305457179	
37	Unknown			1.051032366	0.714414331
38	2-Hexadecanol	$C_{16}H_{34}O$	258	0.225372941	
39	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	30.20114195	18.9487971
40	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.965295347	0.87802988

41	9-Octadecenoic acid (Z)	$C_{18}H_{34}O$	282	5.121527763	0.13122337
42	9,12-Ocadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	32.52949133	
43	9,12,15-Octadecatrienoic acid,methyl ester,(Z,Z,Z)-	$C_{19}H_{32}O_2$	292	12.90471443	
44	Acetic acid, ethyl ester	$C_4H_8O_2$	88		1.850765771
45	2，3-Butanedione	$C_4H_6O_2$	86		0.080573603
46	1-Propanol	C_3H_8O	60		0.15564121
47	Butanoicacid,ethyl ester	$C_6H_{12}O_2$	116		0.066380036
48	1-Propanol, 2-methy1-	$C_4H_{10}O$	74		3.14553018
49	1-Butanol, 3-methyl, acetate	$C_7H_{14}O_2$	130		0.276872926
50	1-Butanol	$C_4H_{10}O$	74		0.17536866
51	3-Butanol, 3-methyl- (impure)	$C_5H_{12}O$	88		28.36631051
52	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144		0.098634851
53	3-Buten-l-ol,3-methyl-	$C_5H_{10}O$	86		0.033330746
54	2-Butanone, 3-hydroxy-	$C_4H_8O_2$	88		0.799835794
55	1-Pentanol,3-methyl-	$C_6H_{14}O$	102		0.019115145
56	Propanoic acid, 2-hydroxy-,ethyl ester	$C_5H_{10}O_3$	118		14.31695908
57	1-Propanol, 3-ethoxy-	$C_5H_{12}O_2$	104		0.026970415
58	Butanoic acid, 2-hydroxy-3-methyl-,ethyl ester	$C_7H_{14}O_3$	146		0.05321375
59	Octanoic acid ,ethyl ester	$C_{10}H_{20}O_2$	172		0.133032084
60	2-Furancarboxaldehyde	$C_5H_4O_2$	96		0.039497641
61	Butanoic acid,3-hydroxy-,ethyl ester	$C_6H_{12}O_3$	132		0.163006155
62	2,3-Butanediol	$C_4H_{10}O_2$	90		1.947305029
63	4-Heptanol, 2,6-dimethyl-	$C_9H_{20}O$	144		0.066666627
64	2,3-Butanediol	$C_4H_{10}O_2$	90		0.486447594
65	Decanoic acid ,ethyl ester	$C_{12}H_{24}O_2$	200		0.053327006
66	Butanedioicacid,diethyl ester	$C_8H_{14}O_4$	174		2.347400209
67	1-Propanol, 3-(methythio)-	$C_4H_{10}Os$	106		0.40646095
68	1,3-Propanediol, diacetate	$C_7H_{12}O4$	160		0.377057632
69	Pentanoic acid, ethyl ester	$C_7H_{14}O_2$	130		1.834074334
70	2-Hexenoic acid, (E)(8CI9CI)	$C_6H_{10}O_2$	114		0.046005906
71	Pantloactone	$C_6H_{10}O_3$	130		0.085335209
72	Butanedioic acid, hydroxyl-,diethyl ester	$C_8H_{14}O_5$	190		0.288233661
73	Octanoic Acid	$C_8H_{16}O_2$	144		0.928585139
74	Thiazoil	C_3H_3NS	85		0.460561421
75	2(3H)-Furanone, 5-ethldihydro-	$C_6H_{10}O_2$	114		0.365149586
76	Octanoic acid	$C_8H_{16}O_2$	144		0.28474671
77	Thuazole	C_3H_3NS	85		1.41677602
78	2-Pyrolidinone	C_4H_7ON	85		0.45539756
79	Decanoic acid	$C_{18}H_{32}O_2$	172		0.08718728
80	Benzeneacetic acid,2-propenyl ester	$C_{11}H_{12}O_2$	176		0.50402828
81	Butanedioicacid,diethyl ester	$C_8H_{14}O_4$	174		0.11917945
82	Benzeneethanol,4-hydroxy-[2-	$C_8H_{10}O_2$	138		0.37191874
83	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280		0.64972338
84	11,14,17-Eicosatrienoic-acid,methyl ester	$C_{21}H_{36}O_2$	320		0.09924711

Esters: The detected fruit aroma and fermented aroma of Cabernet Sauvignon composition were plotted to compare the structure of the compound (Figure 2).Comparative analysis of various aroma components as follows: esters of the fruit of Cabernet Sauvignon contained four categories, the relative content in the fruit is 14.06%, which included two kinds of fatty acid esters and two types of lactones, their relative content respectively were 13.39%and 0.67%.2(5H)-Fran one,5-ethyl was detected for the first time in the fruit of Cabernet Sauvignon (Force *et al*., 1997; Tomas Herraiz *et al*., 1991) and it was an unique chemical compound with special fruit aroma, but the relative content of esters was 16.74%in dry red wine, which contained 16 kinds. Two kinds of higher fatty acid esters and two kinds of lactones were particular chemical compound in fruit of Cabernet Sauvignon, which were converted to 16 kinds of lower fatty acid esters during wine fermentation; this reflected the relative content of kinds of total esters in dry red wine that were more than the relative content of the fruit.

Esterification reaction was always engaged in a slow in the entire aging process of wine, the content of esters was also increasing, which is why aged wine is more fragrant than the new wine (Zhang Yingli, 2011).

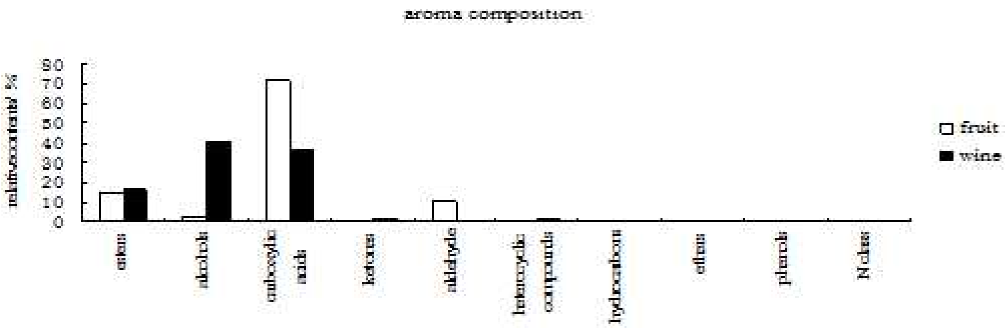


Fig.2. Aroma components and relative contents in the grape and dry red wine of Cabernet

Alcohols: Compared with dry red wine, alcohols content was relatively small in the fruit. In the wine after fermentation, the type and content of the fatty alcohol and aromatic alcohols had shown an increasing trend. As can be seen in the fermentation process, the yeast fermentation to make much aroma components generated Alcohols, which eventually constituted the primary aroma components in the wine (Rodríguez H*et al*., 2008). As Figure 2 shows, in the fruit, the lower saturated and unsaturated fatty alcohols and the higher fatty alcohol were converted to an alcohol component order a lower saturated aliphatic alcohol central. Aromatic alcohol content in red wine in general rose by 0.27 percent to 0.90 percent, the species from two kinds into three types. Aromatic alcohols had an essential role in the overall aroma formation of wine, and its aroma value was (concentration / Threshold) high. Benzeneethanol was the principal products of the metabolism of phenylalanine, and it was formed by itself yeast fermentation (HanJing, 2008). The content of Benzenethanol in the wine had a higher, accounting for 10.21%, olfactory threshold was very low, it probably constituted the characteristics of the species of wine olfactory sense substances and demonstrated sweet fragrance of roses, and it had a particular effect on mellow and pleasant of dry red wine.

Carboxylic acids: The content of carboxylic acids was relatively high in the fruit, there were eight categories, the relative content was 72.03%, it relied mainly on higher fatty acid, and the relative content was 71.12%; the content of carboxylic acids compounds was relatively small in dry red wine, it were mainly the lower fatty acid with the relative content of 15.63% and the higher fatty acid with the relative content of 20.69%. This reflected the variation feature of the higher fatty acids was significantly high levels degradation and transformation during the fermentation process from fruit to wine.

Ketones, Aldehydes: Cabernet Sauvignon grapes contained more types and quantities of aldehydes, and its content was 10.54%, 13 kinds. Did not detect the presence of aldehydes in dry red wine, it indicated aldehydes were the principal component of features compounds of grapes. Ketones were detected in the fruit and wine, the relative content of the fruit was 0.32%, there were three kinds of fatty ketones, relative content of wine rose to 1.47 percent, four kinds.

Heterocyclic compounds: Cabernet Sauvignon fruit contained one kind of furans of heterocyclic, accounting for 0.05% relative to the total peak area, and there were two types of furans and thiazoles of heterocyclic compounds in dry red wine, the relative content was 1.44%, heterocyclic compounds of wine (mainly furans, thiazoles, thiophenes) elevated in both the type and content relative to the fruit.

Other compounds: Cabernet Sauvignon grapes failed to detect the presence of hydrocarbons, but the wine was detected one kind of such substances with the relative content of 0.01%. Did not detect the presence of phenolic compounds in the fruit and wine, but in the wine, two kinds of ethers compound with the relative content of 0.05% were detected. Nitrogenous compounds of Cabernet Sauvignon fruit were not detected, but as the progress of fermentation, these substances were produced.

Foreign studies suggested that β- ionone, Malaysia ketones, terpenes could be identified as Cabernet aroma characteristic material (Eva Maria Diaz-Plaza, 2002; Cabrita M J*et al*., 2006), in this experiment, and none of the species were measured in the wine. The compound of 1-Propanol, 3-lmethylthiol (3MMB) in dry red wine was unanimous together with reported (Catherine Peyrot Des Gachons, 2002) and its content was 0.69%. Their odor threshold was very low, and they had herbaceous, fruity nuances, green pepper, boxwood, broom, grape fruit, passion fruit and smoke. Some reports also thought that it had black currant, guava and other flavors. This compound was considered to be a characteristic odor compound of Cabernet Sauvignon wine.

Conclusion: Grapes ripe fruit volatile substances were mainly 9,12-Octadecadienoic acid, Hexadecanoicacid, 9,12,15-Octadecatrienoic acid, methyl ester, 9-Octadecenoic, 2-Heptenal (E), Octadecanoic acid, 2,4-Nonadienal, Hexanal, 2-Decenal and other ingredients. Volatile substances of wine through fermentation were mainly 1-Butanol,3-methyl-, Benzenethanol, Propanoic acid,2-hydroxy, 2,3-Butanediol, 1-Propanol,2-methyl-, Butanoic acid, diethyl ester, Butanoic acid, ethyl ester and other ingredients. The results showed that in the fermentation process, the yeast metabolism transformed ingredients of sugar, organic acids of fruit and flavor precursor into alcohols, esters and other products, so that the ingredients of fruit of the fruitfully released and reflected in particular the feature of wine in red wine.

This test had detected the type and content of volatile aroma components in the grape and dry red wine of Cabernet Sauvignon from geographic origin of Ningxia Helan Mountain Eastern Region in China. Respectively, 43 and 56 compounds were separated from the grape and wine, and 42 compounds from grape and 55 compounds from wine were identified. They were mainly fatty alcohol, aromatic alcohols, lower fatty acid, fatty ketones, aldehydes and Heterocyclic compounds and so on. Aroma substances of wine mainly originated from the process of the fermentation of yeast metabolism and biochemical chemical reaction apart from the aroma of grape berry. Compared with the rest places of the world of Cabernet Sauvignon fruit and wine, the main content of aroma component was not very different, but micro-aroma components were quite different, such as heterocyclic compounds and so on. Identification of characteristic aroma components must be completed by combining taste and sensory analysis (Ferreira V*et al*., 2000; Margaret diff*et al*., 2002), the results of this test provided a reliable reference data in determining the components of active odor substances of wine of Ningxia Helan Mountain Eastern Region, and it also provided important practical values on production technology of wine and quality evaluation.

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