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# Characterisation of volatile components of red and sparkling wines from a new wine grape cultivar 'Meili' (*Vitis vinifera* L.)

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## Summary

**'Meili' (*Vitis vinifera* L.) is a new wine grape cultivar from China. Volatile profiles of red and sparkling wines made from 'Meili' grapes were analysed using stir bar sorptive extraction-gas chromatography-mass spectrometry in this study. Fiftyfive volatile compounds were quantified in both wines, and quantitative differences for most of the volatile compounds between 'Meili' wines were observed. 'Meili' sparkling wine had a greater content of esters, fatty acids and shikimic acid derivatives than 'Meili' red wine, although 'Meili' red wine had higher concentrations of alcohols, terpenoids and $C_{13}$-norisoprenoids. On the basis of odour activity values, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, octanoic acid, isoamyl alcohol, 2-phenyl ethanol, linalool, β-damascenone and β-ionone were considered as important aroma compounds in 'Meili' wines. For these compounds, 'Meili' sparkling wine had higher content of ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate and isoamyl acetate than 'Meili' red wine, while 'Meili' red wine had higher levels of isoamyl alcohol, 2-phenylethanol, linalool, β-damascenone and β-ionone. The concentration differences of aroma compounds due to the differential vinification procedures suggested the differences in sensory characteristics of the two types of wines. In particular, 'Meili' red wine had more rose aroma than 'Meili' sparkling wine.**

K e y w o r d s : aroma compounds, sparkling wine, odour activity value; stir bar sorptive extraction (SBSE)

## Introduction

'Meili' (*Vitis vinifera* L.), a new red grape cultivar, was released in 2010 by Northwest A&F University in China. The cultivar was developed within European species (*V. vinifera* L.) including 'Merlot', 'Riesling' and 'Muscat', using recurrent selection strategies, which was originally used for breeding disease resistant cultivars. Contrary to its parents, this red grape cultivar is highly disease (*Plasmopara viticola* and *Sphaceloma ampelinum*) resistant and cold resistance, and produces a medium, round and a relatively thick skin grape (Li *et al*. 2007 b, Zhang 2000). This cultivar has been planted in North and Northwest China (Zhang 2000) and is used for the production of quality wines with distinct flavour in China.

The study of volatile composition of 'Meili' wine is of great interest, as volatile compounds are responsible for its aroma characteristic, which is a key attribute for consumers. Several hundred aroma compounds with different chemical and physical properties have been identified in wines (Aznar *et al*. 2001, Ebeler 2001). They can be divided into grape-derived primary compounds, fermentation-derived aroma compounds and ageing aroma compounds (Ebeler 2001). The concentrations of grape-derived compounds such as monoterpenes, $C_{13}$-norisoprenoids and shikimic acid derivatives can be affected by several factors including climate, site, grape variety, ripeness, viticultural practices, etc. (Ferreira *et al*. 2000, Ribéreau-Gayon *et al*. 2000). Fermentative volatile compounds like esters, fatty acids and alcohols are, among others, dependent on grape composition (Karagiannis and Lanaridis 2002, Louw *et al*. 2010), yeast strains (Robinson *et al*. 2011), fermentation conditions like oxygen and temperature (Girard *et al*. 1997, Louw *et al*. 2010) and wine-making processes (Piñeiro *et al*. 2006, Selli *et al*. 2006).

A good understanding of aroma chemistry of the wines from 'Meili' will provide valuable information to modify wine-making techniques for making wines with differentiated characteristics. Previous studies show that the vast majority of volatiles have no aroma activity and only relative few volatiles are aroma-active, based on odour activity values (OAVs) and GC-olfactometry studies (Aznar *et al*. 2001, Ferreira *et al*. 2000, Guth 1997). Li *et al*. analysed the volatile composition in 'Meili' dry red wine using liquid-liquid extraction, but only performed qualitative identification of volatiles (Li *et al*. 2007 a). Distinctive compounds involved in the aroma of 'Meili' wine have not been characterised until now.

As aroma compounds are found in wide range of concentrations and covering a wide range of polarity, solubility and volatility (Ebeler 2001, Perestrelo *et al*. 2006), quantifying aroma compounds in wines has always been a challenging task. Solvent-free extraction methods, such as solid phase micro-extraction (SPME) and stir bar sorptive extraction (SBSE), are growing in popularity, as they are easy to use, have high sensitivity and reproducibility (Perestrelo *et al*. 2009, Zalacain *et al*. 2007). These two

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methods based on the similar theory that the extraction efficiency is proportional to sorbent volume, but SBSE has been shown to be more sensitive than SPME (ALVES *et al* 2005, PERESTRELO *et al*. 2009), as the quantity of sorbent phase on stir bar is much greater than that coated on SPME fiber. SBSE can be done in headspace mode (WELDEGERGIS *et al*. 2007), although it is usually directly introduced into the aqueous sample to extract volatiles. SBSE followed by thermal desorption-gas chromatography-mass spectrometry (GC-MS) has been used several times to quantify volatile compounds in wines (ALVES *et al*. 2005, FANG and QIAN 2006, ZALACAIN *et al*. 2007).

The objective of this work was to define the profiles of volatile compounds present in 'Meili' red and sparkling wines by SBSE-GC-MS and compare the concentration differences of aroma compounds and sensory attributes of the monovarietal wines.

## Material and Methods

Vineyard site: 'Meili' grapevines were grown in Yangling district, Shaanxi Province, Northwest of China, with row and vine spacing of 2.5 m and 1.0 m, respectively. Vines were trained on a vertical shoot positioning system with a pair of wires, and shoots were trimmed twice manually, between bloom and veraison, to a height of about 1.0 m. In addition, soils in the vineyard were relatively uniform, typified by loam, with organic matter of 1.2 % and pH 8.3.

Vinification: Wine making was conducted in the Experimental Center located in Northwest A&F University in 2010 using homogeneous grape samples of 'Meili'. The grapes were harvested at 20 °Brix corresponding to wines containing 11 % ethanol (v/v). Two kinds of wines including sparkling wine and red wine were made using the same commercial dry yeast AWRI 796 (*Saccharomyces cerevisiae*, Lallemand Inc., Quebec, Canada).

Sparkling wines were produced through the use of the secondary fermentation in the same tank. About 100 kg grape berries were gently pressed to avoid skin contact and make sure the juice yield was less than 66 % (v/m, L of juice per kg of grape). The juice was added with 40 $mg \cdot L^{-1}$ of $SO_2$, and clarified by natural settling with the must in repose for 24 h at 15 °C. The juice (16 L) was fermented in 20 L-pressure-resistant stainless steel tanks in triplicate with activated dry yeast at a rate of 20 $g \cdot hL^{-1}$. During fermentation, the temperature was maintained at 18 °C, and the decrease in density was checked at intervals of 8 hours. Sugar content was determined by alkaline cupric solutions at intervals of two hours when its content was about 40 $g \cdot L^{-1}$. The fermentation tanks were sealed until the residual sugar in the juice was about 24 $g \cdot L^{-1}$. After fermentation (reducing sugar < 4 $g \cdot L^{-1}$), the wines were stored at 4 °C for 5 months prior to analysis.

For red wine making, the grapes were processed following the traditional protocol, and three independent winemaking replications were performed. 'Meili' grapes (16 kg) were destemmed and crushed on an experimental destemmer-crusher and transferred to 20 L stainless-steel containers. The paste was inoculated with 20 $g \cdot hL^{-1}$ of the same activated dry yeast for alcohol fermentation. Maceration was carried out at the same time as fermentation, which took place over a 8-day period at 26 °C. After fermentation (reducing sugar < 4 $g \cdot L^{-1}$), the wine was separated from the skins, then decanted to another tank. Wine samples with the addition of 40 $mg \cdot L^{-1}$ $SO_2$ were cold stabilized at 4 °C until analysis.

Chemical and reagents: Ethyl 2-methylpropanoate, isobutyl acetate, ethyl isobutyrate, octyl propanonate and octyl 2-methylpropanoate were obtained from K & K Laboratories (Jamaica, NY). Methyl octanoate, ethyl octanoate,ethyl nonanoate, methyl decanoate, ethyl decanoate, nonanoic acid, heptan-1-ol, octan-1-ol, nonan-1-ol and decan-1-ol were from Eastman Chemical Products, Inc. (Kingsport, TN). Geranyl acetone and nerolidol were supplied by Hoffman-La Roche (Nutley, NJ). Benzaldehyde, octyl acetate and β-damascenone were supplied by Polyscience Corp. (Niles, IL), Compagnie Parento, Inc. (Lenoir, NC) and Firmenich (Princeton, NJ), respectively. Methyl dihydrojasmonate was purchased from TCI Japan (Tokyo, Japan). Ethyl cinnamate was from Alfa Aesar (Ward Hill, MA). Isobutyl alcohol was from Mallinckrodt, Inc. (Maywood, NJ). 2-nonanone was obtained from White Label (New York, NY). All other chemical standards were obtained from Sigma–Aldrich Chemical Co., Inc. (Milwaukee, WI). All standard solutions were prepared individually in methanol at a concentration around 10,000 $mg \cdot L^{-1}$. An internal standard solution (IS) of 3-heptanone, octyl propanoate and hexyl formate in methanol was prepared at a concentration of 96.1 $mg \cdot L^{-1}$, 118.1 $mg \cdot L^{-1}$ and 104.2 $mg \cdot L^{-1}$ respectively. A synthetic wine was prepared as described by FANG and QIAN (2006).

Wine volatile analysis: The extraction method of volatile compounds was conducted according to FANG and QIAN (2006) with minor modification. A 10 mL wine sample was diluted with 10 mL of saturated salt water in a 20 mL vial, in which 20 μL of IS solution were added. A pre-conditioned stir bar (Twister) coated with polydimethylsiloxane (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was used to extract volatile compounds. The sample was extracted for 3 h at a speed of 1000 rpm. After extraction, the stir bar was rinsed with distilled water, dried with a tissue paper, and placed into a sample holder for GC-MS analysis.

GC-MS analyses were performed using an Agilent 6890 gas chromatograph with a 5973 mass selective detector (Agilent, Santa Clara, CA). Samples were loaded into a thermal desorption unit (TDU) by a multi-purpose auto-sampler (Gerstel). A cooled injection system (CIS4, Gerstel) was used in the GC-MS system. The TDU had an initial temperature of 25 °C. After the sample was loaded, the TDU was heated at a rate of 100 °C $min^{-1}$ to a final temperature of 250 °C and held for 2 min. The TDU injection was in splitless mode during thermal desorption, while the CIS4 was in a solvent vent mode with a venting flow of 50 $mL \cdot min^{-1}$ for 4.7 min, at a venting pressure of 36.8 psi. After the solvent vent, the CIS4 was switched to

splitless mode for 3.0 min, then changed to split mode with a venting flow of 50 mL·min$^{-1}$. The initial temperature of the CIS4 was kept at -80 °C for 0.2 min then ramped at a rate of 10 °C s$^{-1}$ to a final temperature of 250 °C and held for 10 min.

A RTX-1 column (60 m length, 0.25 mm i.d., 0.25 µm film thickness, Resteck Inc., Bellefonte, PA) was used to separate the volatiles. The oven temperature was programmed at 40 °C for a 2 min holding, then to 210 °C at 3 °C min$^{-1}$, and to 270 °C at 5 °C min$^{-1}$ with 5 min holding. A constant helium column flow of 2.5 mL min$^{-1}$ was used. A column splitter was used at the end of the column, 1 mL min$^{-1}$ column flow was introduced to the MS, and the other 1.5 mL·min$^{-1}$ was vented out. The MS transfer line and ion source temperature were 280 and 230 °C, respectively. Electron ionization mass spectrometric data from *m/z* 35~350 were collected using a scan rate of 5.27/s, with an ionization voltage of 70 eV.

Volatile compounds were identified by comparing their mass spectra with those in the Wiley 275.L Database (Agilent Technologies Inc.) and linear retention indexes (LRIs) with those of authentic standards available in the laboratory using the same instrument. LRIs were calculated after analysing $C_6$-$C_{20}$ n-alkane series (Supelco, Bellefonte, PA) under the same chromatographic condition.

For quantification, the standard solutions were prepared by diluting the stock solution in synthetic wine to give a range of concentrations (The standard solutions were analysed using the same procedure as described for wine samples). The calibration curve for each target compound was built up by plotting the selected mass ion abundance ratio of target compound with their respective internal standard against the concentration ratio. Standard calibration curves were obtained through Chemstation Software and were used to calculate the concentrations of volatile compounds in wine samples. Duplicate analysis was performed for the wine samples 5 months after winemaking.

Sensory evaluation of wines: All the wine samples presented in a random order were evaluated by trained panelists. A panel of 10 judges (five males and five females) had been trained with "Le Nez du Vin" aroma kit over 70 d as described by Tao *et al*. (2009). A list of seven descriptors (Figure) that describe the aroma of 'Meili' wines were previously determined by the panelists and subsequently used to describe the wines. The intensity of each term was scored using a 5-point scale (1) very weak; (2) weak; (3) moderate; (4) strong; (5) very strong.

Statistical analysis: Student's t test with $p < 0.05$ as significant level was used to compare the differences of the concentrations of the aroma compounds using statistical software SPSS (version 16.0; Chicago, IL).

## Result and Discussion

Fifty five compounds were quantified in 'Meili' rose and sparkling wines, and were listed in the Table. The concentrations of most of the compounds are in the range of the µg·L$^{-1}$ with the exception of those of isoamyl alcohol,

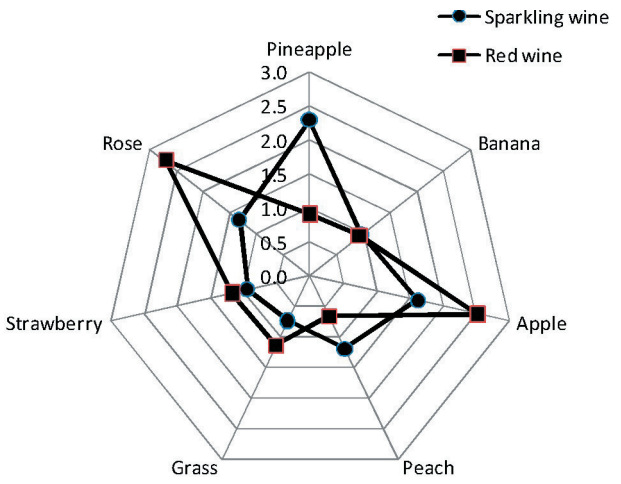


Figure: Sensory evaluation of 'Meili' red and sparkling wines.

2-phenyl ethanol, ethyl acetate, which are in the range of the mg L$^{-1}$. Alcohols were the most dominant compounds, followed by esters and fatty acids. Other minor volatiles quantified were shikimic acid derivatives, terpenoids, $C_{13}$-norisoprenoids, aldehydes, ketone and lactone. The concentrations of alcohols, terpenoids and $C_{13}$-norisoprenoids were higher in 'Meili' red wine as compared to 'Meili' sparkling wine, while 'Meili' sparkling wine had higher concentrations of esters, fatty acids and shikimic acid derivatives. Overall, the volatile profiles in both red and sparkling wines were similar. However, the concentrations of some volatile compounds varied greatly.

Alcohols accounted for 73 % and 84 % of the total volatile compounds for 'Meili' red and sparkling wine, respectively. The main higher alcohols were isobutyl alcohol, isoamyl alcohol, and 2-phenyl ethanol in 'Meili' wines and they had 1.7 to 3.5 times higher concentrations in 'Meili' red wine than in 'Meili' sparkling wine ($p < 0.001$). These compounds can be synthesized by yeast through anabolic pathway from glucose or catabolic pathway from their corresponding amino acids (valine, leucine, phenylalanine) (Perestrelo *et al*. 2006). Higher content of fusel alcohols in 'Meili' red wine could be due to skin contact treatment, insoluble solids present in must (Edwards *et al*. 1990, Karagiannis and Lanaridis 2002, Selli *et al*. 2006) and higher fermentation temperature (Girard *et al*. 1997). It is noticeable that higher alcohols apart from 2-phenylethanol have a negative effect at high concentrations, but contribute to the complexity of wine aroma when present at less than 0.30 g L$^{-1}$ (Ribéreau-Gayon *et al*. 2000). The total concentration of higher alcohols in 'Meili' wines was below 0.3 g·L$^{-1}$. 2-Phenyl ethanol exists in grapes in a small amount and is mainly produced by yeast action through catabolic pathway (Fang and Qian 2006, Perestrelo *et al*. 2006) and gives rosy and honey aromas (Ferreira *et al*. 2000).

Other alcohols quantified including $C_6$-$C_{10}$ alcohols also had higher content in 'Meili' red wine with the exception of heptan-1-ol. They have been associated with herbaceous, intense citrus, sweet, green and orange flowery aroma.

Table

Concentrations of volatile compounds in 'Meili' (*Vitis vinifera* L.) wines (μg·L$^{-1}$, average ± SD)

| No. | LRI[a] | Compound name | OT[b] | Description | Sparkling wine | | Red wine | | p value[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration | OAV[c] | Concentration | OAV | |
| | | **Ethyl esters of straight-chain fatty acid** | | | | | | | |
| 1 | 603 | Ethyl acetate[e] | 7500 (GUTH 1997) | Pineapple, fruity, balsamic | 30.5±3.9a | 4.1 | 25.0±2.9a | 3.3 | 0.123 |
| 2 | 702 | Ethyl propanoate | 1800 (PEINADO *et al.* 2004) | Banana, apple | 272.0±30.9b | 0.2 | 164.2±27.3a | 0.1 | 0.011 |
| 3 | 788 | Ethyl butanoate | 20 (GUTH 1997) | Strawberry, apple, banana | 346.9±23.4b | 17.3 | 120.2±8.9a | 6.0 | 0.000 |
| 4 | 889 | Ethyl pentanoate | > 200 (AZNAR *et al.* 2001) | Fruity, ester | 0.6±0.03a | 0.0 | 1.1±0.1b | 0.0 | 0.001 |
| 5 | 985 | Ethyl hexanoate | 5 (GUTH 1997) | Fruity, green apple, floral, violet | 443.2±27.2b | 88.6 | 167.9±9.8a | 33.6 | 0.000 |
| 6 | 1084 | Ethyl heptanoate | 220 (ANTALICK *et al.* 2010) | Pineapple, fruity | 1.0±0.03a | 0.0 | 1.1±0.02b | 0.0 | 0.002 |
| 7 | 1183 | Ethyl octanoate | 2 (GUTH 1997) | Fruity, pineapple, pear, floral | 314.8±14.9b | 157.4 | 90.7±3.3a | 45.4 | 0.000 |
| 8 | 1282 | Ethyl nonanoate | 1300 (LI 2006) | Waxy, fruity | 0.2±0.1a | 0.0 | 0.1±0.03a | 0.0 | 0.129 |
| 9 | 1382 | Ethyl decanoate | 200 (FERREIRA *et al.* 2000) | Fruity, fatty, pleasant | 54.2±2.8b | 0.3 | 10.2±0.5a | 0.1 | 0.000 |
| | | Subtotal (mg·L$^{-1}$) | | | 31.9±4.0 | | 25.6±3.4 | | |
| | | **Higher alcohol acetates** | | | | | | | |
| 10 | 760 | Isobutyl acetate | 1600 (PEINADO *et al.* 2004) | Waxy, fruity, apple, banana | 41.4±3.5a | 0.0 | 69.8±4.7b | 0.0 | 0.001 |
| 11 | 866 | 2-Methylbutyl acetate | - | Fruity, fatty, pleasant | 136.7±6.2a | - | 169.4±8.5b | - | 0.006 |
| 12 | 862 | Isoamyl acetate | 30 (GUTH 1997) | Banana, fruity, sweet | 878.8±62.4a | 29.3 | 860.9±74.1a | 28.7 | 0.765 |
| 13 | 999 | Hexyl acetate | 670 (PEINADO *et al.* 2004) | Pleasant fruity, pear, cherry | 21.0±0.6b | 0.0 | 4.2±0.1a | 0.0 | 0.000 |
| | | Subtotal (μg·L$^{-1}$) | | | 1077.9±72.7 | | 1104.3±87.4 | | |
| | | **Ethyl esters of branched-chain fatty acid** | | | | | | | |
| 14 | 746 | Ethyl 2-methylpropanoate | 15 (FERREIRA *et al.* 2000) | Fruity, banana | 32.1±1.7a | 2.1 | 124.6±7.2b | 8.3 | 0.001 |
| 15 | 839 | Ethyl 2-methylbutanoate | 18 (FERREIRA *et al.* 2000) | Sweet fruit | 3.8±0.2a | 0.2 | 10.0±0.5b | 0.6 | 0.000 |
| 16 | 842 | Ethyl 3-methylbutanoate | 3 (FERREIRA *et al.* 2000) | Berry, blackberry | 4.1±0.2a | 1.4 | 10.8±0.5b | 3.6 | 0.000 |
| | | Subtotal (μg·L$^{-1}$) | | | 40.0±2.1 | | 145.4±8.2 | | |
| | | **Aromatic esters** | | | | | | | |
| 17 | 1226 | Ethyl 2-phenylacetate | 73 (ANTALICK *et al.* 2010) | Floral, honey | 1.7±0.1a | 0.0 | 2.1±0.1b | 0.0 | 0.019 |
| 18 | 1239 | Phenethyl acetate | 250 (GUTH 1997) | Pleasant, floral, flowery | 58.0±2.5a | 0.2 | 68.4±4.5b | 0.3 | 0.026 |
| 19 | 1332 | Ethyl dihydrocinnamate | 1.6 (FERREIRA *et al.* 2000) | Strawberry, plum, flowery | 0.1±0.001a | 0.1 | 0.4±0.02b | 0.3 | 0.000 |
| 20 | 1450 | Ethyl cinnamate | 1.1 (FERREIRA *et al.* 2000) | Strawberry cream | 0.1±0.001a | 0.1 | 0.1±0.01b | 0.1 | 0.000 |
| 21 | 1568 | Ethyl vanillate | 990 (AZNAR *et al.* 2001) | Vanilla, chocolate | 60.6±3.0a | 0.1 | 875.4±138.9b | 0.9 | 0.001 |
| | | Subtotal (μg·L$^{-1}$) | | | 120.5±5.6 | | 946.4±143.5 | | |
| | | **Other esters** | | | | | | | |
| 22 | 1111 | Methyl octanoate | - | Waxy, apple skin, fruity | 1.1±0.03a | - | 1.2±0.01b | - | 0.013 |
| 23 | 1156 | Diethyl succinate | 6000 (PERESTRELO *et al.* 2006) | Light fruity, Wine | 373.3±30.7a | 0.1 | 911.5±75.2b | 0.2 | 0.000 |
| 24 | 1311 | Methyl decanoate | 1200 * | Waxy, soap, fruity | 0.6±0.05 b | 0.0 | 0.2±0.01a | 0.0 | 0.003 |
| 25 | 1335 | Octyl 2-methylpropanoate | - | Fruity, sweet | 0.4±0.02a | - | 0.5±0.04b | - | 0.000 |
| 26 | 1633 | Methyl dihydrojasmonate | - | - | 0.5±0.1a | - | 0.4±0.1a | - | 0.594 |
| | | Subtotal (μg·L$^{-1}$) | | | 375.9±30.9 | | 913.8±75.4 | | |
| | | **Total esters** (mg·L$^{-1}$) | | | 33.5±4.1 | | 28.7±3.3 | | |
| | | **Fatty acids** | | | | | | | |
| 27 | 1165 | Octanoic acid | 500 (FERREIRA *et al.* 2000) | Rancid, harsh, cheese, fatty acid | 1610.9±55.2b | 3.2 | 49.6±14.3a | 0.1 | 0.000 |
| 28 | 1262 | Nonanoic acid | - | Cheese, waxy flavour | 441.8±98.1a | - | 355.0±53.4a | - | 0.249 |
| 29 | 1366 | Decanoic acid | 1000(FERREIRA *et al.* 2000) | Fatty, unpleasant | 509.4±61.4b | 0.5 | 93.3±33.5a | 0.1 | 0.001 |

Table, contineud

| No. | LRI[a] | Compound name | OT[b] | Description | Sparkling wine | | Red wine | | p value[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Concentration | OAV[c] | Concentration | OAV | |
| 30 | 1553 | Dodecanoic acid | 1000 * | Dry, metallic, laurel oil flavour | 29.2±8.1b | 0.0 | 4.5±0.4a | 0.0 | 0.006 |
| | | Subtotal (μg·L$^{-1}$) | | | 2591.3±222.8 | | 502.4±101.6 | | |
| | | **Alcohols** | | | | | | | |
| 31 | 617 | Isobutyl alcohol | 40000 (Guth 1997) | Fusel, alcohol | 285.6±17.0a | 0.0 | 1013.1±70.7b | 0.0 | 0.000 |
| 32 | 727 | Isoamyl alcohol[e] | 30000 (Guth 1997) | Whiskey, nail polish | 84.5±5.3a | 2.8 | 140.1±5.8b | 4.7 | 0.000 |
| 33 | 859 | Hexan-1-ol | 8000 (Guth 1997) | Green, grass | 165.3±41.0a | 0.0 | 252.9±16.9b | 0.0 | 0.027 |
| 34 | 963 | Heptan-1-ol | 1000 (Antalick *et al*. 2010) | Grape, sweet | 160.8±11.2b | 0.2 | 84.2±3.0a | 0.1 | 0.000 |
| 35 | 1104 | 2-Phenylethanol[e] | 14000 (Ferreira *et al*. 2000) | Roses | 14.3±0.4a | 1.0 | 24.0±1.5b | 1.7 | 0.000 |
| 36 | 1022 | 2-Ethyl hexanol | 8000 * | Mushroom, sweet fruity | 3.2±0.3a | 0.0 | 6.5±0.7b | 0.0 | 0.002 |
| 37 | 1062 | Octan-1-ol | 900 (Li 2006) | Intense citrus, roses | 11.2±0.7a | 0.0 | 15.5±3.7a | 0.0 | 0.119 |
| 38 | 1162 | Nonan-1-ol | 600 * | Green | 3.1±0.1a | 0.0 | 3.8±0.3b | 0.0 | 0.017 |
| 39 | 1264 | Decan-1-ol | 400 (Ferreira *et al*. 2000) | Orange flowery, special fatty | 0.7±0.04a | 0.0 | 2.5±0.2b | 0.0 | 0.000 |
| | | Subtotal (mg·L$^{-1}$) | | | 99.4±5.7 | | 165.5±7.4 | | |
| | | **Shikimic acid derivatives** | | | | | | | |
| 40 | 1301 | 4-Vinyl-2-methoxy-phenol | 1100 (Ferreira *et al*. 2000) | Phenolic, pleasant | 154.3±17.7b | 0.1 | 5.7±3.7a | 0.0 | 0.000 |
| 41 | 1345 | Eugenol | 6 (Ferreira *et al*. 2000) | Clove | 0.3±0.1a | 0.1 | 1.5±0.05b | 0.3 | 0.000 |
| 42 | 944 | Benzaldehyde | 2000 (Peinado *et al*. 2004) | Almond | 1.5±0.8 | 0.0 | 1.1±0.6 | 0.0 | 0.594 |
| | | Subtotal (μg·L$^{-1}$) | | | 156.1±18.6 | | 8.3±4.4 | | |
| | | **Aldehydes** | | | | | | | |
| 43 | 782 | Hexanal | - | Apple, green, grassy | 4.4±0.1a | - | 11.6±8.7a | - | 0.226 |
| 44 | 1088 | Nonanal | - | Green, slightly pungent | 1.8±0.2a | - | 1.7±0.1a | - | 0.511 |
| | | Subtotal (μg·L$^{-1}$) | | | 6.2±0.3 | | 13.3±8.8 | | |
| | | **Terpenoids** | | | | | | | |
| 45 | 1029 | Limonene | 15 (Zalacain *et al*. 2007) | Flowery, green, citrus | 0.2±0.1a | 0.0 | 0.3±0.1a | 0.0 | 0.120 |
| 46 | 1092 | Linalool | 25 (Ferreira *et al*. 2000) | Flowery, fruity, muscat | 15.6±0.1a | 0.6 | 59.6±1.8b | 2.4 | 0.000 |
| 47 | 1219 | Citronellol | 100 (Ferreira *et al*. 2000) | Green lemon | 0.0±0.0a | 0.0 | 3.7±0.1b | 0.0 | 0.000 |
| 48 | 1245 | Geraniol | 30 (Ferreira *et al*. 2000) | Citric | 1.9±0.1a | 0.1 | 10.5±0.5b | 0.4 | 0.000 |
| 49 | 1438 | Geranyl acetone | 60 (Antalick *et al*. 2010) | Floral | 0.5±0.04a | 0.0 | 0.5±0.1a | 0.0 | 0.345 |
| 50 | 1558 | Nerolidol | 700 * | Rose, apple, green, citrus | 5.2±1.2b | 0.0 | 2.7±0.2a | 0.0 | 0.024 |
| | | Subtotal (μg·L$^{-1}$) | | | 23.4±1.6 | | 77.3±2.8 | | |
| | | **$C_{13}$-norisoprenoids** | | | | | | | |
| 51 | 1285 | Vitispirane [f] | 800 (Ebeler 2001) | Eucalyptus, woody, spicy | 0.3±0.01a | 0.0 | 1.3±0.2b | 0.0 | 0.001 |
| 52 | 1378 | β-Damascenone | 0.05 (Ferreira *et al*. 2000) | Floral, sweet, honey, apple | 2.4±0.1a | 48.0 | 3.4±0.2b | 68.0 | 0.003 |
| 53 | 1480 | β-Ionone | 0.09 (Ferreira *et al*. 2000) | Balsamic, rose, violet | 0.1±0.003a | 1.1 | 0.2±0.02b | 2.2 | 0.000 |
| | | Subtotal (μg·L$^{-1}$) | | | 2.8±0.1 | | 4.9±0.4 | | |
| | | **Ketone and lactone** | | | | | | | |
| 54 | 1077 | 2-Nonanone | - | Fruity, floral, fatty | 4.3±0.1b | - | 0.7±0.02a | - | 0.000 |
| 55 | 1685 | δ-Dodecalactone | - | Coconut-fruity | 11.6±0.4b | - | 3.3±0.3a | - | 0.000 |
| | | Subtotal (μg·L$^{-1}$) | | | 15.9±0.5 | | 4.0±0.3 | | |

[a] LRI, linear retention index calculated on RTX-1 capillary column. [b] OT, Odour threshold, OTs of the volatiles were presented in μg·L$^{-1}$. The reference from which the OT has been taken is given in parentheses. [c] OAV, Odour activity value. [d] p values for statistical significance according to t test. [e] The concentrations of ethyl acetate, isoamyl alcohol and 2-phenyl ethanol in 'Meili' wines were measured in mg·L$^{-1}$. [f] Vitispirane was tentatively identified by MS and LRI from literature and estimated on the basis of β-damascenone. * Odor thresholds were calculated in the wine sensory laboratory, College of Enolgy, Northwest A&F University, China and they were calculated in 12 % ethanol/water mixture containing 5 g·L$^{-1}$ tartaric acid at pH 3.2.

Esters were the major volatile compounds in 'Meili' wines. These compounds can be divided into ethyl esters of straight-chain fatty acid, higher alcohol acetates, ethyl esters of branched-chain fatty acid, aromatic esters, etc. The total concentration of esters was higher in 'Meili' sparkling wine than in 'Meili' red wines, mainly due to higher content of ethyl esters of straight-chain fatty acid. Among them, ethyl acetate was the most abundant ester in both wines with the aroma of pleasant fruity (GIL *et al*. 2006). A large amount of ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate were also detected, and their concentrations were two to five times higher in 'Meili' sparkling wine than in 'Meili' red wine. Higher amounts of these compounds in sparkling wine could be attributed to lower fermentation temperature (GIRARD *et al*. 1997, PERESTRELO *et al*. 2006, PIÑEIRO *et al*. 2006), as well as stressful fermentation conditions for yeast like clarified must (EDWARDS *et al*. 1990). In addition, PIÑEIRO *et al*. (2006) found that vinification without maceration resulted in an increase in ethyl esters of straight-chain fatty acid.

For higher alcohol acetates, isobutyl acetate, 2-methylbutyl acetate, isoamyl acetate and hexyl acetate were quantified, with isoamyl acetate found in the highest concentration in both wines. 'Meili' sparkling wine had higher concentrations of isoamyl acetate and hexyl acetate than 'Meili' red wine, which could be due to lower fermentation temperature and must clarification during sparkling wine making (GIL *et al*. 2006, GIRARD *et al*. 1997). However, 'Meili' red wine had significantly higher levels of isobutyl acetate and 2-methylbutyl acetate than 'Meili' sparkling wine. All of these compounds are important contributors to the fruity and sweet aroma of wines, notably isoamyl acetate with banana-like note (ANTALICK *et al*. 2010, FANG and QIAN 2006).

The three ethyl esters of branched-chain fatty acid (ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate) quantified had 2.6 to 3.9 times higher concentrations in red wine than in sparkling wine, in accordance with the result of ANTALICK *et al*. (2010), who reported that ethyl esters of branched-chain fatty acids were more abundant in red wines than in white wines. Ethyl esters of branched-chain fatty acids are formed from the corresponding amino acids by Strecker degradation or from some amino acid derivatives (DIAZ-MAROTO *et al*. 2005, FERREIRA *et al*. 2000), which are important contributors to the fruity and sweet aroma of wines (AZNAR *et al*. 2001).

For aromatic esters investigated including ethyl 2-phenylacetate, phenylethyl acetate, ethyl dihydrocinnamate, ethyl cinnamate and ethyl vanillate, their concentrations were significantly higher in 'Meili'' red wine with the exception of ethyl cinnamate. These compounds have been considered as important flavour contributors to wine aroma and they give the aroma of floral, cherry, stone-fruit and dry plum (FANG and QIAN 2006).

Some other esters were also quantified, although they had low concentrations in both wines with the exception of diethyl succinate, whose concentration was approximately 2.5 times higher in 'Meili' red wine than in 'Meili' sparkling wine. Higher content of diethyl succinate in 'Meili' red wine could result from skin contact treatment (SELLI *et al*. 2006) and be due to malolactic fermentation, which more frequently takes place in red-wine making (GIL *et al*. 2006).

The fatty acids quantified were octanoic acid, nonanoic acid, decanoic acid and dodecanoic acid. Trace amount of hexanoic acid was also detected in this study, while octanoic acid and decanoic acid were abundant in the wines. 'Meili' sparkling wine had five times higher content of total fatty acids compared to 'Meili' red wine, and octanoic acid showed the greatest difference between the two types of wines ($p < 0.001$). These straight medium-chain fatty acids, derived from the grape and the yeast, originate from lipid metabolism and can also be formed from catabolism of long chain fatty acids. Fermentation conditions like reduced oxygen, low fermentation temperature, clarification of the must, vinification without maceration, etc. could be responsible for the higher concentrations of fatty acids in 'Meili' sparkling wine (EDWARDS *et al*. 1990, NICOLINI *et al*. 2011, PIÑEIRO *et al*. 2006). These fatty acids are described as the aroma of rancid, cheesy and vinegar-like aromas, but they are usually present low concentration in healthy wines (LOUW *et al*. 2010). The fatty acids in both wines could give positive effect on the global aroma quality, since their concentrations are below 20 mg $L^{-1}$, beyond which their impacts on wine become negative (SHINOHARA 1985) and indirectly affect wine aroma by leading to the production of ethyl esters of fatty acid.

In terms of shikimic acid derivatives, low concentrations of benzaldehyde and eugenol were determined in both wines. 4-Vinyl-2-methoxy-phenol, however, had high concentration, which was significantly higher in 'Meili' sparkling wine than in 'Meili' red wine. It has been shown that the formation of 4-vinyl-2-methoxy-phenol was related to the non-oxidative decarboxylation of some phenolic acids (p-coumaric and ferulic acid) and is catalyzed by cinnamate decarboxylases, produced by *S. cerevisiae* during alcohol fermentation. The action of these enzymes can be inhibited by some tannic substances, resulting in the lower content of 4-vinyl-2-methoxy-phenol in red wine (CHATONNET *et al*. 1993). This compound has a pleasant spicy aroma at low and moderate concentration (GIL *et al*. 2006).

Grape derived terpenoids are responsible for fruity and flowery notes in wine and contribute significantly to varietal aroma character (EBELER 2001). In this study, the terpenoids quantified were limonene, linalool, citronellol, geraniol, geranyl acetone and nerolidol. The concentrations of limonene, linalool, citronellol, geraniol were higher in 'Meili' red wine than in 'Meili' sparkling wine. Geraniol and linalool were found in concentrations four and six times higher in red wine than in sparkling wine, respectively ($p < 0.001$), although only linalool had high concentration above its odour threshold. Linalool has been reported as one of the most important aroma compounds in wines, especially in Muscat wines (SELLI *et al*. 2006). Higher levels of linalool suggested that linalool could be an important varietal aroma compound responsible for rose aroma of 'Meili' red wine (RIBÉREAU-GAYON *et al*. 2000).

Three $C_{13}$-norisoprenoids including vitispirane, β-damascenone and β-ionone were quantified and their concen-

trations were higher in 'Meili' red wine than in sparkling wine. $C_{13}$-norisoprenoids are reported from photochemical and enzymatic degradation of carotenoids such as β-carotene and lutein (Mendes-Pinto 2009). Moreover, $C_{13}$-norisoprenoids predominantly occur in grapes as glycosidically bound precursors and are formed by complex chemical rearrangements of the odourless aglycones during winemaking and ageing (Ebeler 2001). $C_{13}$-norisoprenoids contribute characteristic aroma to many varieties of *V. vinifera* L., because they have low odour thresholds. β-Damascenone has the sensory perception of floral, sweet, and cooked apple, β-Ionone has typical raspberry, violet note, whereas vitispirane has a eucalyptus or camphoraceous aroma (Mendes-Pinto 2009).

Higher content of terpenoids and $C_{13}$-norisoprenoids in 'Meili' red wine could be due to maceration during red wine making, which can enhance the presence of free and glycosilated form of terpenoids and $C_{13}$-norisoprenoids in the final wine. The glycosilated form of aroma compounds is predominant in grape skins and can be released by hydrolysation to the corresponding free form during fermentation and ageing (Mateo and Jiménez 2000).

Some other compounds were aldehydes, one ketone and one lactone. There were no significant differences for the concentrations of aldehydes between the two wines. However, the levels of 2-nonanone and δ-dodecalactone were significantly higher in 'Meili' sparkling wine than in red wine, which might be attributed to high pressure during second fermentation of sparkling wine. The odour thresholds of these two compounds were unknown, but they may give fruity aroma in 'Meili' sparkling wine.

OAV calculate and sensory characteristics: OAV for each compound was calculated as the ratio between the concentration of a compound and its odour threshold in synthetic wine or wine matrix reported in the literatures. Thirteen aroma compounds should be considered as odour-active compounds of 'Meili' wines because their concentrations exceeded their sensory thresholds (Ferreira *et al*. 2000, Guth 1997). These compounds were composed of ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, octanoic acid, isoamyl alcohol, 2-phenylethanol, linalool, β-damascenone and β-ionone (Table).

The volatiles with high OAVs (OAV > 10) were mainly esters including ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate and isoamyl acetate. The OAVs of these compounds were higher in 'Meili' sparkling wine as compared to 'Meili' red wine, resulting in higher global OAVs of esters in 'Meili' sparkling wine, although 'Meili' red wine had higher OAVs of ethyl 2-methylpropanoate and ethyl 3-methylbutanoate. β-Damascenone also had high OAV, but its OAV was higher in 'Meili' red wine rather than 'Meili' sparkling wine. The OAVs of isoamyl alcohol, 2-phenyl ethanol, linalool and β-ionone were also higher in 'Meili' red wine than in 'Meili' sparkling wine.

Sensory evaluation was conducted to compare the aroma differences of 'Meili' wines and relate aroma compounds with sensory attributes. Fruity aromas including pineapple, apple, banana, strawberry, peach and grassy were observed in 'Meili' wines by the panelists (Figure). Both wines had similar intensity of banana and peach aroma. 'Meili' sparkling wine had higher score in pineapple ($p < 0.05$), while 'Meili' red wine had higher score in apple aroma ($p > 0.05$). These sensory attributes might be explained by the concentration differences of the esters with higher OAVs and their synergetic interactions. It should be noticeable that 'Meili' red wine had higher intensity of rose aroma than sparkling wine ($p < 0.05$), which could be due to higher OAVs of 2-phenyl ethanol and linalool in those wines. However, "grassy" is a term more difficult to correlate with volatile compounds.

## Conclusion

Volatile profiles of 'Meili' red and sparkling wines were determined by SBSE-GC-MS. Although aroma profiles of 'Meili' red and sparkling wines were common, there were quantitative differences for individual aroma compounds between the two wines. Thirteen compounds were considered as important volatile compounds in 'Meili' wines based on OAVs. The OAV differences for odour-active compounds of 'Meili' wines suggest the differences in sensory characteristics between the two types of wines.

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