Effect of Grapevine Age on the Aroma Compounds in 'Beihong' Wine

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The main aim of this study was to determine the influence of grapevine age (3, 6 and 12 years) on the aroma compounds in 'Beihong' wine. Aroma compounds in wine were analyzed by solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC/MS). Thirty-three (33) volatile compounds were identified and quantified. The majority of aroma compounds were esters (20) and the concentration of these totaled 90.63-92.82% (w/w) of the total aroma compounds; particularly, ethyl octanoate and ethyl decanoate. Through the descriptive analysis aroma profile for 'Beihong' wine, the highest aroma contribution was from the fruity and floral series. As the age of the grapevine increased, the concentrations of total volatiles and total odor activity values (OAVs) of the wines significantly increased ($p < 0.001$). This suggests that grapevine age could affect berry composition, enhance the content of wine aroma compounds and improve wine quality.

INTRODUCTION

The 'Beihong' grape is a late-ripening red wine cultivar derived from 'Muscat Hamurg' × '*V. amurensis*'. The 'Beihong' berry matures by the end of September in Beijing. This variety is attractive to Chinese organic wine-making industries. There are several reasons for the interest in 'Beihong': (a) It has a high soluble solid (23.8-27%) (w/w) and appropriate acid content (pH 3.2-3.4); (b) it is highly resistant to cold and can grow in most places in Northern China without needing to be covered in winter when the temperature is usually very low; (c) it has high resistance to disease and can grow in some of the warm humid areas of Southern China; (d) 'Beihong' wine has a balanced taste and delightful odor, in particular a Muscat aroma.

When buying wine, aroma is one of the most important quality attributes to consumers (Lorenzo *et al*., 2008; Tao *et al*., 2009). To date, several hundred volatile aroma compounds such as alcohols, esters, organic acids, aldehydes, ketones and terpenes have been identified in wine and their combination forms the character of wine and distinguishes one wine from another. Over 800 volatile compounds have been found in wines, with a wide concentration range varying from hundreds of mg/l to the µg/l or ng/l level (Marti *et al*., 2003).

The aroma profile of a wine is influenced by many factors: grape variety, climatic conditions, soil, region, cultivation techniques, yeast, enological techniques and aging (Jackson and Lombard, 1993; Moret *et al*., 1994; Dirninger *et al*., 1998; Fischer *et al*., 1999; Jones and Davis 2000; Falqué *et al*., 2001; Gómez-Míguez *et al*., 2007). However, few studies have investigated the effect of grapevine age on the aroma compounds in wine, especially in 'Beihong' wine. Thus, the objective of this study was to determine the influence of grapevine age (3, 6 and 12 years) on the major volatile compounds in 'Beihong' wine.

MATERIALS AND METHODS

Grape sampling

The grapevines were grown at the Institute of Botany, the Chinese Academy of Sciences, Beijing, China. The soil in the vineyard was sandy (yellow-brown-soil) and the row and vine spacing were 2.5 × 1.0 m, respectively. Grape samples were harvested at 24°Brix on the basis of similar size and absence of physical injuries or infections. Sixty (60) grapevines were selected from ten (10) rows and 1 grape sample each from 3, 6 and 12 year-old grapevines, respectively.

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Yeast strains

Two different strains of *Saccharomyces cerevisiae* were tested. The commercial strain *S. cerevisiae* R_2 (Lallemand, France) was used as reference standard. *S. cerevisiae* wine yeast strain BH8 was isolated from the 'Beihong' wine grape variety, cultivated by the Institute of Botany, the Chinese Academy of Sciences, Beijing, China (Li *et al*., 2010). This strain was identified as *S. cerevisiae* by color and colony topography on WL Nutrient Agar (Pallmann *et al.*, 2001) as well as DNA sequence analysis, conducted in the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. These commercial yeast strains are widely used in Chinese wine factories with good results.

Fermentation experiments

After harvesting, the 'Beihong' grapes were immediately destemmed, softly crushed and sulfur dioxide added (sulfited) to a final concentration of 20 mg/L. The musts were clarified by cold-settling for 18 h at 10°C to separate the clear juice from the sediment. Finally, the pH was adjusted to 3.3 by aseptically adding tartaric acid (85%, w/v). This organic acid was selected because it is normally found in grapes and wines and is very rarely metabolized by ascomycetous yeasts. The 'Beihong' grape must was sterilized with 0.2% (v/v) dimethyl dicarbonate for 48 h at 4°C to allow decomposition (Pérez-Torrado *et al*., 2002).

Fermentations were carried out in 1L flasks with 800 ml medium and fitted with lids that excluded the atmospheric oxygen, but enabled the carbon dioxide to escape and samples to be removed. The initial yeast inoculums were 1×10^6 cells/ml from cultures grown overnight in YPD medium (1%, w/v, yeast extract, 2%, w/v, peptone and 2%, w/v, glucose).

Fermentations with two different yeast strains in the grape must of 3, 6 and 12 year-old grapevines, respectively, were derived in triplicate at 15°C, without shaking. The weight loss of the fermenters was monitored and the fermentation stopped when the CO_2 release was less than 0.2 g/day.

General enological parameters

The general parameters of the wine were analyzed according to the Office International de la Vigne et du Vin. (1990). The following parameters were analyzed: alcohol percentage, reducing sugar, pH, total acidity, free SO_2 and dry extract.

Reagents and standard solutions

All standard solutions were purchased from Sigma-Aldrich (China sector) and Fluka (Buchs, Switzerland). Sample solutions were prepared using the methods reported by Howard (Howard *et al*., 2005). The internal standard was 4-Methyl-2-pentanol. For quantification, 5-point calibration curves for each compound were prepared using the method described by Ferreira *et al*. (2000). This was also used as a reference to determine the concentration range of standard solutions. The regression coefficients of the calibration curves were above 97% (w/w).

HS-SPME procedure

Aroma compounds were extracted from the wine samples by HS-SPME and analyzed using gas chromatography/mass spectrometry as described by Zhang *et al*. (2007). A five milliliter sample of wine and 1g NaCl were placed in a 15 mL sample vial. The vial was tightly capped with a PTFE-silicon septum and heated at 40°C for 30 min on a heating platform with agitation at 400 rpm. The SPME (50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA, USA), preconditioned according to the manufacturer's instructions, was then inserted into the headspace, where extraction occurred for 30 min with continued heating and agitation, using a magnetic stirrer. The fiber was subsequently desorbed in the GC injector for 25min.

GC-MS analysis

The GC-MS system used was an Agilent 6890 GC equipped with an Agilent 5975 mass spectrometer. The column used was a 60m × 0.25mm HP-INNOWAX capillary with 0.25 µm film thickness (J & W Scientific, Folsom, CA, USA). The carrier gas was helium at a flow rate of 1 mL/min. Samples were injected by placing the SPME fiber at the GC inlet for 25min with the splitless mode. The oven's starting temperature was 50°C, which was held for 1min, then raised to 220°C at a rate of 3°C/min and held at 220°C for 5 min. The mass spectrometry in the electron impact mode (MS/EI) at 70eV was recorded in the m/z range 20 to 450 U. The mass spectrophotometer was operated in the selective ion mode under auto tune conditions and the area of each peak was determined by ChemStation software (Agilent Technologies). The analyses were repeated three times.

Statistical analysis

All statistical procedures were performed through SPSS version 16.0 statistical package for Windows (SPSS Inc., USA). A one-way analysis of variance (ANOVA) was used to gauge differences between means of volatiles amounts employing Duncan's multiple range tests at a level of $p < 0.001$, and the results were presented as mean ± SD of triplicate runs.

RESULTS AND DISCUSSION

General composition of wine

After approximately three weeks, all the fermentations were completed (final concentration of sugar < 4 g/l). The general composition of the wine is shown in Table 1. 'Beihong' wine made from grapevines of three different ages and two yeast strains did not show significant differences ($p < 0.05$) in dry extract, total acidity, pH, free SO_2, reducing sugar and ethanol concentration.

Aromatic profile analysis

The volatile compounds isolated from 'Beihong' wine made from grapes taken from grapevines of different ages and using two different yeast strains are listed in Table 2. Odor descriptors and thresholds are also listed in Table 2. In this study, the olfactory perception threshold and odor descriptors of the compounds in wine were taken from information available in the literature (Guth, 1997; López *et al*., 1999; Ferreira *et al*., 2000; Culleré *et al*., 2004; Peinado *et al*., 2004; Gómez-Míguez *et al*., 2007; Li *et al*., 2008; Liberatore *et al*., 2010; Pino & Queris, 2011). A total of 33 compounds were identified and quantified in 'Beihong' wine, including alcohols (4), aldehydes (3), terpenes (2), acids (4) and esters

TABLE 1
General composition of wines

Composition of wine	Type of wine[a]					
	3B	3R	6B	6R	12B	12R
Alcoholicity (v/v, %)	12.77±0.17	12.69±0.17	12.83±0.27	12.75±0.19	12.87±0.21	12.81±0.15
Reducing sugar (g/L)	2.67 ± 0.07	2.91 ± 0.08	2.12 ± 0.05	2.31 ± 0.06	1.92 ± 0.04	2.03 ± 0.05
Free SO_2 (mg/L)	5.42 ± 0.13	5.47 ± 0.15	5.33 ± 0.13	5.37 ± 0.15	5.28 ± 0.17	5.34 ± 0.14
Total acidity[b] (g/L)	6.23 ± 0.14	6.22 ± 0.15	6.19 ± 0.13	6.18 ± 0.16	6.17 ± 0.24	6.16 ± 0.21
pH	3.42 ± 0.17	3.43 ± 0.16	3.41 ± 0.18	3.42 ± 0.19	3.44 ± 0.13	3.45 ± 0.15
Dry extract (g/L)	25.41±1.24	25.22±1.32	26.13±2.07	26.32±2.15	26.52±2.37	26.37±2.21

Results are the mean values for three independent fermentations

[a]3B: 3-year-old grapevine, strain BH8; 3R: 3-year-old grapevine, strain R2; 6B: 6-year-old grapevine, strain BH8; 6R: 6-year-old grapevine, strain R2; 12B: 12-year-old grapevine, strain BH8; 12R: 12-year-old grapevine, strain R2.

[b]Measured as H_2SO_4

(20). According to the quantitative data, the concentration of total volatiles in the wines ranged from 5470 to 13680 µg/L. As the age of the grapevine increased, the concentrations of total volatiles significantly increased ($p < 0.001$). This could be attributed to the effects of grapevine age on berry composition which enhanced the content of wine aroma compounds. The concentration of total aroma compounds from the wines made with strain BH8 (5960.05, 7605.22 and 13682.93 µg/L for 3, 6 and 12 year-old grapevines, respectively) were higher than the wines made with strain R_2 using the same grapevine (5470.96, 6756.04 and 11022.27 µg/L for 3, 6 and 12 year-old grapevines, respectively).

Quantitatively, esters were the most abundant group of aromatic components in the wines, followed by alcohols and acids. The subtotal concentration of esters in the wines was in the 4958.38-12638.16 µg/L range, being 90.63-92.82% (w/w) of the total volatile compounds detected. With increased grapevine age, the concentration of esters in the wines significantly increased ($p < 0.001$). The concentration of esters in the wines made with strain BH8 were higher than that in the wines made with strain R_2 using the same grapevine. This was attributed to a much higher concentration of ethyl octanoate and ethyl decanoate in the wines made from grapes taken from the 12 year-old grapevine and the absence of ethyl heptanoate, ethyl acetate, propyl octanoate, ethyl tridecanoate and ethyl octadecanoate in the wines made from grapes taken from the 3 or 6 year-old grapevines. Esters are largely responsible for the fresh and fruity aroma of wine and can be produced during the alcoholic fermentation (Rocha *et al*., 2004). Ethyl esters of fatty acids are formed from ethanolysis of acyl-CoA during fatty acid synthesis or degradation (Lee *et al*., 2004); these compounds appear mainly during the alcoholic fermentation phase (Gil *et al*., 2006). On the other hand, the formation of acetate esters is the result of the reaction between acetyl-coenzyme A (acetyl-CoA) and alcohols (Lee *et al*., 2004).

The subtotal concentration of alcohols in the wines was in the 370.95-638.53 µg/L range, being 4.67-6.61% (w/w) of the total volatile compounds detected. Alcohols are formed from the degradation of amino acids, carbohydrates and lipids (Antonelli *et al*., 1999). As the age of the grapevine increased, the concentration of alcohols in the wines significantly increased ($p < 0.01$). The concentration of alcohols in the wines made with strain BH8 was higher than that in the wines made with strain R_2 using the same grapevine. 1-Decanol was only detected in the wine made from grapes taken from the 3-year-old grapevine with yeast strain BH8. Alcohols were reported to contribute more to the intensity of the odor of the wine than to its quality (Etiévant, 1991).

The concentration of acids detected in the wines was between 91.35 and 393.12 µg/L, which was 1.21-2.87% (w/w) of the total aroma compounds. The production of fatty acids has been reported to be dependent on the composition of must and fermentation conditions (Schreirer, 1979). There were also significant differences in the type and amount of acids present in the wines ($p < 0.001$) in this study. The concentration of acids was higher in the wines made from grapes taken from the 12 year-old grapevine than in the wines made from grapes taken from the 3 or 6 year-old grapevines. Acetic acid and octanoic acid were only detected in the wines made from grapes taken from the 12 year-old grapevine. Acetic acid is produced during alcoholic and malolactic fermentation. Fatty acids are associated with fatty notes (Rocha *et al*., 2004).

The concentration of terpenes and aldehydes were much lower than the other aroma compounds. Numerous studies have reported that terpenoid compounds can be used analytically for varietal characterization. Terpene compounds belong to secondary plant constituents, of which the biosynthesis begins with (acetyl Co-A). Microorganisms are also able to synthesize terpene compounds, but the formation of terpenes by *Saccharomyces cerevisiae* has not yet been observed. Terpenes are an important component of varietal aroma and are not changed by the metabolism of yeast during fermentation (Mateo & Jiménez, 2000). Benzaldehyde was only detected in the wines made from grapes taken from the 6 year-old grapevine. Decanal was absent in the wines made from grapes taken from the 12 year-old grapevine.

Evaluation of the aromatic profile of the wines was based on the study of "odor activity values" (OAVs), because this aromatic index permits the evaluation of the degree of participation of each compound in the final aroma. In this sense, only compounds with OAVs > 1 are considered as

TABLE 2
The impact of grapevine age on aroma compounds in 'Beihong' wine

Aroma compound	Type of wine[a] (µg/L)						Odor descriptors[b]	Odorant series	Threshold (µg/L)
	3B	3R	6B	6R	12B	12R			
Alcohols									
3-Methyl-1-butanol	376.74 ± 13.15*	324.47 ± 11.23*	418.52 ± 16.47*	354.97±10.11*	631.65±21.24*	548.41±15.27*	Floral	2	30
1-Hexanol	12.4 ± 0.87*	18.71 ± 0.54*	9.91 ± 0.25*	11.21 ± 0.21*	6.88 ± 0.43*	9.7 ± 0.32*	Green, grass	4	8000
1-Decanol	1.82 ± 0.07*	—	—	—	—	—	Floral, fruity, alcohol	1, 2, 3	400
2-Nonanol	3.46 ± 0.11*	3.7 ± 0.12*	2.25 ± 0.08*	4.77 ± 0.15*	—	7.39 ± 0.14*	Cucumber	4	58
Subtotal (µg/L)	394.42	346.88	430.68	370.95	638.53	565.5			
Subtotal (w/w, %)	6.61	6.34	5.66	5.49	4.67	5.13			
Aldehydes									
Benzaldehyde	—	—	12.03 ± 1.17*	3.7 ± 0.21*	—	—	Almond, fragrant, cherry	6	2000
Nonanal	8.71 ± 0.24*	9.82 ± 0.74*	6.34 ± 0.42*	5.47 ± 0.13*	7.17 ± 0.15*	1.79 ± 0.15*	Green	4	1
Decanal	1.48 ± 0.15*	1.52 ± 0.11*	1.53 ± 0.13*	1.33 ± 0.11*	—	—	Grass	4	1000
Subtotal (µg/L)	10.19	11.34	19.9	10.5	7.17	1.79			
Subtotal (w/w, %)	0.18	0.21	0.26	0.16	0.056	0.02			
Terpenes									
4-Terpinenol	1.34 ± 0.04*	1.52 ± 0.03*	1.99 ± 0.03*	2.51 ± 0.07*	2.12 ± 0.06*	2.32 ± 0.07*	Sweet, green, citrus, floral	2, 3, 4, 6	250
β-Damascenone	3.06 ± 0.21*	4.31 ± 0.31*	1.79 ± 0.08*	2.24 ± 0.12*	3.83 ± 0.07*	2.13 ± 0.12*	Flowers, apple, rose and honey	2, 3, 6	0.05
Subtotal (µg/L)	4.4	5.83	3.78	4.75	5.95	4.45			
Subtotal (w/w, %)	0.07	0.11	0.05	0.07	0.044	0.04			
Acids									
Acetic acid	—	—	—	—	9.14 ± 0.32*	19.8 ± 0.35*	Vinegar, fatty	5	200 000
Hexanoic acid	51.16 ± 3.12*	58.95 ± 3.34*	36.18 ± 1.21*	49.61 ± 2.11*	76.74 ± 3.43*	34.15 ± 1.21*	Cheese, fatty, grass, fruity	5, 6	140
Octanoic Acid	—	—	—	—	107.23± 1.03*	173.99 ± 11.15*	Fatty, rancid	5	500
n–Decanoic acid	65.41 ± 2.27*	89.58 ± 2.32*	55.17 ± 1.23*	84.93 ± 2.53*	200.01 ± 3.13*	54.17 ± 1.43*	Fatty	5	15 000
Subtotal (µg/L)	116.57	148.53	91.35	134.54	393.12	282.11			
Subtotal (w/w, %)	1.96	2.71	1.21	1.99	2.87	2.56			
Esters									
Ethyl butyrate	26.37 ± 1.21*	23.5 ± 0.76*	24.18 ± 0.87*	24.12 ± 0.86*	42.29 ± 1.54*	31.72 ± 0.37*	Fruity	6	20
Isoamyl acetate	1381.13 ± 7.43*	784.65 ± 7.35*	1350.84±12.17*	711.01 ± 8.14*	1932.34±14.32*	860.23 ± 3.54*	Banana, fruity, sweet	6, 3	30
Ethyl caproate	17.01 ± 0.67*	6.35 ± 0.07*	6.85 ± 0.09*	2.51 ± 0.05*	19.46 ± 1.06*	2.36 ± 0.04*	Fruity, banana	6	5
Ethyl hexanoate	1066.63 ± 7.53*	914.41 ± 4.32*	1287.56 ± 6.32*	1189.39±4.21*	1495.13 ± 8.43*	942.47 ± 2.11*	Green apple, banana	6	14
Hexyl acetate	333.19 ± 2.11*	392.43 ± 1.32*	276.69 ± 2.97*	273.17 ± 1.32*	312.09 ± 1.32*	280.29 ± 3.12*	Green, fruity, fatty, fresh	4, 5, 6	670
Hexenyl acetate	11.76 ± 0.45*	14.47 ± 0.85*	5.58 ± 0.14*	4.9 ± 0.15*	8.98 ± 0.31*	3.15 ± 0.11*	Fruity	6	2
Ethyl heptanoate	1.91 ± 0.06*	2.24 ± 0.06*	—	—	—	7.07 ± 0.21*	Fruity, green	4, 6	14

TABLE 2 (CONTINUED)

Aroma compound	Type of wine[a] (μg/L)						Odor descriptors[b]	Odorant series	Threshold (μg/L)
	3B	3R	6B	6R	12B	12R			
Ethyl acetate	—	4.42 ± 0.21*	—	—	14.35 ± 0.32*	27.82 ± 1.14*	Fruity, sweet	3, 6	7500
Methyl octanoate	2.6 ± 0.13*	2.49 ± 0.21*	2.42 ± 0.07*	2.62 ± 0.09*	5.79 ± 0.21*	2.79 ± 0.22*	Fruity, green	4,6	200
Ethyl octanoate	1830.21±10.21*	1744.43 ± 0.17*	2690.84±12.17*	2407.85±13.27*	5118.42±34.78*	4977.42±22.43*	Floral, fruity, banana, pear	2, 6	5
Octyl acetate	81.19 ± 1.35*	73.24 ± 1.15*	74.62 ± 2.32*	114.25 ± 4.27*	50.94 ± 2.11*	76.16 ± 1.37*	Orange floral, jasmine, pear	2, 6	50 000
Propyl octanoate	—	—	1.03 ± 0.07*	0.88 ± 0.003*	8.11 ± 0.16*	2.61 ± 0.12*	Fruity	6	—
Methyl decanoate	1.24 ± 0.07*	1.22 ± 0.04*	4.56 ± 0.16*	3.56 ± 0.17*	14.62 ± 0.43*	9.43 ± 0.24*	Fruity, floral	6, 2	1200
Ethyl decanoate	283.25 ± 3.34*	454.4 ± 4.13*	800.07 ± 6.24*	954.99 ± 7.84*	2286.91±21.34*	1873.23±12.43*	Fruity	6	200
Ethyl 9-decenoate	39.23 ± 1.21*	153.45 ± 3.41*	8.85 ± 0.47*	10.81 ± 0.24*	229.32 ± 21.32*	190.99 ± 2.43*	Fruity, fatty	6, 5	100
Isoamyl Caprylate	2.67 ± 0.11*	3.7 ± 0.11*	26.4 ± 0.17*	24.71 ± 0.21*	59.88 ± 2.13*	45.53 ± 0.32*	Sweet, fruity	3, 6	580
Phenylethyl acetate	314.08 ± 2.15*	302.09 ± 2.07*	267.62 ± 1.67*	286.08 ± 2.01*	430.15 ± 3.12*	333.44 ± 1.75*	Floral	2	250
Ethyl laurinate	42 ± 0.32*	80.89 ± 1.43*	225.02 ± 3.43*	191.29 ± 1.43*	490.91 ± 5.31*	417.17 ± 3.17*	Fruity	6	1500
Ethyl tridecanoate	—	—	4.19 ± 0.08*	14.49 ± 0.27*	29.73 ± 1.21*	11.92 ± 0.84*	Sweet, green	3, 4	—
Ethyl octadecanoate	—	—	11.19 ± 1.01*	18.67 ± 1.32*	88.74 ± 3.24*	72.62 ± 2.31*	Waxy	5	3000
Subtotal (μg/L)	5434.47	4958.38	7059.51	6235.3	12638.16	10168.42			
Subtotal (w/w, %)	91.18	90.63	92.82	92.29	92.36	92.25			
Total (μg/L)	5960.05	5470.96	7605.22	6756.04	13682.93	11022.27			

*Mean value and S.D. for three independent fermentations

— no data

[a]3B: 3-year-old grapevine, strain BH8; 3R: 3-year-old grapevine, strain R2; 6B: 6-year-old grapevine, strain BH8; 6R: 6-year-old grapevine, strain R2; 12B: 12-year-old grapevine, strain BH8; 12R: 12-year-old grapevine, strain R2.

[b], 1, solvent; 2, floral; 3, sweet; 4, green; 5, fatty; 6, fruity.

active odorants. OAVs are calculated by using the equation OAV = *c/t* (Falqué *et al*., 2001), where *c* is the concentration of each compound in the wine and *t* is the olfactory perception threshold of the compound in wine. Among 33 volatile compounds identified and quantitatively determined in the wines, only twelve (12) compounds were detected at the levels above their odor thresholds (OAVs > 1), which included alcohols (1), aldehydes (1), terpenes (1) and esters (9). Ethyl octanoate had the highest OAV (> 900), followed by (OAVs > 100) ethyl hexanoate, then 3-methyl-1-butanol, β-damascenone, isoamyl acetate and ethyl decanoate reached concentrations above the perception threshold (OAVs > 10), followed by nonanal, ethyl butyrate, hexenyl acetate, ethyl caproate, ethyl 9-decenoate and phenylethyl acetate reached concentrations above the perception threshold (OAVs > 1).

Aromatic series can be defined as a group of volatile compounds with similar odor descriptors. On the basis of these odor descriptors, each compound also was assigned to one or several aromatic series: solvent, floral, sweet, green (vegetal or herbaceous), fatty or fruity series, which were chosen on account of their extensive use for describing young white table wines (Peinado *et al*., 2004). The value of each aromatic series was obtained by adding the OAVs of the compounds that form such a series. Therefore, it is possible to determine the contribution of a specific compound to each series. This procedure makes it possible to relate quantitative information obtained by chemical analysis to sensory perception, providing a single aroma profile based on an objective. It has recently been used by Peinado *et al*. (2004). The impact of grapevine age on odor activity values (OAVs) in the odorant series for 'Beihong' wine can be seen in Table 3. The highest aroma contribution was from the fruity and floral series, followed by the sweet, green and fatty series. The solvent series was not detected in the wines and the fatty series was only detected in the wines of 3R, 12B and 12R. All the odorant series exhibited significant differences in the wines (floral and fruity: p≤0.001; sweet, green and fatty: p≤0.01), except the solvent series. As the age of the grapevine increased, the OAVs of wines significantly increased ($p < 0.001$). The OAVs of wines made with strain BH8 were higher than those of the wines made with strain R_2 using the same grapevin3e. Normally after being planted, the roots of young grape plants will horizontally and vertically expand in the soil. This growth period will last about 10-15 years, then stabilize. For the wild hybrid type like "Beihong", the first stage will last longer. Compared with 3 year-old grapevines, 6 and 12 year-old grapevines should have stronger root systems, which will help the grape increase the absorption of individual substances, enhancing the finished wine's aroma and taste. Besides the roots, the number of actinomyces, inorganic phosphorus bacteria and nitrogen fixing bacteria in the soil of 6 and 12 year-old grapevines were more than that of 3-year-old grapevines, based on the results of Luan *et al*. (2009). It will also enhance the absorption of nutrients. In this study, it was established that the concentrations of total volatiles and total odor activity values (OAVs) of the wines significantly increased ($p < 0.001$) as the age of the grapevine increased. The mechanics between aroma forming and increased nutrition absorption should be elucidated in a future study.

TABLE 3
The impact of grapevine age on odor activity values (OAVs) of the odorant series for 'Beihong' wine

Series	Type of wine a OAVs [a]						Significant
	3B	3R	6B	6R	12B	12R	
Solvent	0	0	0	0	0	0	NS
Floral	441.06	447.11	588.99	539.35	1123.06	1057.70	***
Sweet	107.24	112.36	80.83	68.50	141.01	71.27	**
Green	8.71	9.82	6.34	5.47	7.17	1.79	**
Fatty	0	1.53	0	0	11.29	1.91	**
Fruity	561.48	540.04	720.33	643.46	1228.11	1148.52	***

[a]3B: 3-year-old grapevine, strain BH8; 3R: 3-year-old grapevine, strain R2; 6B: 6-year-old grapevine, strain BH8; 6R: 6-year-old grapevine, strain R2; 12B: 12-year-old grapevine, strain BH8; 12R: 12-year-old grapevine, strain R2.

NS, no significative interaction

*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

CONCLUSIONS

This is a first study on the influence of grapevine age (3, 6 and 12 years) on volatile compounds in 'Beihong' wine. This grapevine variety was bred by Institute of botany, The Chinese Academy of Sciences. Thirty-three (33) aromatic compounds were identified and quantified. As the age of the grapevine increased, the concentrations of total volatiles and odor activity values (OAVs) of the wines significantly increased ($p < 0.001$). These results can be used for wineries to improve the wine quality.

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