

Quantification of Selected Aroma-Active Compounds in Pinot Noir Wines from Different Grape Maturities

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Effect of grape maturity on aroma-active compounds in Pinot noir wine was investigated using stir bar sorptive extraction–gas chromatograph–mass spectrometry (SBSE-GC-MS). High correlation coefficient (>0.95) and low standard deviation ($<10\%$) were obtained for all aroma-active compounds of interest. Two vintages of Pinot noir wines with three different grape maturities each were analyzed. Statistical analysis showed that both grape maturity and growing year significantly affected the aroma composition of the final wine. Analysis of wine samples from the same vintage indicated that grape maturity could affect aroma compounds in different ways, based on their biochemical formation in the wines. For most short-chain fatty acid esters, there were no obvious trends with grape maturity, however, the concentrations of ethyl 2-methylpropanoate and ethyl 3-methylbutanoate consistently decreased with grape maturity. The decreasing trend was also observed for other esters including ethyl cinnamate, ethyl dihydroxycinnamate, and ethyl anthranilate, with the exception of ethyl vanillate, while C₁₃ norisoprenoids, monoterpenes, and guaiacols had increasing trends with grape maturation.

KEYWORDS: Stir bar sorption extraction (SBSE); aroma; Pinot noir wine; grape maturity

INTRODUCTION

Gas chromatography/olfactometry technique has been used to study important aroma compounds in wines. Using Charm analysis and aroma extract dilution analysis (AEDA), it has been identified that ethyl butanoate, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, 3-hydroxy-4,5-dimethyl-2(5H)-furan-2(3H)-one, β -damascenone, 3-methylbutanol, 2-phenylethanol, 3-ethylphenol, linalool, butane-2,3-dione, ethyl cinnamate, vanillin, guaiacol, 4-vinylguaiacol, dimethyl sulfide, dimethyl trisulfide, and wine lactone (3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one) were the most important odorant in Chardonnay, White Riesling, and several other white wine varieties (1–4). Similar odorants in red wines have also been identified (5–8). In addition, β -ionone, 3-methylthio-1-propanol, ethyl dihydrocinnamate, γ -nonalactone, eugenol, geraniol, guaiacol, 3-isobutyl-2-methoxypyrazine, 4-ethylguaiacol, and whisky lactone have been identified in various red wines (8, 9). It is also reported that additional C₁₃-norisoprenoid compounds including 3-hydroxy- β -damascenone, 3-oxo- α -ionol, vomifoliol, 4-oxo- β -ionol, 3-oxo-7,8-dihydro- α -ionol, 4-oxo-7,8-dihydro- β -ionol, grasshopper ketone, and 7,8-dihydrovomifoliol play important roles in Tannat red wine (10).

Pinot noir is one of the oldest wine cultivars. It originated in the Burgundy region of France and has become popular in the United States, especially in Oregon. It exhibits distinct red fruity aromas evoking particularly the odors of small-stone fruits (plum

and cherry). However, the grape requires a long, cool growing season to develop the right flavor attributes, and it is difficult to turn the grapes into good wine. Both the volatile and aroma compounds in Pinot noir wine have been studied (11, 12). The most important odor-active compounds in Pinot noir include alcohols, short-chain fatty acids, ethyl esters and acetates, 3-ethylthio-1-propanol, methionol, methional, benzaldehyde, benzyl alcohol, 2-phenylethanol, linalool, *trans*-linalool oxide, γ -octalactone, γ -nonalactone, ethyl and methyl vanillate, acetovanillone, whisky lactone, β -damascenone, vanillin, and eugenol (13, 14). In addition, ethyl and methyl anthranilate, ethyl cinnamate, and ethyl dihydroxycinnamate were identified in Burgundy Pinot noir (15). Although later quantification of these four compounds showed that the concentrations were below the sensory thresholds (16), they may still act synergistically with each other or other compounds to contribute to aroma.

Quantification of aroma compounds in wines is challenging due to complexity of the matrix and low concentration of the aroma compounds. Liquid–liquid extraction followed by concentration has been widely used, and the stable-isotope technique has been proved to be the most reliable method for aroma quantification in wine; however, stable isotope compounds are not readily available and the synthesis is typically tedious and time-consuming, thus aroma quantitative studies often report the relative concentration by a semiquantitative method or the concentrations of a few selected aroma compounds (16–18).

Solid-phase microextraction (SPME) has gained popularity for wine aroma analysis due to its simplicity and sensitivity. The most widely used technique is headspace solid-phase

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Table 1. Standard Curve and Quantification of Aroma-Active Compounds in Wine ($n = 6$)

compounds	quantify ion	qualify ion	quantification limit ($\mu\text{g/L}$)	A^a	correlation coefficient	RSD (%)
<i>trans-carveol</i> (IS)	109	84				
guaiacol	109	81, 124	0.88	7.86	0.968	7.49
linalool	71	93	1.06	0.33	0.997	3.60
nerol	69	41, 93	0.84	0.32	0.995	5.26
geraniol	69	41	0.98	0.33	0.998	2.87
eugenol	164	149	1.02	0.63	0.996	4.07
benzyl alcohol	108	79	174	99.4	0.999	3.11
phenylethanol	122	91	146	40.7	0.999	2.51
citronellol	69	81	0.86	0.36	0.968	9.70
4-ethylguaiacol	152	137	0.88	7.71	0.999	5.65
<i>hexyl formate</i> (IS)	56	69				
ethyl 2-methylpropanoate	71	116	3.58	10.5	0.999	7.21
ethyl butanoate	71	88	3.78	11.6	0.999	5.42
3-methylbutyl acetate	70	87	3.82	1.66	0.999	3.95
2-methylbutyl acetate	70	43, 55	3.7	1.65	0.999	3.22
ethyl 3-methylbutanoate	88	57	5.14	1.60	0.998	4.73
<i>octyl propanoate</i> (IS)	112	75				
ethyl hexanoate	88	99	3.94	0.32	0.990	6.56
ethyl octanoate	88	101, 127	5.08	0.16	0.983	4.68
ethyl decanoate	88	101, 155	4.8	0.23	0.981	7.05
<i>2-nonenal</i> (IS)	70	55, 83				
β -damascenone	121	69	1.04	0.26	0.997	3.26
β -ionone	177	135	0.1	0.07	0.995	3.68
γ -nonalactone	85	114	0.96	1.13	0.997	3.98
<i>linalyl 2-methylpropanoate</i> (IS)	93	121				
ethyl phenylacetate	164	91	1.04	1.55	0.998	7.99
ethyl dihydroxycinnamate	178	104	0.88	0.92	0.996	8.98
ethyl anthranilate	165	119	1.18	1.02	0.983	9.94
ethyl cinnamate	131	103	1.38	0.31	0.996	5.70
methyl vanillate	151	182	1.02	23.1	0.987	4.70
ethyl vanillate	196	151	0.8	1.00	0.983	5.88
phenylethyl acetate	104	91	1.52	0.51	0.999	5.57
ethyl 3-phenylpropanoate	104	91	0.88	0.42	0.997	8.46

^a Values for the constant A in the equation $C_{TC} = A(C_{IS}/R_{IS})R_{TC}$. C_{TC} : concentration of target compound. C_{IS} : concentration of internal standard. R_{TC} : MS response of target compound. R_{IS} : MS response of internal standard.

microextraction (HS-SPME) or in-sample immersion solid-phase microextraction (IS-SPME). More recently, a stir bar sorptive extraction (SBSE) technique has been developed (19), and it has much higher sensitivities than SPME due to its much larger volume of polymeric coating (20, 21). SBSE technique has been applied to analyze some aroma compounds in wines recently (22–24).

It is generally recognized that grape maturity will affect the flavor profile of the wine. A preliminary sensory evaluation with seven panelists showed that the wines made from late stage grapes had more complex aroma with more floral, more dried fruit, and more oak-like aroma, while the early stage wines showed the highest fruity aroma. However, the chemical basis for this difference is not clear. The objective of this study is to develop a sensitive SBSE-GC-MS technique to quantify a wide range of important aroma compounds in Pinot noir wine and employ this technique to study the impact of grape maturity on the aroma composition of Pinot noir wines.

MATERIALS AND METHODS

Chemicals. All aroma standards listed in Table 1 were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY), and tartaric acid was from Mallinckrodt Inc. (Paris, KY).

A synthetic wine was made by dissolving 3.5 g of L-tartaric acid in 1 L of 12% ethanol solution, and adjusting the pH to 3.5 with 1 M NaOH (25). Standard stock solutions (10 000 mg/L) were prepared in ethanol first and then diluted to the proper concentrations of working standards in synthetic wine. An internal standard stock solution was made by dissolving 46 mg/L of hexyl formate, 48 mg/L of octyl

propanoate, 7 mg/L of *trans-carveol*, 9 mg/L *trans-2-nonenal*, and 9 mg/L of linalyl 3-methylbutanoate in ethanol, and the mixture was stored at $-15\text{ }^{\circ}\text{C}$.

Wine Samples. Vintage 2003 and 2004 Pinot noir wines were produced from grapes grown at the Oregon State University experimental vineyard planted in 1984 as described previously (26). During each growing season, fruits were harvested when the grape sugar reached around 21 °Brix, and were labeled as “early stage of maturity”. Fruits were also collected in each of the next two weeks, and they were labeled as “middle stage of maturity” (25 °Brix for 2003 and 22 °Brix for 2004) and “late stage of maturity” (33 °Brix for 2003 and 25 °Brix for 2004). Harvested grapes were crushed, destemmed, and fermented separately (1 g/L Lavin RC 212 Bourgorouge yeast). New wines were settled and racked off the primary yeast, followed by malolactic fermentation. The wines were cold stabilized, bottled at 9 months of age, and stored in the pilot winery at $15\text{--}20\text{ }^{\circ}\text{C}$. Each wine was manufactured in triplicate in different fermentors, and three bottles of each wine from different fermentors were combined for analysis.

Aroma Extraction and Analysis. Each wine sample (10 mL) was diluted with 10 mL of water in a 40 mL vial, in which 6 g of sodium chloride and 20 μL of internal standard solution were added. A stir bar (Twister) coated with poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was used to extract the aroma compounds from the sample. The Twister bar was constantly stirred for 12 h at a speed of 1000 rpm. After sampling, the Twister bar was rinsed with distilled water, dried with a Kimwipe tissue paper, and placed into the sample holder of the thermal desorption unit (TDU) (Gerstel, Inc.).

The analyses were performed using a TDU autosampler (Gerstel, Inc., Baltimore, MD) mounted on an Agilent GC-MS system (Agilent 5973 GC-MS, Agilent Technologies, Little Falls, DE). The analytes were thermally desorbed at the TDU in splitless mode, ramping from

35 to 300 °C at a rate of 700 °C/min, and held at the final temperature for 3 min. The desorbed analytes were cryofocused (−60 °C) in a programmed temperature vaporizing (PTV) injector (CIS 4, Gerstel, Inc.) with liquid nitrogen. After desorption, the PTV was heated from −60 to 250 °C at a rate of 10 °C/s and held at 250 °C for 3 min. The solvent vent injection mode was employed. A RTX-1 capillary GC column (60 m, 0.25 mm i.d., 0.5 μm film thickness, Resteck Inc., Bellefonte, PA) was employed to separate the analytes. Carrier gas (helium) was set at a constant flow rate of 1.8 mL/min. The oven temperature was initially set at 50 °C for 2 min, raised to 210 °C at a rate of 2 °C/min, then to 250 °C at a rate of 10 °C/min, and held at 250 °C for 15 min. An Agilent 5973 MSD at scan mode was used. The electron impact (EI) energy was 70 eV, and the ion source temperature was set at 230 °C. Enhanced ChemStation Software (GCA v. D.00.01.08, Agilent Technologies Inc.) was used for data acquisition and analysis.

Standard Calibration Curve. The stock solutions were prepared by dissolving around 10 000 mg/L each target compound individually into ethanol solution. Individual stock solution was added to synthetic wine to make the first level mixed standard solution, which was then diluted at 4:1 (v/v), 3:2 (v/v), 2:3 (v/v), and 1:4 (v/v) ratio with synthetic wine to give a range of concentrations. The standard solutions were analyzed using the same procedure as described for the wine sample. Selective ion-monitoring (SIM) mass spectrometry was used to quantify the aroma-active compounds. The quantify and qualify ions for both the aroma-active compounds and internal standards are listed in **Table 1**. The calibration curve for individual target compounds was built up by plotting the selected mass ion abundance ratio of target compounds with their respective internal standard against the concentration ratio. For each calibration curve, the regression correlation coefficients were calculated using the ChemStation data analysis software, and relative standard errors (RSDs) were calculated based on triplicate analysis of the combined wine samples.

Quantification of Aroma-Active Compounds in Pinot Noir Wine. Aroma-active compounds in six wines from three different maturity grapes in two years were quantified. A 10 mL of wine sample along with 20 μL of internal standard solution were added into a 40 mL vial with 10 mL of water and 6 g of sodium chloride. The SBSE and GC-MS conditions were the same as described previously. The concentrations of aroma-active compounds were calculated based on their calibration curves. Triplicate analysis was performed on all samples, and the average values are reported.

Statistical Analysis. The effect of grape maturity on the aroma composition of Pinot noir wine was investigated using multivariate analysis of variance (MANOVA). In the MANOVA model, year, maturity, and the two-way interaction (year × maturity) were studied.

RESULTS AND DISCUSSION

Quantification of Aroma-Active Compounds in Wines. Both aroma extract dilution analysis (AEDA) and GC-Osme techniques have been used to characterize the aroma profile of Oregon Pinot noir wines (13, 27). The results indicate that Pinot noir aroma is a complex formulation of many aroma compounds, and there is no single compound responsible for the characteristic aroma of Pinot noir wine. Different proportions of these compounds give rise to different perceived odors. Ultimately, concentration of these aroma compounds and their balance in the wine matrix will affect the quality of Pinot noir wines. On the basis of the results of previous GC-olfactometry identification of aroma compounds in Pinot noir (11, 13, 15, 16, 28, 29), 28 key aroma-active compounds were selected for quantification, which included 9 alcohols, 16 esters, 2 ketones, and 1 lactone. Acids and higher alcohols are formed primarily during fermentation, and they have high sensory thresholds, so they were not quantified although they are important to wine aroma (13).

Calibration curves of selected aroma-active compounds were constructed individually using pure aroma compounds and internal standards in synthetic wine. Due to the wide range of

concentration and different chemical and physical properties of the aroma-active compounds, five internal standards, including one alcohol, one aldehyde, and three esters, were used to quantify all aroma-active compounds. The chromatographic conditions were selected to give good resolution for the aroma-active compounds, and the quantifying ions were carefully selected to eliminate any interfering ions from coeluted compounds and give good sensitivity. The correlation coefficients for most of the aroma-active compounds were greater than 0.99 (**Table 1**). The method is reproducible with a RSD less than 10% for most of the aroma-active compounds quantified (**Table 1**).

Ester was the major class of aroma-active compounds analyzed in this study. Ethyl esters of butanoate, hexanoate, octanoate, and decanoate were all quantified, and high concentrations were found for all. Branch-chained esters such as ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, and 2-methylbutyl acetate also had high concentrations (ranging from 0.1 to 1 mg/L), which were consistent with the literature (30). Because sensory thresholds of these esters are at μg/L levels, they should contribute to the characteristic fruity aromas of the wine.

Several aromatic esters were also quantified. Aromatic esters are typically described as floral, cherry, stone-fruit, and dry-plum. Ethyl phenylacetate, 2-phenylethyl acetate, and ethyl 3-phenylpropanoate have been identified as important flavor contributors to wine aroma (22, 31). Ethyl dihydroxycinnamate, ethyl cinnamate, and ethyl anthranilate were pointed out to be important in Pinot noir wines of Burgundy (15). The odor threshold of ethyl cinnamate in water was determined to be 16 ppb (16), and the concentration of this compound in the Pinot noir wine was lower than the sensory threshold. Therefore, its contribution to the wine aroma is probably limited, which is consistent with the quantification results by stable isotope dilution assay (16). There is no sensory threshold data for ethyl dihydroxycinnamate and ethyl anthranilate, thus their aroma contributions are not clear.

Among the aroma-active compounds quantified, phenyl-ethanol, which gives rosy and honey aromas, showed the highest concentration (24 to 37 mg/L). This compound has a sensory threshold of 1 mg/L in water (32), so it is an important aroma contributor. This compound has been reported as a key characteristic aroma compound in Pinot noir wines (13, 14). Benzene alcohol, which was described as floral, also was present at mg/L level in the wine samples.

Guaiacol, typically described as smoky, spicy, and medicine-like, was found to be from 70 to 200 μg/L in the wine samples. Compared to its sensory threshold (20 ppb in white wine) (33), this compound will contribute to the wine aroma. Eugenol and 4-ethylguaiacol, which were also described as smoky and spicy, were detected at μg/L level in the wines. Though generally considered to be faults at high concentration, these phenolic compounds can contribute attractive elements of aroma to a wine's bouquet, and this positive effect may vary based on the grape variety (34).

It had been widely reported that monoterpene alcohols are responsible for the characteristic floral aroma in grapes and wines. In this study, four monoterpene alcohols, linalool, geraniol, nerol, and citronellol, were quantified. The results showed that all of them were present at μg/L levels in the wines. Since the sensory thresholds of these compounds are generally at μg/L levels, they may play roles in contributing floral and cherry flavors to Pinot noir wine.

β-Damascenone, which has a scent reminiscent of exotic flowers with a heavy fruity undertone, is variably described as

Table 2. Concentration ($\mu\text{g/L}$) of Selected Aroma-Active Compounds in Pinot Noir Wines from Different Grape Maturities ($n = 3$)

compounds	wine sample			2004		
	2003			2004		
	early stage	middle stage	late stage	early stage	middle stage	late stage
alcohols						
guaiacol	82 \pm 6	103 \pm 8	177 \pm 6	73 \pm 6	115 \pm 10	140 \pm 12
linalool	12.2 \pm 0.3	11.4 \pm 0.3	10.2 \pm 0.3	14.4 \pm 0.5	11.5 \pm 1.1	8.6 \pm 0.1
nerol	2.38 \pm 0.16	2.80 \pm 0.33	4.43 \pm 0.17	9.19 \pm 0.15	9.43 \pm 0.65	11.23 \pm 0.09
geraniol	5.6 \pm 0.1	9.5 \pm 0.6	12.8 \pm 0.3	14.7 \pm 0.4	19.7 \pm 1.0	24.2 \pm 0.1
eugenol	4.13 \pm 0.12	3.65 \pm 0.19	3.28 \pm 0.08	3.09 \pm 0.13	3.11 \pm 0.28	2.83 \pm 0.03
benzylalcohol (mg/L)	1.11 \pm 0.03	1.15 \pm 0.06	1.56 \pm 0.05	1.80 \pm 0.05	1.86 \pm 0.04	2.01 \pm 0.06
phenylethanol (mg/L)	37.3 \pm 0.7	37.4 \pm 1.2	37.0 \pm 0.3	23.9 \pm 1.0	24.0 \pm 0.8	23.7 \pm 0.5
citronellol	6.25 \pm 0.21	7.09 \pm 0.33	9.41 \pm 0.63	3.70 \pm 0.26	6.92 \pm 2.81	8.93 \pm 0.50
4-ethylguaiaicol	ND ^a	1.96 \pm 0.16	3.49 \pm 0.25	ND ^a	2.08 \pm 0.04	5.62 \pm 0.31
ketones						
β -damascenone	6.22 \pm 0.16	6.76 \pm 0.36	9.42 \pm 0.11	4.49 \pm 0.15	5.14 \pm 0.16	5.88 \pm 0.24
β -ionone	0.44 \pm 0.01	0.47 \pm 0.02	0.63 \pm 0.02	0.23 \pm 0.01	0.23 \pm 0.01	0.30 \pm 0.01
γ -nonalactone	13.0 \pm 0.1	13.7 \pm 0.2	18.0 \pm 1.3	10.3 \pm 0.5	10.6 \pm 0.3	15.1 \pm 1.1
esters						
ethyl 2-methylpropanoate	769 \pm 34	549 \pm 46	430 \pm 37	361 \pm 23	209 \pm 22	132 \pm 6.0
ethyl butyrate	212 \pm 2	201 \pm 12	215 \pm 29	145 \pm 3	148 \pm 8	114 \pm 5
3-methylbutyl acetate	463 \pm 23	547 \pm 23	480 \pm 40	267 \pm 4	287 \pm 11	240 \pm 2
2-methylbutyl acetate	114 \pm 5	130 \pm 5	113 \pm 5	70 \pm 1	71 \pm 2	53 \pm 1
ethyl 3-methylbutanoate	79.4 \pm 4.5	56.1 \pm 3.9	47.9 \pm 2.8	24.0 \pm 0.8	14.8 \pm 0.5	9.0 \pm 0.3
ethyl hexanoate	227 \pm 7	296 \pm 19	288 \pm 27	236 \pm 11	179 \pm 18	239 \pm 14
ethyl octanoate	283 \pm 14	237 \pm 9	246 \pm 6	191 \pm 11	182 \pm 13	191 \pm 7
ethyl decanoate	100 \pm 6	108 \pm 5	142 \pm 18	79 \pm 6	79 \pm 6	94 \pm 3
ethyl phenylacetate	6.10 \pm 0.40	3.83 \pm 0.26	3.81 \pm 0.23	2.18 \pm 0.14	1.61 \pm 0.16	1.25 \pm 0.16
ethyl dihydroxycinnamate	1.21 \pm 0.09	0.89 \pm 0.09	0.52 \pm 0.04	0.82 \pm 0.05	0.48 \pm 0.04	0.35 \pm 0.04
ethyl anthranilate	0.80 \pm 0.07	0.60 \pm 0.04	0.64 \pm 0.01	0.33 \pm 0.02	0.19 \pm 0.02	0.16 \pm 0.04
ethyl cinnamate	2.96 \pm 0.09	2.68 \pm 0.05	1.92 \pm 0.17	6.36 \pm 0.10	4.29 \pm 0.44	4.01 \pm 0.33
methyl vanillate	43.2 \pm 0.13	32.9 \pm 1.2	33.5 \pm 0.7	38.8 \pm 1.6	32.0 \pm 2.2	26.8 \pm 2.3
ethyl vanillate	13.0 \pm 0.5	13.1 \pm 0.5	17.3 \pm 0.9	10.5 \pm 0.2	13.7 \pm 1.5	17.4 \pm 1.5
phenylethyl acetate	25.0 \pm 1.5	23.7 \pm 0.6	19.2 \pm 1.3	11.8 \pm 0.1	10.8 \pm 0.9	7.81 \pm 0.69
ethyl 3-phenylpropanoate	1.51 \pm 0.09	1.11 \pm 0.11	0.68 \pm 0.05	0.94 \pm 0.10	0.62 \pm 0.05	0.38 \pm 0.03

^a ND: not detected by this method.

Table 3. Multivariate Tests^a for Aroma Compounds in Wine Samples from Different Grape Maturities

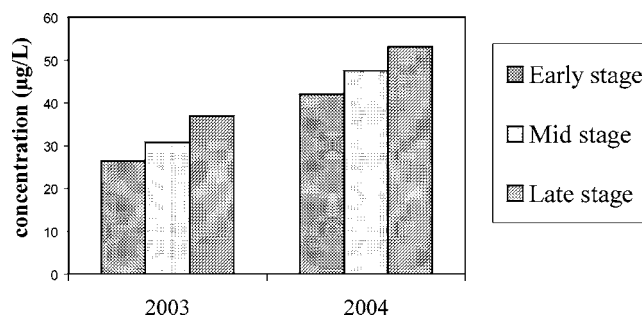
effect	Wilks' λ	F value	hypothesis df	error df	sig.
YEAR	.000	647.660	12	1	0.031
MATURITY	.000	62.291	24	2	0.016
YEAR * MATURITY	.000	5.710	24	2	0.160

^a Model: YEAR + MATURITY + YEAR * MATURITY.

apple, rose, and honey. It had concentrations from 5 to 10 $\mu\text{g/L}$ in the wines, while its sensory threshold was reported as low as 0.002 $\mu\text{g/L}$ (32). β -Ionone has a distinct berry and violet-like aroma. It had a high concentration from 0.2 to 0.6 $\mu\text{g/L}$. The low sensory threshold (0.007 $\mu\text{g/L}$) (32) of β -ionone makes it a very important aroma compound for Pinot noir wine. γ -Nonalactone, which is usually described as coconut and peach, was detected at 10–18 $\mu\text{g/L}$ in wine samples.

Effect of Grape Maturity on Wine Aroma Composition. MANOVA analysis was performed on all quantified aroma-active compounds. As shown in **Table 3**, both grape harvest maturity and producing year could affect the aroma composition of Pinot noir wine ($p < 0.05$), and these effects were independent of each other ($p = 0.16$). To further investigate the effects of grape maturity on wine aroma, the aroma composition of wine samples in the same year were compared.

Among the terpene alcohols studied, the concentration of geraniol, nerol, and citronellol increased with grape maturity (**Table 2**). However, the linalool concentration decreased slightly. Terpene compounds belong to the secondary plant

**Figure 1.** Concentration of total terpene alcohols (linallol, nerol, geraniol, and citronellol) in Pinot noir wines from different grape maturities.

constituents. Generally, 90% of the terpenes were present as nonvolatile glycosides that can be hydrolyzed (enzymatically or chemically) to the free form during fermentation and aging (35). Except for hydrolysis, acid-catalyzed rearrangements during wine processing and aging also can result in changes in concentration and formation of new compounds that were not present in the original grapes and young wines (36). Moreover, linalool can be further transformed to geraniol and nerol during wine producing, and geraniol and nerol can be further changed to citronellol through enzymatic reactions (37), and the latter one has a much lower sensory threshold than other two (38), which partially explained why the late stage wine presents more floral-aroma than the early one. The total terpene alcohols increased with grape maturity in both years (**Figure 1**).

Low levels of guaiacol and 4-ethylguaiaicol were also detected, and their concentrations dramatically increased along

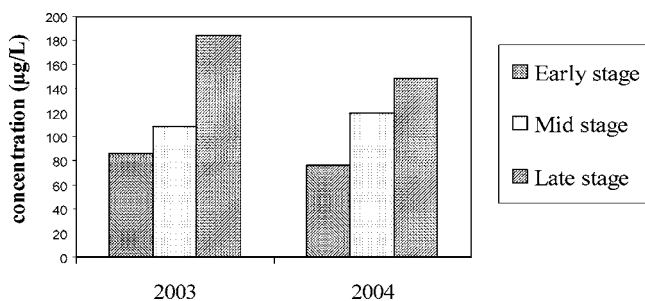


Figure 2. Concentration of total phenols (eugenol, guaiacol, and 4-ethylguaiacol) in Pinot noir wines from different grape maturities.

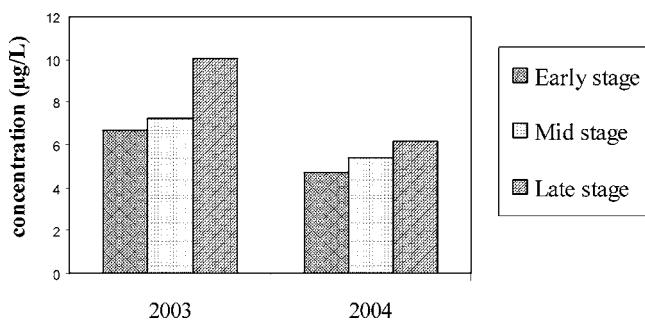


Figure 3. Concentration of C13-norisoprenoids (β -damascenone, β -ionone) in Pinot noir wines from different grape maturities.

with grape maturation (Table 2). Eugenol, however, showed a decreased trend with maturity. Most of the guaiacol and 4-ethylguaiacol in red wines were related to oak barrel aging. It has been reported that toasting of the oak barrels leads to thermal degradation of lignin and produces the volatile phenols, which could be extracted into the wine (39, 40). In addition, they could be associated with spoilage by *Brettanomyces* (41) in red wine. Tannins in red wine can be degraded to 4-vinylphenol and 4-vinylguaiacol, and *Brettanomyces* can convert them to 4-ethylphenol and 4-ethylguaiacol, respectively. However, phenolic compounds were also detected in a non-oak aged alcoholic beverage (42), which indicates another pathway. Since the experimental wines were not aged in oak barrels, guaiacol and 4-ethylguaiacol were probably formed from grape phenolic acid degradation. As more tannins are formed during grape maturation, more phenols can be generated (Figure 2).

β -Damascenone and β -ionone, two C13 norisoprenoids quantified in this study, are arised from carotenoid degradation during grape ripening (35). Predominantly occurring in grapes as glycosidically bound precursors, those compounds could be released in wine by enzyme and acid hydrolysis. For both years, the late maturity wines had much higher concentrations of these two compounds than the early stages (Figure 3), which could contribute a more berry-like aroma in the late maturity wine sample.

The result also showed that the late stage wines had higher concentrations of γ -nonalactone (Table 2). Lactones are widely distributed in the fruit of plants, although some of them could originate from aging in oak barrels.

It is widely known that esters are especially important to wine flavor. Esters are usually considered secondary aromas, and they are formed from acyl-SCoA by yeast during fermentation. Ester formation can be affected by many factors such as yeast strain, fermentation temperature, oxygen availability, and nitrogen level during fermentation (30, 43).

Two acetate and six fatty acid esters, including 2-methylbutyl acetate, 3-methylbutyl acetate, ethyl 2-methylpropanoate, ethyl

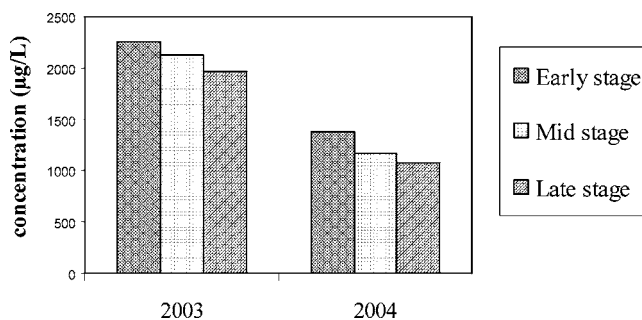


Figure 4. Concentration of short chain fatty acid esters (ethyl 2-methylpropanoate, ethyl butanoate, 3-methylbutyl acetate, 2-methylbutyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate) in Pinot wines from different grape maturities.

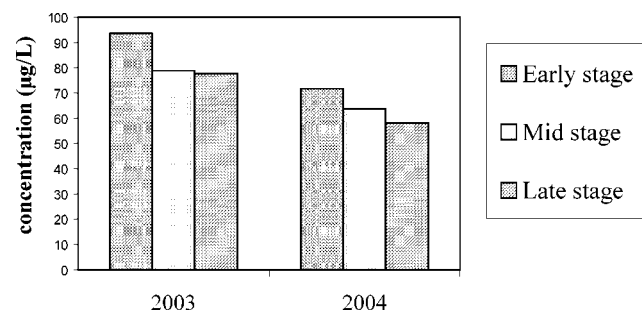


Figure 5. Concentration of aromatic esters (ethyl phenylacetate, ethyl dihydrocinnamate, ethyl anthranilate, ethyl cinnamate, methyl vanillate, ethyl vanillate, phenethyl acetate, and ethyl 3-phenylpropanoate) in Pinot noir wines from different grape maturities.

butanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were analyzed in this study. Though those esters had high concentrations in wines, there was no obvious correlation with grape maturity for most of the esters. However, it was observed that the concentrations of ethyl 2-methylpropanoate and ethyl 3-methylbutanoate consistently decreased with grape maturity. The total concentration of short chain fatty acid esters decreased with grape maturity for both years (Figure 4). Obvious decreasing trends with grape maturity were observed for aromatic esters including ethyl anthranilate, ethyl cinnamate, ethyl dihydrocinnamate, ethyl phenylacetate, phenethyl acetate, ethyl 3-phenylpropionate, and methyl vanillate (Table 2). The opposite trend was observed for ethyl vanillate. The total concentrations of aromatic esters also decreased with maturity (Figure 5). The decreasing trend of both total short-chain fatty acid esters and aromatic esters explained why the late stage of wines showed less fruity aroma. Further research needs to be done to understand the formation mechanism of those compounds during grape maturity and the wine making process.

In conclusion, a rapid method using SBSE-GC/MS was developed to quantify the aroma-active compounds in wine. The correlation coefficient and RSD of calibration curves showed that this method could be used to accurately analyze most key aroma-active compounds in Pinot noir wines. Moreover, this method was applied to investigate how grape maturity affects the aroma-active compounds in wine. The results further verify that grape maturity affects aroma composition of wine. The concentration of most grape-derived aroma-active compounds increased along with grape maturity, while the opposite trend occurred for esters.

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Received for review May 17, 2006. Revised manuscript received August 28, 2006. Accepted September 3, 2006.

JF061396M