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# Effect of Peduncle on Aroma of Cabernet Sauvignon Dry Red Wine

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**Abstract.** The aim was to evaluate effect of peduncle on aroma of Cabernet Sauvignon dry red wine by changes in alcohols, acids, esters, alkane, benzene and unsaturated hydrocarbon including olefine, aldehyde, ketone, sugar and phenolic. Zero stalk (CK) was taken as control to compare effect of stalk ratio, including retaining 20% stalk (R20), 40% stalk (R40) and all stalk (R100), on aroma varieties and values of wine by gas chromatography-mass spectrometry (GC-MS). Obviously, total aromas in CK and R40 surpassed 80 species, respectively. However, the higher content came from R20 (15.64 mg) and CK (7.52 mg), respectively. Esters occupied 118 species (14.398 mg) in whole volatiles, while R40 of which incorporated the most 32. The next were alkanes, 68 (3.053 mg) and alcohols, 48 (17.457 mg), respectively. Variety and number of organic acids (1.845 mg) were very poor, which was a sign of wine maturation. Overall, R20 affected obviously wine aroma.

## INTRODUCTION

The wine with stem could increase ethanol, total phenolic, rotundone and total antioxidant capacity [1-2]. Fact has shown that there are various phenolics in the grapy carpopodium. Mature level of plants and fruits in different region influenced discrepancy in that of fruit stalk, which was accompanied by the variation on the varieties and contents of flavors, including acid, tannin, phenolics in the pedicel. These phenolic substances would interact with flavor ingredients in the skin and pulp by some critical processes, including steep, fermentation and aging, which ameliorated inevitably aromatous characteristic in wine. The methoxypyrazine content of stems was higher than those of grape berry or leaf, though the aliphatic carbonyl compound content of stems was less than the other parts. The blended stems increased the methoxypyrazine content, but the aliphatic green odorants of macerated stems were not detected in wine[3]. Hashizume et al. [4] detected 2-hydroxy-3-isobutylpyrazine and 2-hydroxy -3-isopropylpyrazine in unripe berries, and S-adenosyl-L-methionine -dependent O-methyltransferase activity toward 2-hydroxy-3- alkylpyarzine in crude extracts from young shoots and unripe grapes that accumulated 2-methoxy -3-alkylpyrazine. Undeniably, the more content of stem can not better quality of wine during the fermentation and adultness, which means usually the worse bitter, especially for the stronger bitter wine owe to the low maturity of stalk. Therefore, the mature level of the fruit and stem endowed the feature of wine with the affluent, complex and fruity flavor. The bitter wine would patronize the degustator of consumers. Based on the fact above, the proportion of stem was an important characteristic indicator of the wine flavor during the fermentation. It was necessary for the winemakers to master the influence of

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grape stem to different varieties of vinaceous quality according to the ecological characteristics in the region, so as to make an appropriate decision on whether or how to add the pedicel. Meanwhile a nuclear study on the aroma focused on improving elegant and rich degree of vinous aroma, and prolonging shelf life of wine through the stem.

The vineyard is located at Huami in Xinjiang, China (91°11'-96°33' W, 40°45' - 45°09' N). The region is characterized by a typically continental climate, with an abundant sunshine and the larger diurnal temperature difference during the growing season, hot and dry summer/cold winter, mean sunshine hours of approximate 3357 per year, and average annual temperature and annual precipitation about 9.9 °C and 33.2 mm, while there are typically 184 frost-free days per year. Compared with the same latitude, there are the more favorable light and heat resources. These advantages could avoid fewer diseases and insect pests, provid the advantaged ecological condition for the development on high quality of Cabernet Sauvignon [5]. Inevitably, Hami was evolved into a high-quality appellation for Cabernet Sauvignon grape [6]. On the one hand, the excessively hot climate promoted the inadequate accumulation of nutrient in the fruit, especially the aromatic substance. On the other hand, some flavorful volatiles from the stem could make up for the defect of insufficient accumulation from aroma - tique constituent during the frugivorous maturation. Cabernet Sauvignon, as an eurytopic variety, had been planted widely in Hami before 5 years old. There was excellent performance of the berry quality nowadays, which, therefore, was thought of as an investigative focus in this test. The fruit without peduncle (CK) was taken as control to compare aromatic components in R40, R20 and R100 wines, and determine the optimal reserving ratio of stalk embodying fully the potential aroma of Cabernet Sauvignon dry red wine, which provided theoretical basis for accumulation of volatile substance in the wine of the region.

## MATERIALS AND METHODS

### Condition of Vineyard

The experimental design consisted of two areas with three replicates (60 plants each) each in a completely randomized block in 2014 and 2015. North - south orientated grapevines, 3.0 m × 1.0 m on the silty loam soil, were trained to the horizontal trellises with the inclined trunks along.

### Winemaking

Healthy grapes, Cabernet Sauvignon, were harvested manually, and micro-vinification was performed with 20 kg of grapes per replication at Shihezi University on September 24, 2014 and 2015, respectively. Technological processes of every sample could be duplicated entirely. 5 mL of 6% sulphurdioxide was immediately added after extracted manually musts were barreled in 20 L of vitreous vessels. Following 4 h of blend, 0.02 g/L of pectinase (Lallzyme Ex) was also added according to commercial specifications. After another 4 h, 0.2 g/L of dried active yeast (Saccharomyces cerevisiae RC 212, Lallemand, Danstar Ferment AG, Switzerland) was replenished onto the supernatant and liquid temperature was monitored between 25 and 29 °C during the whole fermentation. After alcoholic fermentation (AF) finished (residual sugar < 4 g/L), wines were transferred respectively to 2-10 L of vitric containers by siphonage to get rid of air in vessels. Afterwards, lactic acid fermentation was finished favorably by adding Lactobacillus. In addition, some conventional parameters were assessed after alcoholic fermentation and a month of aging (Table 1), respectively. Subsequently, wine was stored under the aphotic surrounding at 4 - 6 °C for 12 months until final analyses.

### Volatile components analysis

Aromatous components were analyzed by GC-MS with especial configuration: one injector was connected to capillary column with a flow splitter. Aromatic ingredients were extracted by solid-phase micro-extraction technology. A 16-mL of sample was extracted for 30 min by the oscillator with a 16-μL of internal standard. Then the filtrate by 0.4 μm of membrane was injected in the GC. All samples were analyzed in triplicate.

The injector temperature was set at 250 °C. The oven temperature was kept at 40 °C for 3 minutes, then heated up to 120 °C at a rate of 5 °C/min, following 230 °C at a rate of 8 °C/min for 10 minutes, respectively.

The temperatures for transfer line and ionic source were set at 230 °C, respectively. Mass range (m/z) was 33 - 450 Da. Helium was used as the carrier gas (continuous flow 1.0 mL/min), and 2-octanol (99% optical purity, Aldrich Milwaukee, WI, USA) as the internal standard.

Identification of components was taken from the retention indices of one column and comparation with those of referential components, meanwhile volatile contents were determined by quantitative method. The mass spectra were obtained from the NIST2.0 MS library database, and in literature.

## Content of Volatile Components (CVC)

CVC is calculated using the following equation:

$$CVC = (\text{Peak area of aroma components}/2434943.02) \times 0.4914 \times 16 \times 0.001 \quad (1)$$

Where 2434943.02 indicates peak area of 2 - octanol; 0.4914 stands for concentration of 2 - octanol, g/L; 16 represents sample volume, mL.

## Statistical Analysis

Statistical analyses were performed using Microsoft Excel 2000 software (Microsoft Corporation). The results obtained from each treatment were compared by Duncan analysis of variance test, and least significant difference (LSD) at the 5% level was used to identify variant mean values.

# RESULTS AND DISCUSSION

## General Analysis

General properties of wines, including alcohol degree, pH, total soluble solid (TSS), total acid (TA), and residual sugar after alcoholic fermentation (AF) and a month of aging including malolactic fermentation (MLF), were determined according to GB 15037 2006. All parameters were analyzed in triplicate (Table 1). Therefore, each index determinated by GC/MS was eligible.

**TABLE 1.** General parameters in different stalks of Cabernet Sauvignon dry red wine.

| Treatment methods | Alcoholicity (%) | pH | TSS(%) | TA (g/L) | Residual sugar (g/L) |
|---|---|---|---|---|---|
| | | After AF | | | |
| CK | 12.6±0.01a | 3.86±0.02a | 10±0.03a | 6.56±0.05b | 3.98±0.04c |
| R20 | 12.1±0.02a | 3.92±0.02a | 8.2±0.02a | 6.35±0.02b | 3.89±0.02b |
| R40 | 12.2±0.03a | 3.98±0.01a | 8.0±0.05a | 6.14±0.01 | 3.66±0.03a |
| R100 | 12.2±0.01a | 4.03±0.04a | 8.0±0.04a | 6.22±0.06a | 3.60 ±0.01a |
| | | After a month of aging | | | |
| CK | 12.4±0.02a | 3.92±0.02a | 8.5±0.05a | 4.96 ±0.02a | |
| R20 | 11.9±0.04b | 3.96±0.05c | 8.5±0.03b | 4.96±0.03b | |
| R40 | 12.0±0.02a | 4.11±0.02b | 8.5±0.02a | 4.62±0.01c | |
| R100 | 12.0±0.03c | 4.21±0.03a | 9.0±0.04c | 5.66±0.02ab | |

**Note**: Different letters in the same column indicate significant differences ($P < 0.05$). These datum are from the means ± standard deviation (SD) in 2014 and 2015, respectively. Similarly hereinafter, except for Table 2.

From Table 2-8, 71 types of volatile compounds, 0.001 mg - 2.065 mg, the highest summation of 7.432 mg, was quantified in CK wine, which covered alcohols (12 varieties, 4.531 mg), acids (3 kinds, 0.013 mg), esters (28, 1.862 mg), unsaturated hydrocarbon including olefine, aldehyde, ketone, sugar, and phenolic (OAKSP, 8, 0.078 mg), alkane (17, 0.94 mg), benzene (3, 0.008 mg). Similarly, volatile constituent of R20 wine was detected 69 species, from 0.003 mg to 4.527 mg with the highest total value of 15.49 mg. These ingredients concentrated seven species of alcohols (7.586 mg), two kinds of acids (0.016 mg), 27 varieties of esters (6.734 mg), 7 types of OAKSPs (0.131 mg), 17 constituents of alkanes (0.918 mg), 9 classes of benzenes (0.105 mg). R40 wine was also analyzed successfully 78 volatile components from 0.001 mg to 1.681 mg with the highest total of 5.493 mg, such as 15 alcohols (2.684 mg), 1 acid (0.002 mg), 31 esters (1.791 mg), 5 OAKSPs (0.073 mg), 14 alkanes (0.796 mg), 12 benzenes (0.129 mg). It was noticed that R100 wine was found 62 compounds from 0.001 mg to 2.737 mg, the highest total was 6.868 mg, which incorporated 12 alcohols (2.616 mg), 2 acids (0.003 mg), 28 esters (3.997 mg), 6 OAKSPs (0.049 mg), 12 alkanes (0.201 mg), 2 benzenes (0.004 mg). These characteristic constituents could not be ignored although less.

**TABLE 2.** Varieties and quantity of volatile components in different stalks of Cabernet Sauvignon dry red wine

| Treatment methods | Acohols | Acids | Eters | OAKSPs | Akanes | Bnzenes | Subtotal |
|---|---|---|---|---|---|---|---|
| CK | 12 | 3 | 28 | 8 | 17 | 3 | 71 |
| R20 | 7 | 2 | 27 | 7 | 17 | 9 | 69 |
| R40 | 15 | 1 | 31 | 5 | 14 | 12 | 78 |
| R100 | 12 | 2 | 28 | 6 | 12 | 2 | 62 |
| Subtotal | 46 | 8 | 114 | 26 | 60 | 26 | 280 |

## Variety and Content of Volatile Alcohols

Alcohols in wine originated mainly from alcoholic fermentation, conversion of amino acid as well as oxidative degradation of linolenic acid [7]. From Table 3, there were 12, 7, 15 and 12 sorts of alcohols detected in 4 various Cabernet Sauvignon dry red wine, respectively, the most values of which belonged to R40 wine. Three alcohols, such as 3-methyl-1-butanol (isoamyl alcohol), 1-Hexanol and phenylethyl alcohol, were detected simultaneously in four wines, which were identified as inherent components for Cabernet Sauvignon dry red wine. There were all 3-methyl -1-butanol in four wines, especially in R20 sample upto the highest level of 4.527 mg. Therefore, R20 was the most favorable to the accumulation of isoamyl alcohol under a certain amount of oxygenous condition. The quantities of alcoholic esters in R20 wine, further, were the highest compared with other treatments (Table 5). 3-(methylthio)-1-propanol indwelled in three wines including CK, R20 and R40 (0.003, 0.012, and 0.006 mg, in turn), thus R20 could accumulate the most 3-(methylthio)-1-propanol. Also, CK, R40 and R100 samples were detected 2-methyl-1- butanol, 2-hexadecanol and 2,3-butanediol, which implied their outright advantage to the accumulation. In addition, 1-octanol was detected only in CK and R100 wines, benzyl alcohol, 2-nonanol and (S)-3-ethyl-4-methylpentanol were merely reserved in R40 and R100, 3-methyl- 2-hexanol in R20 and R40, 1-heptanol in CK and R40, which were manifested in small amount in wine, and suitable ratio of stem could be counted as a path accumulating more aromatic substances. Others was just found in any handling, which had much to do with the appended proportion of stalk during the wine fermentation. Stem, therefore, could influence the metabolic pathway and promote the self-aggregation of alcohols by adjusting progenitive and metabolic conditions of microorganism, such as pH, temperature and oxygen during the fermentation. It was noticed that some volatile constituents still existed in CK after a year of aging, such as 2-hexyl-1-decanol, tert-hexadecanethiol, 2-methyl-1-hexadecanol, and 1-heptanol, which originated from the transformation, esterification, synthesis and decomposition of internal components or precursors during the aging, or even the anaerobic conditions. Although less species, there was important effect on the winy flavor and clinical remedy. Another, 3-(methylthio)-1-propanol could endow wine the special flavor with the raw potato and garlic. Research of Tao et al. [8] demonstrated that the better the maturity was, the higher the number was, but it was not the better wish to the higher ripeness. The experiment found that the reservation of the suitable stem (R20) was beneficial to the accumulation of 3-(methylthio)-1- propanol compared to R100.

Alcohols in wine, especially $C_7$ - $C_{10}$ saturated alcohols, often exerted the fragrance of flowers. According to Djegui et al. [9], alcohols, fatty acids and esters were products of fermentation. Therefore, the species and contents of alcohols were associated closely with the fermentation stage. Ethanol was one of catabolic product in yeasts during the fermention. Lower alcohols were derived from the hydrolysis of esters during the fermentation [10]. While higher alcohols from yeast metabolism were related to the nitrogenous contents of grapes as well as homologous vinification [11-12]. Lower higher alcohols vested pleasant aroma to wine, however, undesirable flavor would emerge from higher concentrations of higher alcohols [12]. Therefore, contents of higher alcohols were important for evaluation of quality in wine [13]. Previous studies had confirmed that some alcohols, such as benzyl alcohol, phenylethyl alcohol, isoamyl alcohol and hexyl alcohol, had definite contribution to wine aroma [14]. Different alcohols could ensure complexity of aroma in wine. Benzyl alcohol with cheerful potpourri played an important role in the aromatous formation of whole wine [15], which was obtained only by brewing technology with R40 and R100 (Table 3). Benzene ethanol with typical aroma was mainly formed by the corresponding metabolism of amino acid under yeasty command or by metabolic pathway of glucose, which contributed the diversity of vinous relish with rose fragrance, violet, jasmine, lilac or peach. Therefore, it was not neglect of a positive role on fragrance of wine [16]. Isoamyl alcohol with fresh fragrance and bitter almond as well as 1-hexanol with herby aroma and toast constituted rich and elegant characteristic of representative aroma [17]. 2, 3 - Butanediol afforded butter and cream, and 2-methyl-1-butanol endowed the banana's flavor or bouquet for the wine. It was alarming that the suitable levels played positive effect on wine quality compared with the higher ones [10, 18]. In addition, lower 3-(methylthio)- 1-propanol could ensure unique flavor, including raw potato and garlic, to Cabernet Sauvignon dry red wine, which was more appropriate in a certain proportion of the stem (Table 3). (E)-2-nonen-1-ol with fat and violet odor was only in R20. 1-Octanol with jasmine was only detected in CK and R100 wines. 1-Hexadecanol with rose fragrance could inhibit greasy taste. Hexadecanoic acid, ethyl ester with nutty flavor was just from esterification of 1-hexadecanol with acetic acid in this experiment (Table 3).

**TABLE 3.** Varieties and contents of volatile alcohols in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK (mg) | R20 (mg) | R40 (mg) | R100 (mg) |
|---|---|---|---|---|
| 3-Methyl-1-butanol | 2.065±0.01a | 4.527±0.03a | 1.681±0.03a | 1.997±0.04a |
| 1-Hexanol | 0.054±0.01a | 0.080±0.04a | 0.043±0.04a | 0.090±0.02ab |
| Phenylethyl alcohol | 0.874±0.02a | 2.879±0.05a | 0.727±0.03a | 0.376±0.02a |
| 3-(Methylthio)-1-propanol | 0.003±0.03a | 0.012±0.01a | 0.006±0.04a | |
| 2-Methyl-1-butanol | 0.255±0.02a | | 0.183±0.01a | 0.076±0.02a |
| 2-Hexadecanol | 0.040±0.04a | | 0.003±0.01a | 0.002±0.03a |
| 2,3-Butanediol | 0.420±0.04a | | 0.007±0.02a | 0.040±0.06a |
| 1-Octanol | 0.060±0.03a | | | 0.007±0.04a |
| Benzyl alcohol | | | 0.003±0.05a | 0.007±0.04a |
| 2-Nonanol | | | 0.005±0.03a | 0.004±0.03ab |
| (S)-3-Ethyl-4-methylpentanol | | | 0.002±0.02a | 0.012±0.06a |
| 3-Methyl-2-hexanol | | 0.070±0.01a | 0.016±0.02a | |
| 1-Heptanol | 0.170±0.02a | | 0.005±0.01a | |
| Others | | | | |
| 2-Hexyl-1-decanol | 0.500±0.02a | | | |
| n-Heptadecanol-1 | | | 0.015±0.03a | |
| 1-Octen-3-ol | | | | 0.001±0.01a |
| Tert-hexadecanethiol | 0.080±0.05a | | | |
| (E)-2-Nonen-1-ol | | 0.007±0.02a | | |
| 1-Hexadecanol | | | 0.004±0.04a | |
| 3,7-Dimethyl-2-octen-1-ol | | | | 0.004±0.01a |
| 2-Methyl-1-hexadecanol | 0.030±0.04a | | | |
| 1-Dodecanol | | 0.011±0.01a | | |
| 3-Methyl-4-penten-1-ol | | | 0.002±0.02a | |



## Variety and content of organic acids

Organic acids in wine, mostly from yeasty fermentation excluding few from grape berries, were usually characterized by an unpleasant smell [19]. However, a small amount of organic acids played an important role in aroma balance because of inhibiting role from hydrolysis of aromatic ester [20].

**TABLE 4.** Varieties and contents of volatile organic acids in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK(mg) | R20(mg) | R40(mg) | R100(mg) |
|---|---|---|---|---|
| Phenyl-butanedioic acid | 0.004±0.02a | | | |
| 4-Hydroxy-butanoic acid | | 0.010±0.04a | | |
| Trans-13-octadecenoic acid | | | 0.002±0.02ab | |
| 4,4'-Dithiobis-butanoic acid | | | | 0.002±0.02a |
| Benzyl oxy tridecanoic acid | 0.006±0.03a | | | |
| o-Acetyl-L-serine | | 0.006±0.05a | | |
| 3-Hydroxy-dodecanoic acid | | | | 0.001±0.03a |
| 2-Hydroxypentadecyl propanoate | 0.003±0.02a | | | |

There were different species and quantities of organic acids in every wines (Table 4), which demonstrated obviously importance of pedicel to volatile organic acids, because consequence had much to do with not only proportion of carpopodium, but also aging technology. Alcohols were mainly derived from alcoholic fermentation, acids were principally accumulated during the fruit maturation, while esters were chiefly esterifiable product of alcohols and acids in wines. From Table 3-5, esters were the most components (Table 5), alcohols were followed (Table 3), while the least organic acids were listed in Table 4 as for varieties and contents, which indicated that grapes accumulated a lot of sugars owe to higher temperature in the region, so there generated plentiful varieties and higher contents of alcohols after fermentation; at the same time, the abundant solar - thermal resources resulted in clipping degradation of acidity in Hami during the maturation. Therefore, there was just the fewer accumulation of acid. Esters were abundantly accumulated after the fermentation and aging for a long time, thus the esterification between acids and alcohols reduced greatly varieties and contents of acids, including content of total acid (Table 1). Less organic acids were a sign of berries ripeness, and even wine's maturity and aging. Most organic acids detected in CK were born in grape berries, fermentation and aging of wine. Therefore, there had obvious effect of stem on organic acid in wine, which had a direct relationship with effect of mature degree, composition and organizational structure of stem on steep, fermentation and aging of wine, and manifested also that the inherent composition of grape berries (including precursors in grapy berries and stems) had not been completely transformed, synthesized, decomposed and esterified during the aging period. In addition, there was also firsthand relationship with organic acid created by anaerobic environment of berries and juices in CK wine. Despite the fewer varieties and amounts, these acids were important awfully for some characteristics of wine.

## Variety and content of esters

The largest percentage of esters (40.71%), totaling 118 species, were detected in aromatic components. Ethyl esters, mainly 13 species of alcohol esters just from 4 treatments, accounted for the largest proportion in esters (Table 5), which suggested that alcohol esters were the main characteristic esters in Cabernet Sauvignon wine during the aging period. There were many ethyl esters including octanoic acid ethyl ester, decanoic acid ethyl ester, hexanoic acid ethyl ester, tetradecanoic acid ethyl ester and palmitic acid ethyl ester in CK wine, which afforded higher levels, such as 0.914, 0.406, 0.131, 0.075 and 0.072 mg, respectively. Octanoic acid ethyl ester in R20 wine, however, reached the highest levels (up to 2.968 mg). The following order were samples R100, R40 and CK, contents of which were up to 2.737, 0.004 and 0.914 mg, respectively. Other esters also presented similar trend, which revealed that stem had conspicuous effect on divers esters, and others were characteristic esters going through corresponding handling. There was distinct difference of accumulative level in the same wine of disparate esters, however, more obvious difference arose in the same esters of disparate wine, which offered a new thought for exploitation of quality wine.

4 kinds of wines were detected out 15 categories of esters, such as ethyl butyrate, ethyl caproate, heptanoic acid ethyl ester, ethyl caprylate, succinic acid diethyl ester, acetate-3-methy- 1-butanol, acetic acid-2-phenylethyl ester, pelargonic acid ethyl ester, decanoic acid ethyl ester, octanoic acid-3-methylbutyl ester, tetradecanoic acid ethyl ester, pentadecanoic acid ethyl ester, hexadecanoic acid ethyl ester, isopentyl hexanoate, and ethyl-9-decenoate, which were characteristic ingredients of Cabernet Sauvignon wines after a long-term aging. Octadecanoic acid ethyl ester and arsenous acid-tris-(trimethylsilyl) ester were extracted in vintage wine except for R100, decanoic acid tetradecyl ester was done in samples besides R40, ethyl iso-allocholate and acetate-2-methyl-1-butanol were done in ones other than R20, 9-octadecenoic acid (Z)- phenylmethyl ester, butyrolactone, ethyl oleate and trans-9-octadecenoic acid (2-phenyl-1,3- dioxolan-4-yl) methyl ester were done in CK and R40, 12, 15 - octadeca diynoic acid methyl ester and cyclopropanedodecanoic acid 2-octyl- methyl ester were done in CK and R20, acetic acid hexyl ester was done in R20 and R100, butanedioic acid, ethyl 3-methylbutyl ester and cis-9-octadecenoic acid (2-phenyl- 1,3- dioxolan-4-yl) methyl ester were done in R40 and R100. The facts above verified that the reserved ratio of pedicel during the fermentation would accumulate different varieties and quantities of esters, which were ultimately determined by three factors, for instance the variety and purity of sugars and acids depending on the maturity, and influence of different proportion of stems to fermentative conditions (temperature, oxygen, and pH) during fermentation, except for the mutual conversion among acids, alcohols and esters during the aging. Whereas, there were different distributive ratios to other esters in every sample, the most of which were distributed to R40 and R100 wines, the second were R20 and CK, which were related to, on the one hand, the proportion of stalk added during the fermentation, on the other hand, by-products by varieties microbial metabolism during the fermentation. Therefore, there were larger discrepancy in vinous style.

**TABLE 5.** Varieties and contents of volatile esters in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK(mg) | R20(mg) | R40(mg) | R100(mg) |
|---|---|---|---|---|
| Butanoic acid, ethyl ester | 0.014±0.01a | 0.028±0.03a | 0.012±0.04a | 0.021±0.04a |
| Hexanoic acid, ethyl ester | 0.131±0.02a | 0.640±0.05a | 0.140±0.05a | 0.329±0.03a |
| Heptanoic acid, ethyl ester | 0.004±0.02a | 0.019±0.04a | 0.227±.0.03a | 0.011±0.05b |
| Octanoic acid, ethyl ester | 0.914±0.01a | 2.968±0.03a | 0.004±0.04a | 2.737±0.06a |
| Butanedioic acid, diethyl ester | 0.041±0.03a | 0.090±0.02a | 0.133±0.02a | 0.080±0.02a |
| Nonanoic acid, ethyl ester | 0.009±0.03a | 0.033±0.05a | 0.012±0.03a | 0.018±0.02b |
| Decanoic acid, ethyl ester | 0.406±0.03a | 0.840±0.02a | 0.647±0.04a | 0.456±0.04a |
| Tetradecanoic acid, ethyl ester | 0.075±0.02a | 0.043±0.04a | 0.078±0.02a | 0.036±0.03a |
| Pentadecanoic acid, ethyl ester | 0.003±0.02b | 0.004±0.05a | 0.009±0.03a | 0.004±0.04a |
| Hexadecanoic acid, ethyl ester | 0.072±0.02a | 0.038±0.03b | 0.160±0.05a | 0.031±0.01a |
| Acetic acid, 2- phenylethyl ester | 0.010±0.04a | 0.040±0.03a | 0.007±0.04a | 0.007±0.02b |
| Ethyl 9- decenoate | 0.035±0.03a | 0.128±0.02a | 0.065±0.02a | 0.076±0.04a |
| Isopentyl hexanoate | 0.006±0.03a | 0.015±0.05a | 0.007±0.03a | 0.015±0.03a |
| Acetate-3-methy-1-butanol | 0.062±0.02a | 1.700±0.03a | 0.058±0.04a | 0.106±0.04a |
| Octanoic acid, 3-methylbutyl ester | 0.040±0.02a | 0.087±0.04a | 0.050±0.06a | 0.030±0.02a |
| Octadecanoic acid, ethyl ester | 0.006±0.03a | 0.004±0.06a | 0.005±0.02b | |
| Arsenous acid, tris (trimethylsilyl) ester | 0.001±0.01a | 0.005±0.04a | 0.002±0.03a | |
| Decanoic acid, tetradecyl ester | 0.004±0.02a | 0.004±0.05a | | 0.003±0.04a |
| Acetate-2-methyl- 1-butanol | 0.006±0.03b | | 0.006±0.05b | 0.005±0.03a |
| Ethyl iso-allocholate | 0.002±0.02b | | 0.003±0.02a | 0.001±0.02a |
| 9-Octadecenoic acid (Z)- phenylmethyl ester | 0.002±0.02a | | 0.004±0.04a | |
| Butyrolactone | 0.003±0.03a | | 0.002±0.03a | |
| Ethyl oleate | 0.007±0.05a | | 0.005±0.05b | |
| Trans-9-octadecenoic acid (2-phenyl-1,3- dioxolan-4-yl) methyl ester | 0.002±0.04a | | 0.001±0.04a | |
| 12,15-Octadeca diynoic acid methyl ester | 0.001±0.03a | 0.004±0.03a | | |
| Cyclopropanedodecanoic acid, 2-octyl-, methyl ester | 0.001±0.02a | 0.003±004a | | |
| Acetic acid hexyl ester | | 0.008±0.03a | | 0.002±0.04a |
| Cis-9-octadecenoic acid (2-phenyl-1,3- dioxolan-4-yl) methyl ester | | | 0.001±0.03a | 0.001±0.03a |
| Butanedioic acid, ethyl 3-methylbutyl ester | | | 0.040±0.04a | 0.004±0.04b |
| Others | | | | |
| Carbonic acid, heptyl methyl ester | | 0.005±0.04a | | |
| Methyl 4-hydroxybutanoate | | | 0.004±0.06a | |
| Octanoic acid, 2- butyl ester | | | | 0.003±0.03a |
| 13-Methyl- tetradecanoate-ethyl | 0.003±0.01a | | | |
| Benzeneacetic acid, ethyl ester | | 0.004±0.05a | | |
| Benzeneacetic acid, 3-tetradecyl ester | | | 0.010±0.02a | |
| Tridecanoic acid, tripropylsilyl ester | | 0.007±0.05a | | |
| (S)-(-)- Lactate ethyl | 0.002±0.03a | | | |
| 2,5-Octadecadiynoic acid, methyl ester | | 0.003±0.01a | | |
| n-Caprylic acid isobutyl ester | | | 0.002±0.04a | |
| Benzeneacetic acid, 3-pentadecyl ester | | | | 0.003±0.04a |
| (L)-Propanoic acid, 2-hydroxy-, ethyl ester | | | | 0.005±0.02a |
| 13,16-Octadecadiynoic acid, methyl ester | | 0.004±0.02b | | |
| Chloroacetic acid 3-methylbutyl ester | | | | 0.003±0.01a |
| Butanedioic acid, ethyl 3- methylbutyl ester | | 0.010±0.04a | | |
| (E)-9-Octadecenoic acid ethyl ester | | | 0.053±0.04a | |
| 3- Hydroxy - dodecanoic acid, ethyl ester | | | | 0.001±0.04a |
| Ethyl 9- hexadecenoate | | | 0.044±0.06a | |
| Oleic acid, eicosyl ester | | | | 0.002±0.03a |
| Hexanoic acid, 2- methylbutyl ester | | | 0.003±0.03a | |
| Propyl octanoate | | | | 0.007±0.01a |

Esters in wine were mainly acquired through three approaches: accumulation of peel during the development, fermentation commanded by yeast or bacteria, and esterification during the storage [21]. Fatty acids were synthesized esters with alcohols by the catalysis of enzyme, acyl CoA combined ethanol into fatty acid ethyl ester, and acetate was composited acetyl CoA with higher alcohol, which contributed fragrance to wine, meanwhile reduced the various characteristic of vinous aroma, promoted the balance, fusion, and coordination among all kinds of smells finally [22], such as ethyl butyrate, ethyl palmitate and octylic acid ethyl ester with typical fruity, and phenyl ethyl acetate with pleasant scent [23]. Based on the above study, R20 was more advantageous to accumulation of ethyl butyrate and ethyl caprylate, nevertheless R40 was more conducive to boost of palmitic acid ethyl ester, which guided the transformation of aroma in wine to a more strong degree, so as to the tendency of balance, fusion and coordination (Table 5). In theory, synthesis of every ester required conjunct participation of an organic acid and an alcohol. So, the reason that there was not stearic acid ethyl ester in R100 relied on the absence of stearic acid in the corresponding wine during the fermentation (Table 4-5). Some ingredients, such as ethyl butyrate, ethyl caproate, ethyl caprylate, decanoic acid ethyl ester and 2 - phenyl ethyl acetate, took on the typical aroma of fresh wine [24]. In this study, the lower content of succinic acid diethyl ester contributed to aromatic characteristic in wine [25].

Rich esters were the main reason for complex aroma, every ester showed distinct characteristics in aroma. Octanoic acid ethyl ester with pleasant sweetness, fruity flavor, pear, apricot, bouquet, and yeast flavor under the lower threshold (5 μg/L) could reflect aroma with generally small content in wine, so the role could not be ignored [26], which displayed obviously in wine owe to extremely high content (Table 5). Ethyl caproate with green apple, strawberry and anise was an important contributor to the fruity aroma of fresh wine, and fermentation under the lower temperature could midwifed this aroma [17]. Decanoic acid ethyl ester could keep fruity aroma and comfortable vinegar; ethyl butyrate could emit aromas, such as sour fruit, strawberry and fruit under the lower threshold. These esters were the characteristic component of aroma in the region.

Lactic acid esters, with flavor of milk and dried fruit, were generated mainly during the malolactic fermentation [27]; succinic acid esters with frugivorous aroma originated from activity of lactobacillus, however, the threshold in red wine was so high that there was not so prominent aroma [23]; lauric acid ethyl ester took on flavor of spices. Enough acetate-2-methyl- 1-butanol could usually induce suncrack of skin. Therefore, the negation was necessary because of the less value in wine.



## Variety and content of OAKSP

2,4-Di-tert-butylphenol and nonanal were detected in four wines (Table 6).

**TABLE 6.** Varieties and contents of volatile olefines, aldehydes, ketones, sugars, and phenols in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK(mg) | R20(mg) | R40(mg) | R100(mg) |
|---|---|---|---|---|
| 2,4-Di-tert-butylphenol | 0.046±0.02a | 0.081±0.04a | 0.052±0.04a | 0.024±0.01a |
| Nonanal | 0.014±0.05a | 0.007±0.02b | 0.003±0.02b | 0.004±0.03a |
| 2-Nonanone | 0.002±0.04a | 0.009±0.03a | | 0.004±0.02a |
| 1- Methyl-4- (1-methylethenyl)- (S)-Cyclohexene | 0.002±0.03a | 0.013±0.04a | | 0.003±0.04a |
| Benzeneacetaldehyde | 0.002±0.02b | 0.009±0.03a | | |
| Decanal | 0.004±0.02a | | 0.005±0.02a | |
| Dodecanal | | 0.008±0.04a | 0.012±0.02a | |
| l-Gala-l-ido-octose | | 0.004±0.03a | 0.001±0.02a | |
| Others | | | | |
| Pentadecanal | | | | 0.003±0.02a |
| Styrene | 0.004±0.02a | | | |
| 1-Nonene | | | | 0.011±0.04a |
| 4-O-methyl mannose | 0.004±0.03a | | | |

The specific ingredients, adjusting only yield without the direct relationship with stem, emerged during the fermentation and aging. Nonanal, derived from oxidation of nonanol during the alcoholic fermentation (Table 3), could be oxidized to nonanone (Table 6), and pelargonic acid further. However, few pelargonic acid was entirely esterified into pelargonic acid ethyl ester (Table 5). So there was not pelargonic acid detected in Table 4. Wines reserving Ck, and R20 and R100 existed 2 - nonanone and 1-methyl-4- (1-methylethenyl)-(S)-cyclohexene without exception, CK and R20 were detected benzene acetaldehyde, CK and R40 discovered decanal, and R20 and R40 were done dodecanal and l-gala-l-ido-octose, which made clear a certain relationship of ingredient with content and proportion of stems. There was no nonanone detected in R40 because the lesser nonanol obtained from the fermentation was transformed into nonanoic acid ethyl ester. The most species of other OAKSPs belonged exclusively to CK wine. The flavor substances, mainly born in grape and wine, were concerned with the fermentation and aging of wine, while the varieties in R100, R40 and R20 wines were reduced, which assured that OAKSP were associated with the adding proportion of stem, in addition to the factors above. For instance, there were assay difference, such as benzene acetaldehyde, decanal, twelve aldehyde, pentadecanal, octadecanal and styrene, of the corresponding alcohols under different fermentation conditions.

## Variety and Content of Volatile Alkanes

In Table 7, 7 alkanes, including octadecamethyl-cyclononasiloxane, decamethyl-cyclopentasiloxane, dodecamethyl-cyclohexasiloxane, pentyl-cyclopropane, 3-ethyl-5-(2-ethylbutyl)-octadecane, hexadecamethyl-cyclooctasiloxane, and tetradecamethyl- cycloheptasiloxane, were detected in each sample, which verified common alkane in Cabernet Sauvignon wines. Three samples except for R100 were detected out 3-methyl- tetradecane and 2,6,10-trimethyl-dodecane, three liquors apart from R40 were checked out nonadecane, hexamethyl-cyclotrisiloxane and octamethyl-cyclotetrasiloxane. Additionally, triethyl (2-phenylethoxy)-silane was also measured in three wines other than R20. Similarly, 2-methyl -pentadecane was merely probed in CK and R20, and tetradecane and heptadecane were nothing but explored in R40 and R20. In addition, every wine was plumbed the corresponding alkane (in the rest, Table 7). It could be seen that 3 species of alkanes emerged from CK, while R20 and R40 were examined 2 breeds of alkanes, respectively, and R100 was done 1.

**TABLE 7.** Varieties and contents of volatile alkanes in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK(mg) | R20(mg) | R40(mg) | R100(mg) |
|---|---|---|---|---|
| Decamethyl-cyclopentasiloxane | 0.037±0.02a | 0.068±0.04a | 0.017±0.04a | 0.037±0.02a |
| Dodecamethyl-cyclohexasiloxane | 0.030±0.04a | 0.052±0.03a | 0.020±0.02a | 0.034±0.03a |
| Tetradecamethyl-cycloheptasiloxane | 0.472±0.05a | 0.262±0.04a | 0.410±0.03a | 0.052±0.01a |
| Hexadecamethyl-cyclooctasiloxane | 0.123±0.03a | 0.142±0.01a | 0.146±0.04a | 0.018±0.02a |
| Octadecamethyl-cyclononasiloxane | 0.013±0.02a | 0.031±0.02a | 0.033±0.01a | 0.007±0.03a |
| Pentyl-cyclopropane | 0.004±0.04a | 0.008±0.01a | 0.003±0.02b | 0.007±0.02a |
| 3-Ethyl-5-(2-ethylbutyl)-octadecane | 0.001±0.03a | 0.005±0.04a | 0.002±0.02a | 0.002±0.02b |
| 2,6,10-Trimethyl-dodecane | 0.040±0.04a | 0.029±0.04a | 0.020±0.03a | |
| 3-Mehyl-tetradecane | 0.010±0.02a | 0.009±0.03a | 0.011±0.03a | |
| Nonadecane | 0.159±0.03b | 0.157±0.02a | | 0.027±0.02a |
| Hexamethyl-cyclotrisiloxane | 0.006±0.02a | 0.031±0.02a | | 0.008±0.04a |
| Octamethyl-cyclotetrasiloxane | 0.002±0.03b | 0.008±0.04a | | 0.002±0.02b |
| Triethyl(2-phenylethoxy)-silane | 0.002±0.02a | | 0.002±0.02a | 0.004±0.03a |
| 2-Methyl-pentadecane | 0.024±0.05a | 0.015±0.03a | | |
| Tetradecane | | 0.033±0.04a | 0.009±0.03a | |
| Heptadecane | | 0.011±0.02a | 0.004±0.04a | |
| Others | | | | |
| 1-(Methylthio)-octadecane | | 0.003±0.03a | | |
| 2,6-Dimethyl-heptadecane | | | 0.005±0.03a | |
| 1-(Ethenylthio)-butane | | | | 0.003±0.02a |
| 4-Methyl-tetradecane | 0.004±0.03a | | | |
| 1-(1-Ethoxyethoxy)-pentane | | 0.054±0.01a | | |
| Hexadecane | | | 0.114±0.03a | |
| 3-Methyl-pentadecane | 0.010±0.02a | | | |
| 1-(Ethenyloxy)-octadecane | 0.003±0.04a | | | |

Methoxy pyrazines were of distinctive compounds in Cabernet Sauvignon wines. The suitable content of methoxy pyrazines shouldered a significant contribution to the typical feature and complexity of wine. On the contrary, characteristic of green and immature fruit depended on excessive methoxy pyrazine [28]. Methoxy pyrazines were dived into wine through harvesting and processing process of grapes, and disturbed wine flavor further[29]. Silicas could balance methoxy pyrazine [28]. Therefore, methoxy pyrazines were not detected in four samples after a long-term aging, which was a symbol of wine maturation.

Hydrocarbons in grapes decreased significantly owe to esterification of alcohol and acid by oxidization of hydrocarbons during the fermentation [30]. Paraffin's contribution to wine aroma was smaller [26]. For instance, low-grade $C_6$ alkanes, n-hexane and cyclohexane, would present special fresh aroma to wine owe to slight petrol. The higher level of alkanes (more than $C_{10}$) would emit strong petrol. However, there was little effect on the overall flavor of wine because of higher boiling point as well as less content in wine [26]. Therefore, higher amount of nonadecane was not enough to affect the overall flavor of wine in this experiment. Tetradecamethyl-cycloheptasiloxane level was higher, but it's silica structure could balance methoxy pyrazine, therefore, flavor of wine was not actually affected.

## Variety and content of volatile benzenes

From Table 8, three benzenes, such as (1-butylheptyl)-benzene, (1-pentylheptyl)-benzene and (1-butyloctyl)-benzene, could be detected in CK, R20 and R40, respectively. Therefore, the more stems might not be always good news for the accumulation of benzenes. (1-butylhexyl)-benzene appeared in R20, R40 and R100, which implied that birth of (1-butylhexyl)-benzene depended on a certain oxygen. Therefore, a proportion of stems were in favor of augment of (1-butylhexyl)- benzene in wine. Four benzenes, including (1-propylnonyl)-benzene, (1-ethyldecyl)-benzene, (1-butylnonyl)-benzene and (1-propylheptyl)-benzene were successfully detected in R20 and R40 wines, which suggested that microaerophilic environment created by an appropriate proportion of stems was conducive to accumulating 4 benzenes above. Benzenes in other were only excavated in one of wines except for CK. In Table 8, R40 wine was detected 4 categories of benzenes simultaneously, while other two samples were only traced one, respectively. Therefore, every benzene needed correspondingly environmental conditions, which was intimately related to chemical properties themselves.

**TABLE 8.** Varieties and contents of volatile benzenes in different stalks of Cabernet Sauvignon dry red wine.

| Compound Name | CK (mg) | R20 (mg) | R40 (mg) | R100 (mg) |
|---|---|---|---|---|
| (1-Butylheptyl)-benzene | 0.004±0.02a | 0.029±0.02a | 0.033±0.02a | |
| (1-Pentylheptyl)-benzene | 0.002±0.03a | 0.014±0.01a | 0.010±0.01a | |
| (1-Butyloctyl)-benzene | 0.002±0.02a | 0.012±0.01a | 0.010±0.01a | |
| (1-Butylhexyl)-benzene | | 0.008±0.03a | 0.009±0.02a | 0.001±0.01a |
| (1-Propylnonyl)-benzene | | 0.007±0.02b | 0.005±0.02a | |
| (1-Ethyldecyl)-benzene | | 0.004±0.02a | 0.004±0.03a | |
| (1-Butylnonyl)-benzene | | 0.004±0.03a | 0.002±0.01a | |
| (1-Propyldecyl)-benzene | | 0.003±0.02a | 0.011±0.02a | |
| Others | | | | |
| (1-Propylheptadecyl)-benzene | | 0.024±0.01a | | |
| (1-Propyloctyl)-benzene | | | 0.033±0.03a | |
| 1,2-Bis(trimethylsilyl)-benzene | | | | 0.003±0.02a |
| (1-Methyldecyl)-benzene | | | 0.009±0.02a | |
| (1-Methylundecyl)-benzene | | | 0.002±0.03a | |
| (1-Methylnonadecyl)-benzen | | | 0.001±0.01a | |

Derivatives of benzenes were derived from grape fruit [31], which could be turned into fatty benzene by secondary metabolism, and form a variety of acids, alcohols, flavonoids and aromatic benzenes further [32]. Therefore, less and less volatile benzene was assembled in wine after the fermentation and aging. The similar result was also observed in the experiment. R100 was not detected out (1-butylheptyl)-benzene, (1-pentylheptyl)-benzene and (1-butyloctyl)-benzene(Table 8), which was in connection with the higher volatility from whole stem besides transformation between relevant ingredients during the fermentation and aging. CK didn't detect (1-butylhexyl)-benzene owe to anaerobic fermentation. In Table 8, R40 and R20 wines centralized the largest number of volatile benzenes. Therefore, the two methods were more conducive to the accumulation of volatile benzenes.

## CONCLUSIONS

In conclusion, some results were obvious: There was obvious impact of stem on aromatic accumulation in wine. In all the volatile substances, the most abundant esters met 118 species, while R40 wine contributed the peak value of 31 esters in proprietary samples. But aroma content each in R20 wine was relatively higher. All in all, R20 was the most obvious method to influence of aroma-tique feature. Emergence of individual aromatic constituents in designated wine had directly something to do with stem's compositions and fermentative conditions, such as temperature and ventilation adjusted by stalks. After the aging, Si-O compounds balanced or even eliminated methoxy pyrazine. So there were not methoxy pyrazine compounds in four Cabernet Sauvignon wines, which was also a symbol of wine maturation. More esters were synthesized in all aromatic substances, which rooted mainly in significant esterification between alcohol and acid from the oxidization of hydrocarbons during the fermentation. In a word, effect of carpopodium on special flavor was obviously prominent, relish of wine could be readily adjusted by varieties and proportions of pedicels soaked in wine according to demand of drinker.

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