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Full Length Research Paper

Changes in aroma composition of blackberry wine during fermentation process

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The study aimed at investigating the influence of fermentation (primary and secondary) on aroma composition of blackberry wine. Gas chromatography-mass spectrometry (GC-MS) was applied to quantify the compounds relevant to sparkling wine aroma. Investigation on this study revealed that a number of aroma components in raw material (55 in numbers), raw wine (54 in numbers), and aging wine (50 in numbers) were identified. In addition, 9 new aroma components such as octanoate, benzenepropanoic acid ethyl ester, ethyl benzoate, dodecyl ethyl, *n*-propanol, *n*-butanol, d-citronellol, benzaldehyde, and cedrol were detected in natural aging wine which appeared during secondary fermentation according to total peak areas of 4.69%. These findings reveal that natural aging is very important to aroma components formation of blackberry wine.

Key words: Blackberry, gas chromatography, primary fermentation, secondary fermentation.

INTRODUCTION

Blackberry (*Rubus* sp.) is a perennial vine belonging to the family Rosaceae. The aggregate fruits of this plant are soft, juicy and rich in nutritional value with a unique flavor. Blackberry is a good source of natural antioxidants as it contains different vitamins, anthrocyanin and phenolic compounds. It has also been reported to reduce fatigue, enhance immunity, decrease cholesterol and prevent cardiac diseases and carcinomas (Wu et al., 2000; Sanchez-Moreno et al., 1999).

The quality of wine has been reported to be highly dependent on the aroma constituents of fresh fruits (Li, 2000), and volatile compounds play an important role for the quality of wine. Recent sensory studies based on consumer preference proved that wine flavor was one of the crucial factors (Tao et al., 2008). More than 800 volatiles have been identified in wines, including alcohols, esters, organic acids, aldehydes, ketones and monoterpenes (Howard et al., 2005).

The main aroma components found after a headspace solid-phase microextraction gas chromatography mass spectrometric (HS-SPME-GC–MS) procedure were alcohols, esters acids, aldehydes, ketones and other compounds (2-heptanol, hexylformate, hexane, and 2-hetanone) (Bian et al., 2010). It is well known that during wine maturation and ageing, there are many chemical changes in the volatile composition. These reactions depend on wine composition, pH, storage time and temperature (Marais and Pool, 1980; Ramey and Ough, 1980).

In a previous study, we compared the blackberry wine obtained by fermenting with active dry yeast and different strains of yeast (D21, D254, UV43, DV10) for aroma and sensory components. The fermentation process revealed

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D254 as the best yeast strain for blackberry wine production. However, there is paucity of literatures that explore and demonstrate the changes in aroma composition in blackberry wine during fermentation process.

MATERIALS AND METHODS

Blackberry was harvested from the Jiangsu Provincial Academy of Agricultural Sciences, Nanjing Lishui modern agricultural planting bases. Active dry yeast and different strains of yeast (D21, D254, UV43, and DV10) were purchased from Angel Yeast Co. (Hubei, PR China) and Lallemand (France) respectively. Ascorbic acid was purchased from Xilong Chemical Engineering Factory (Guangdong, China), while pectinase was from Novozymes (Bagsvaerd, Denmark). Other chemicals were of analytical grade.

Fermentation process

Fermentation was carried out according to our previously reported method (Wang et al., 2008; Li, 2000). Briefly, blackberry wine was prepared as follows: Fully mature blackberry without rot was harvested, pressed, and treated with pectinase (5%, w/w) at 30 - 35°C for 4 h. Aliquot of juice were fermented with cultured yeast for 12 days at 22°C. When the first fermentation process was completed, wine was passed through a filter. The wine was again kept at 18°C for 25 days for secondary fermentation. The liquor was stored for 3 months at a low temperature of 10°C in order to reach maturity. Finally, mass fraction of 0.10% gelatin was added to clarify the wine.

Extraction of aroma components

Fermented samples were placed into a 20-ml glass vial containing 3 g of sodium chloride and incubated for 10 min at 55°C in a water bath. Later, the solid phase micro-extraction were performed at 30°C for 40 min followed by desorption done into the injector for 5 min.

GC-MS analytical conditions

All gas chromatography-mass spectrometry (GC-MS) analyses of aroma compositions were carried out on an Agilent 7890A/5975C GC−MS. The chromatographic condition was set as follows: 2:1 split injection; injector temperature 260°C; transfer line 240°C; ion source 230°C; quadrupole 150°C; full scan acquisition mode; scan mass range: 29-350. The temperatures were programmed as follows: 38.5°C start, held for 2 min, then the rate of 3°C rise 170 min, no reservations, and then to 15°C to 215°C, retention for 10 min and the running time was for 58.85 min.

Data analysis

Data was collected by Agilent ChemStation software. All tests were carried out independently in triple replication (n = 3).

RESULTS AND DISCUSSION

In total, 55 kinds of aroma components were detected in blackberry fruit juice before fermentation (Table 1). The major aroma ingredients found and observed before fermentation process were ethyl acetate, formic acid methyl ester, ethyl-2-methylbutyrate, 2-heptanol, linalool, acetic acid, hexanal, and trans-2-hexanal, with a proportion of 15.50, 6.42, 6.10, 9.96, 4.53, 3.08, 10.34, and 7.38%, respectively. After primary fermentation of juice by D254 yeast, 54 types of aroma materials were obtained in blackberry wine. The aroma composition after primary fermentations was ethyl caprate, ethyl acetate, isoamyl alcohol, phenethyl alcohol, and acetic acid, which showed 8.91, 7.07, 16.43, 7.71, and 3.29%, respectively. However, secondary fermentation resulted in 50 aroma materials in blackberry wine. The proportion of different molecules was ethyl caprate (8.41%), ethyl acetate (7.04%), ethyl octanoate (6.3%), acetic acid (3.23%), isoamyl alcohol (16.34%), and phenethyl alcohol (7.59%).

A comparison between juice and wine showed 27 common aroma materials such as hexyl formate, ethyl acetate, isoamyl acetate, phenethyl alcohol, 2-heptanol, and acetic acid, which accounted for 83.97 and 71.74% aroma compositions of juice and wine, respectively. Results reveal that some substances from fruit juice were carried over to new aroma components after the fermentation process. Comparison between primary and secondary fermentation revealed 41 kinds of common constituents. These common constituents were ethyl caprate, ethyl acetate, ethyl octanoate, acetic acid, isoamyl alcohol, and phenethyl alcohol, which was 91.77% of total primary fermented aroma and 84.93% of secondary fermented aroma. The profile of aroma composition in blackberry wine at different stages of fermentation has been illustrated in Figure 1. The common observation made out of the results was that the formations of most of the aroma materials in blackberry wine develop particularly during primary fermentation.

Intriguingly, we observed disappearance of 28 aroma components during primary fermentation (11.52% of total juice aroma) and generation of 27 new aroma materials (accounting for 26.29%) during secondary fermentation (Table 1). The lost aroma constituents were butyl acetate, ethyl 3-hydroxybutyrate, n-butyl butyrate, ethyl 3-phenylpropionate, 1-nonanol, benzyl alcohol, 1-amyl alcohol,1-hexanol alcohol, lemonol, 6-methyl-5-hepten-2-ol, cis-3-hexenol, 1-octen-3-ol, hexanoic acid, butyric acid, benzaldehyde, phenylacetaldehyde, 2-heptanone, 4-carene, d-limonene, copaene, r-terpinene, 3-methyl-4-heptanone, 2-nonanone, 3,7-dimethyl-2,6-octadienal, α-cadinene, guaiacol, 4-ethyl guaiacol, and α-terpinene. The newly produced aroma components were ethyl pelargonate, isopentyl n-octanoate, isobutyl decanoate, ethyl caprate, methyl n-caprate, ethyl oleate, ethyl linoleate, ethyl ester, isopentyl laurate, ethyl laurate, ethyl cinnamate, diethyl succinate, ethyl phenylacetate, ethyl stearate, methyl hexadecanoate, ethyl-9-hexadecenoate, γ-butyrolactone, ethanol, 3-methylthiopropanol, 2,3-butylene glycol, capric acid, palmitic acid, benzoic acid, α-cadinene, α-pinene, 2,4-di-tert-butylphenol, and

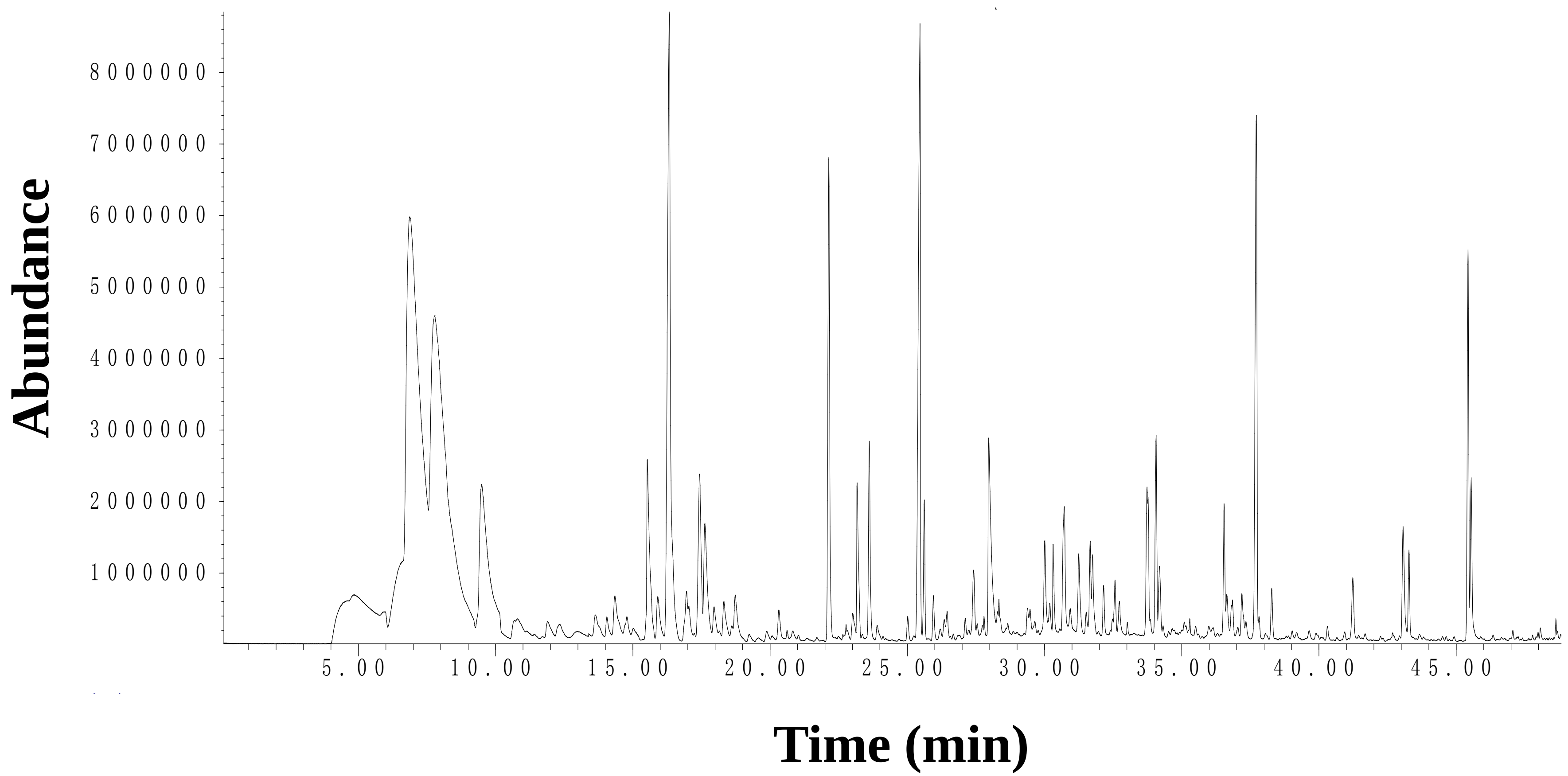

Figure 1a. GC-MS total ion of chromatogram of flavor components in raw material.

styrene. Additionally, 13 aroma ingredients disappeared during the secondary fermentation compared to primary fermentation, and it was almost 6.26% of the total aroma components.

Meanwhile, 9 new aroma compositions were generated during the secondary fermentation, which accounted for 4.69%. The aromas that disappeared were ethyl 3-hydro-xybutyrate, methyl n-caprate, ethyl ester, isopentyl lau-rate, ethyl laurate, phenycholon, 3-methylthiopropanol, 2,3-butylene glycol, palmitic acid, benzoic acid, hexanal, α-pinene, and 2,4-di-tert-butylphenol. The 9 new aroma compounds were methyl octanoate, ethyl 3-phenylpropionate, ethylbenzoate, ethyl laurate, 1-propanol, 1-butanol,

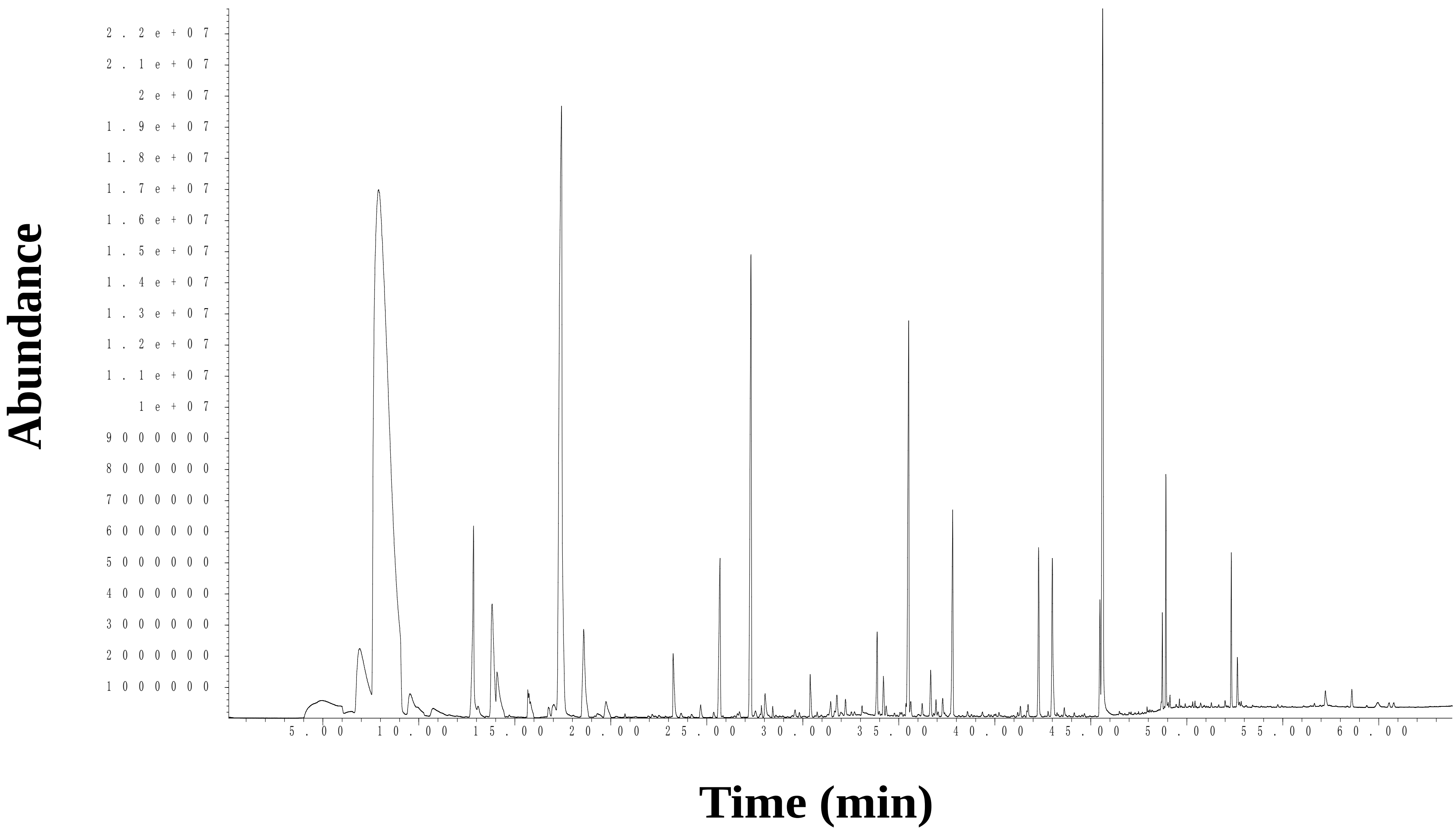

Figure 1b. GC-MS total ion of chromatogram of flavor components in raw wine.

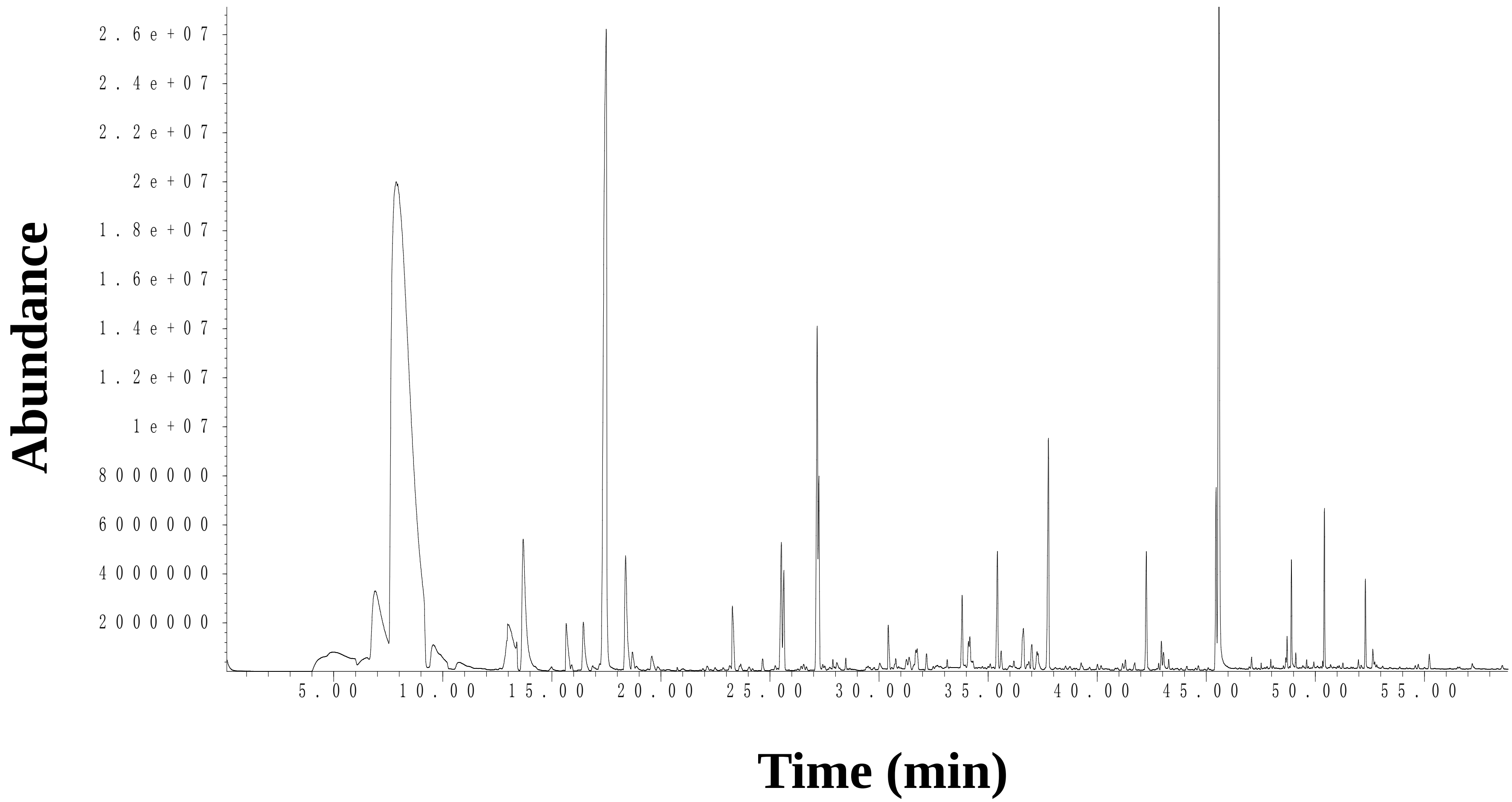

Figure 1c. GC-MS total ion of chromatogram of flavor components in aging wine.

d-citronellol, benzaldehyde, and cedrol. Small amount of aroma compositions were produced during the secondary fermentation, which is normally assumed to balance the wine aroma as the other constituent aroma gets lower during the process of fermentation.

Figure 2 reflects the changes of aroma components in blackberry juice and blackberry wine after primary fermentation and secondary fermentation. Based on Table 1, the content of main aroma in blackberry juice such as esters, alcohols, acids and other types were 38.88, 28.36,

Table 1. The main aroma compositions in blackberry fruit juice after primary and secondary fermentation.

Alcohol	Molecular formula	Molecular weight	Percentage (%)		
			1#	2#	3#
1-Nonanol	$C_9H_{20}O$	144	0.09	-	-
1-Octanol	$C_8H_{18}O$	130	0.27	0.05	0.1
Ethanol	C_2H_6O	46	-	9.7	9.18
Benzyl alcohol	C_7H_8O	108	0.01	-	-
Phenycholon	$C_9H_{12}O$	136	0.03	0.01	-
Phenethyl alcohol	$C_8H_{10}O$	122	1.33	7.71	7.59
2-heptanol	$C_7H_{16}O$	116	9.96	2.08	2.03
Linalool	$C_{10}H_{18}O$	154	4.53	1.31	0.89
Isobutyl alcohol	$C_4H_{10}O$	74	0.85	1.73	1.61
Isoamyl alcohol	$C_5H_{12}O$	88	1.85	16.43	16.34
1-Amyl alcohol	$C_5H_{12}O$	88	0.06	-	-
1-Hexanol alcohol	$C_6H_{14}O$	102	1.09	-	-
1-Propanol	C_3H_8O	60	-	-	0.02
1-Butanol	$C_4H_{10}O$	74	-	-	0.02
Lemonol	$C_{10}H_{18}O$	154	0.45	-	-
A-terpineol	$C_{10}H_{18}O$	154	3.27	0.63	0.5
D-Citronellol	$C_{10}H_{20}O$	156	-	-	0.03
2,3-Butylene glycol	$C_4H_{10}O_2$	90	-	0.04	-
1-Octen-3-ol	$C_8H_{16}O$	128	0.06	-	-
L-a-Cadinol	$C_{15}H_{26}O$	238	0.06	0.03	0.05
3-Methylthiopropanol	$C_4H_{10}OS$	106	-	0.01	-
Trans-2-hexenol	$C_6H_{12}O$	100	3.26	1.06	0.09
6-Methyl-5-hepten-2-ol	$C_8H_{16}O$	128	0.15	-	-
Cis-3-hexenol	$C_6H_{12}O$	100	1.08	-	-
Ester					
Hexyl formate	$C_7H_{14}O_2$	130	6.42	1.47	1.32
Acetic ether	$C_4H_8O_2$	88	15.5	7.07	7.04
Butyl acetate	$C_6H_{12}O_2$	116	0.65	-	-
Isoamyl acetate	$C_7H_{14}O_2$	130	1.4	3.09	2.98
Phenethylacetate	$C_{10}H_{12}O_2$	164	0.1	3.09	3.1
N-butyl butyrate	$C_8H_{16}O_2$	144	1.38	-	-
Ethyl butyrate	$C_6H_{12}O_2$	116	1.15	0.13	0.16
Ethyl-2-methylbutyrate	$C_7H_{14}O_2$	130	6.1	2.19	0.93
Methyl-2-methylbutyrate	$C_6H_{12}O_2$	116	3.39	1.04	1
Ethyl 3-hydroxybutyrate	$C_6H_{12}O_3$	132	1.12	-	-
Ethyl pelargonate	$C_{11}H_{22}O_2$	186	-	0.09	-
Ethyl caproate	$C_8H_{16}O_2$	144	1.44	1.68	1.45
Ethyl octanoate	$C_{10}H_{20}O_2$	172	0.13	6.52	6.3
Methyl octanoate	$C_9H_{18}O_2$	158	-	-	0.05
Isopentyl n-octanoate	$C_{13}H_{26}O_2$	214	-	0.17	0.08
Isobutyl decanoate	$C_{15}H_{30}O_2$	242	-	0.22	0.11
Ethyl caprate	$C_{12}H_{24}O_2$	200	-	8.91	8.41
Methyl n-caprate	$C_{11}H_{22}O_2$	186	-	0.07	-
Ethyl oleate	$C_{20}H_{38}O_2$	310	-	1.29	1.04
Ethyl linoleate	$C_{20}H_{36}O_2$	308	-	0.19	0.01
Ethyl ester	$C_{20}H_{34}O_2$	306	-	0.04	-
Isopentyl laurate	$C_{17}H_{34}O_2$	270	-	0.07	-
Ethyl laurate	$C_{14}H_{28}O_2$	228	-	4.31	-

Table 1. Continued.

Ethyl cinnamate	$C_{11}H_{12}O_2$	176	-	0.04	0.08
Diethyl succinate	$C_8H_{14}O_4$	174	-	0.02	0.02
Ethyl 3-phenylpropionate	$C_{11}H_{14}O_2$	178	0.03	-	0.03
Ethyl phenylacetate	$C_{10}H_{12}O_2$	164	-	0.01	0.05
Ethyl benzoate	$C_9H_{10}O_2$	150	-	-	0.06
Ethyl stearate	$C_{20}H_{40}O_2$	312	-	0.06	0.01
Palmitic acid ethyl ester	$C_{18}H_{36}O_2$	284	0.06	3.71	3.52
Ethyl myristate	$C_{16}H_{32}O_2$	256	0.04	2.13	2.14
Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	-	0.04	0.02
Ethyl laurate	$C_{14}H_{28}O_2$	228	-	-	4.19
Γ-butyrolactone	$C_4H_6O_2$	120	-	0.05	0.11
Ethyl9-hexadecenoate	$C_{18}H_{34}O_2$	282	-	0.12	0.05
Acid					
Acetic acid	$C_2H_4O_2$	60	3.08	3.29	3.23
Octanoic acid	$C_8H_{16}O_2$	144	0.12	1.53	1.42
Capric acid	$C_{10}H_{20}O_2$	172	-	0.28	0.11
Hexanoic acid	$C_6H_{12}O_2$	116	0.86	-	-
Butyric acid	$C_4H_8O_2$	87	0.07	-	-
Palmitic acid	$C_{16}H_{32}O_2$	256	-	0.07	-
Benzoic acid	$C_7H_6O_2$	122	-	0.02	-
Aldehyde and ketone					
Hexanal	$C_6H_{12}O$	100	10.34	1.14	-
Benzaldehyde	C_7H_6O	106	1.1	-	0.08
Phenylacetaldehyde	C_8H_8O	120	0.13	-	-
Trans-2-hexenal	$C_6H_{10}O$	98	7.38	1.17	1.12
3,7-dimethyl-2,6-octadienal	$C_{10}H_{16}O$	152	0.14	-	-
2-nonanone	$C_9H_{18}O$	142	0.15	-	-
3-methyl-4-heptanone	$C_8H_{16}O$	128	0.2	-	-
2-heptanone	$C_7H_{14}O$	114	1.23	-	-
Terpene					
Styrene	C_8H_8	104	-	0.22	0.16
α-Pinene	$C_{10}H_{16}$	136	-	0.14	-
4-carene	$C_{10}H_{16}$	136	0.36	-	-
α-Terpinene	$C_{10}H_{16}$	136	0.12	-	-
D-limonene	$C_{10}H_{16}$	136	0.31	-	-
R-terpinene	$C_{10}H_{16}$	136	0.25	-	-
α-Cadinene	$C_{15}H_{24}$	204	0.13	0.08	0.04
Copaene	$C_{15}H_{24}$	204	0.29	-	-
Phenol					
Phenol	C_6H_6O	94	0.05	0.01	0.02
Guaiacol	$C_7H_8O_2$	124	0.04	-	-
4-ethylguaiacol	$C_9H_{12}O_2$	152	0.02	-	-
2,4-di-tert-butylphenol	$C_{14}H_{22}O$	206	-	0.03	-
2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	220	1.93	1.44	0.68
Other					
(+)-Cedrol	$C_{15}H_{26}O$	222	-	-	0.05

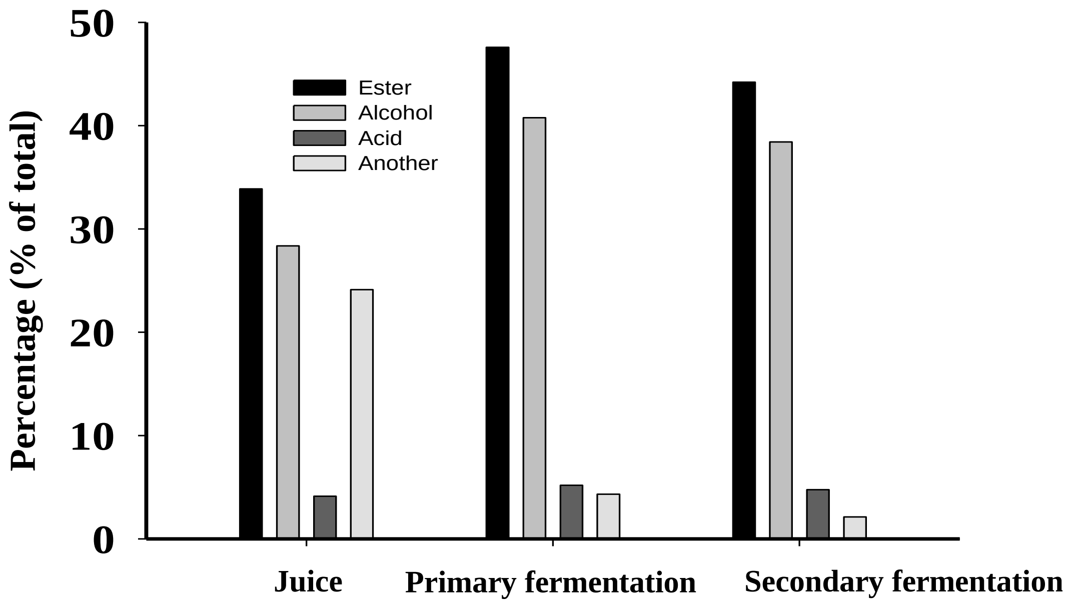

Figure 2. Types and relative contents of flavor components in raw material, raw wine, and aging wine.

4.13 and 24.13%, respectively; the content of main aroma in blackberry wine after primary fermentation were 47.58, 40.76, 5.18 and 4.32%, respectively; the content of main aroma in blackberry wine after secondary fermentation were 44.21, 38.42, 4.76 and 2.13%, respectively.

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