Development of aroma compounds in Pinot noir Grapes and their Relative Importance in Wine (Year 1)

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Introduction

Color stability and flavor are two of the most important aspects of red wine quality. The development of anthocyanins and tannins in Pinot noir grapes and their relative importance in wine has been under investigation by Dr. James Kennedy. Flavor development, however, is not well understood. Several gas chromatography/olfactometry techniques have been used to study important aroma compounds in wines. By using an Osme technique(1), Miranda-Lopez et al. (2) investigated the volatile compounds in Pinot noir wines of different vantages. They found that the aroma character varies significantly with vintage and with maturity within a given vintage. It has been noticed that late grapes produce more flavored wines (2), however, it is not clear that this flavor change is due to wine making practice, flavor or flavor precursor changes in the grapes, or enzyme activity changes. In addition, flavor analysis in the wine poses technical difficulties to obtain reliable quantitative data due to its complexity after fermentation.

The objectives of this research project are to understand the most important flavor compounds formed during berry development in Pinot noir fruit to understand their effects on Pinot noir wine flavor and wine quality. The project starts with the identification of important flavor compounds responsible for Oregon Pinot noir wine, and determine which compounds are generated from grapes (first year). After the grape flavor compounds are identified, the effect of berry development on the selected flavor and flavor precursors composition will be investigated (second year). Lastly, the effect of berry development on the flavor and flavor precursor composition on experimental wines will be investigated (third year).

The information generated from this study will supplement flavor and flavor precursor information for other grape components (anthocyanins, sugar and acids) and correlate grape composition with wine quality. The ultimate results are to investigate possible optimum fruit harvest time based on flavor, flavor precursors, tannins, sugars and acid development in grapes to consistently make high quality Pinot noir wines.

Material and Methods

Identification of important aroma compounds in Oregon Pinot noir

Wine aroma extraction and fractionation: The 2000 vintage Pinot noir wines from Oregon State University viticulture trials at Benton Lane Vineyard were analyzed in this study. One liters of Pinot noir wine was extracted three times with 200ml diethyl ether/pentane (1:1)

mixture in a separatory funnel. Distillation of these combined extracts was performed with solvent assisted flavor evaporation device (SAFE) to remove the nonvolatile constituents (mainly pigments and sugar) at 50°C. The distillate were dried over anhydrous sodium sulfate, and concentrated to 10ml. The aroma extract was fractionated into acidic, water-soluble and neutral fraction. The acidic and water-soluble fractions were concentrated to 500 ul for GC/O-MS analysis. The neutral fraction was further fractionated on a silica gel SPE column into 4 fractions (pentane, pentane-ether (95:5), pentane-ether (90:10) and ether described by Qian (3) and each fraction was concentrated to 100 ul for analysis.

Gas Chromatography/Olfactometry (GC/O)-mass spectrometry: Capillary gas chromatography-mass spectrometry (GC-MS) analysis was carried out using an Agilent GC 6890 fitted with a 30m DB-Wax fused silica column (0.25 mm i.d. and 0.5 um film thickness, J&W Scientific). The sample (2ul) was injected into a split/splitless injection port heated to 250 ^{0}C at a split ratio of 1:1. Helium was used as the carrier gas with constant flow rate of 2ml min^{-1}. The oven temperature program was 40°C (for 2min), then increased at 4°C/min ramp to 230°C, and held at this temperature for 10 min. The column effluent was split 1:1 to the MSD and a sniffing port via a fused silica outlet splitter, and the port effluent was mixed with humidified air. Osme technique was used to select the most important aroma compound sin wines. An Agilent 5973 Mass Selective detector (MSD) was used for identification. The electron impact (EI) energy was 70eV, and the ion source temperature was set at 230°C. System software control and data management/ analysis was performed through Enhanced ChemStation Software, GCA v. C.00.01.08 (Agilent Technologies Inc.). Mass spectra of unknown compounds were compared with those in the Wiley 275.L (G1035) Database (Agilent Technologies Inc.).

Analysis of flavor and flavor precursors in wine grapes

Grape collection and experimental wine: The grapes will be from the Oregon State University experimental vineyard located in Alpine (Woodhall vineyard, maturity/C block, Pommard clone), planted in 1984. Vineyard operations will be in keeping with commercially accepted practice. From the Aug. 19th to Oct. 7th in 2002, about one kilogram of Pinot noir grape samples were randomly collected every week, and were transported on ice to the laboratory, where they were stored at -10F. At three times during fruit ripening (22, 24 and 26 °Brix), sufficient fruits were harvested for experimental wine production. The samples were frozen for 3 months before analysis.

Analysis of free volatiles from mature grape: Ripe Pinot noir grape samples (500 g, collected on Sep. 26th , 2002) were smashed by blender and continuously extracted with 300ml diethyl ether/pentane (1:1) for 8 hours at was 38 ^{0}C in an extraction apparatus. The crude extracts obtained from grape were distilled with SAFE, the distillate was dried over anhydrous sodium sulfate, and then concentrated to 100ul. The final concentrated sample was analysis by GC/O-MS within two weeks.

Flavor and flavor development in berries. 150 gram of grape fruits from various harvesting time were thawed at room temperature for 3 hours. The grapes were blended by pulsing for a total of 2 min at high speed. 15 gram smashed fruity was immediately transferred to a glass tube for analysis. Tekmar ALS 2016 and LSC 2000 purge-and-trap equipment (Terkar Co.) was used for dynamic headspace sampling of volatile compounds. The sample was preheated for 3 min at 50°C, and volatiles purged by nitrogen at a flow of 40 ml/min for 30min. Volatiles

were adsorbed by a Tenex trap (#12-0083-003, Tekmar Co.). After the purge process, the trap was dry purged with nitrogen for 3 min. Volatiles were then thermally desorbed (250°C for 2min) and transferred by helium carrier gas directly to the GC injection port by a 1.5m*1.6 mm id transfer line. The sample was injected at splitless mode. A DB-FFAP column (30 m length, 032 mm i.d and 0.5 ul film thickness, J&W Scientific) was used for separation. The GC/O-MS conditions were the same as described previously.

Results and Discussion:

Identification of important aroma compounds in Oregon Pinot noir

Aroma compounds in Pinot noir wine was extracted and fractionated into acidic, water-soluble and neutral fraction. The neutral fraction was further fractionated on a silica gel SPE column into 4 fractions (pentane, pentane-ether (95:5), pentane-ether (90:10) and ether) described by Qian(3). Gas chromatography/olfactometry (Osme) were performed on all of the fractions. Important aroma compounds were identified by gas chromatography/mass spectrometry.

The most important aroma compounds in the acidic fraction are hexanoic, 3-methylbutanoic, 2-methylpropanoic, acetic, benzeneacetic and butanoic acids (Table 1). Most of these acids contribute to rancid notes. Due to their high Osme values, their concentrations will be measured in wine grapes and corresponding wines.

Table 1. Important Aroma Compounds in acidic fraction of 2000 Vintage Pinot noir Wine

Compound	Descriptor	Intensity	RI
hexanoic acid	sour acid	14	1841
3-methyl butanoic acid	rancid cheesy sweaty	12	1666
acetic acid	acidic	7	1443
2-methylpropanoic acid	woody sour	7	1564
benzeneacetic acid	musty fruity	7	2560
butanoic acid	cheesy, rancid	6	1626
octanoic acid	goaty	5	2068
t-2-hexenoic acid	acidic	4	1939
benzoic acid		3	2422
propanoic acid		2	1538

Water-soluble fraction contains most alcohols (Table 2). Among them, benzeneethanol and benzyl alcohol are very important, giving rosy, floral aroma; isoamyl alcohol and isobutyl alcohols are the most important wine alcohols; 1-hexanol, cis-3-hexenol are responsible for the green, fruity notes; methionol gives vegetable note. Vanillin will also be determined due to its unique aroma contribution.

Table 2. Important Aroma Compounds in water-soluble fraction of 2000 Vintage Pinot noir Wine

Compound	Descriptor	Intensity	RI
benzenethanol	rose	14	1947

Benzyl alcohol	floral, leaf	13	1898
isoamylalcohol	unpleasant musty	13	1262
methionol	potato, vegetable	12	1740
	cooked vegetable herb	10	1480
isobutyl alcohol	alcoholic	8	1136
?	green grass earthy	8	1667
1-hexanol	vegetable fruity	7	1381
cis-3-hexenol	green	6	1408
vanilline	vanilla powder	6	2585
	dry grass woody	6	2195
propanol	floral tulip	5	1074
ethyl lactate	cooked fruity	5	1374
ethyl-2-hydroxybutyrate	floral tulip	5	1541
diethyl malate	green fruity caramel	5	2066
3-hydroxy-4-phenyl-2-butanone	floral, fruity, berry	5	2283
3-Et-2-pentanol	floral, dry grass	5	1595
2,6-dimethyl-4-heptanol	leaf, tea	4	1563
trans-3-hexenol	green	3	1389
Indene ?	cooked herb medicine, sweet	3	1498
butyrolactone	cheese	3	1645
3-(ethylthio)-1-propanol	roasted	3	1802
ethyl-4-hydroxybutanoate	red tea, sweet	3	1831
Guaiacol	tea leaf, sweet, floral	3	1877
ethyl-2-hydroxy-3-phenylpropanoate	blackpepper	3	2300
Benzoic acid, 4-hydroxy-3-methoxy, methyl ester	ripe fruity	3	2567

Silica gel chromatography separates the neutral compounds into four fractions to simplify identification. In the Pentane fraction, there is seldom odorants been identified by OSME. The Pentane-ether (95:5) fraction mainly contains b-damascenone and esters, they contribute to the fruity aroma (Table 3). _-Damascenone was identified to be very important due to its high Osme value.The most important esters are ethyl caproate, isoamyl acetate, ethyl isobutyrate, ethyl 3-phenylpropanoate, ethyl isovalerate, phenylethyl acetate, ethyl butyrate.

Table 3. Important Aroma Compounds in 2000 Vintage Pinot noir Wine-Pentane-ether (95:5) fraction

Compound	Descriptor	Intensity	RI
damascenone	green fruity	12.0	1839
ethyl caproate	fermentation sweet fruity	10.0	1248
ethyl phenylacetate	floral pungent sweet	10.0	1801
isoamyl acetate	banana	9.0	1136
ethyl isobutyrate	floral fruity sweet	8.0	978
ethyl 3-phenylpropinoate	floral	8.0	1902
ethyl isovalerate	sweet grass	7.0	1081
phenethyl acetate	wine floral	7.0	1833
ethyl butyrate	floral berry	6.0	1047

ethyl 2-methylbutyrate	fruity orange sweet	5.0	1063
ethyl n-valerate	fruity floral	5.0	1147
ethyl E-2-hexenoate	acidy green	5.0	1360
ethyl caprylate	green fruity	5.0	1453
a-terpinolene?	sweet nutty	5.0	1504
ethyl 3-methyl thiopropionate	smoky	5.0	1584
ethyl caprate	fruity grape	5.0	1654
ethyl benzoate	tea	5.0	1705
ethyl 9-hexadecanoate	powder dusty	5.0	2297
1-ethoxy-1-pentoxy-ethane	metal burning	4.0	1119
isoamyl isobutyrate	fruity sweet	4.0	1202
a-terpinene	green lemon	4.0	1530
isoamyl hexanoate	fruity green	3.0	1472
Indene?	penetrate	3.0	1662
tetradecanal	fatty oily	3.0	1727
β-ionone	floral fwoody	3.0	1958

The pentane-ether (90:10) (Table 4) and ether (Table 5) fractions revealed many interesting aroma compounds such as eugenol, ethyl anthranilate, benzothiazole, nonalactone, ethyl 2-hydroxy-3-phenylpropanoate, trans-linalool oxide, trans-geraniol. These compounds contributed roasty, smoky, nutty, woody characteristics.

Table 4. Important Aroma Compounds in 2000 Vintage Pinot noir Wine-Pentane-ether (90:10) fraction

Compound	Descriptor	Intensity	RI
diethyl octanedioate	stimulate	8.0	2132
Diethyl azeleate	smoky floral	8.0	2235
ethyl 2-hydroxycaproate	floral jasmine	7.0	1565
ethyl 3-methyl butyl butanedioate	roasted	7.0	1920
eugenol	gravy	6.0	2185
ethyl anthranilate	Smoky, stimulate	6.0	2291
	floral ester	5.0	1550
benzothiazole	dry grass	5.0	1973
5-methyl-thiazole	smoky earthy	5.0	1995
	fruity sweet	4.0	1485
di-isobutyl succinate	fruity	4.0	1811
	woody roasted	4.0	1880
hydroxydihydroedulan	ester floral	4.0	1942
1-octen-3-ol	earthy herb	3.0	1469
3-ethyl-pentanol	floral ester	3.0	1534
fenchyl alcohol	fresh grass floral	3.0	1733

Table 5. Important Aroma Compounds in 2000 Vintage Pinot noir Wine-ether fraction

Compound	Descriptor	Intensity	RI
nonalacone	floral coconut	9.0	2048

octanoic acid	sweet powder	9.0	2084
	grass woody	9.0	2196
	floral sweet ester	8.0	2225
ethyl 2-hydroxy 3-phenylpropanoate	black pepper	7.0	2300
ethyl 2-hydroxy isovalerate	fatty milky sweet	6.0	1443
ethyl-2-hydroxybutyrate	floral	6.0	1562
	roasted smoky woody	6.0	1589
	roasted	6.0	1878
	pepper spicy meat	6.0	2315
trans-linalool oxide	wet woody grass	5.0	1490
trans-geraniol	green floral	5.0	1869
1-octanol	floral tea	3.0	1581
ethyl-3-hydroxyoctanoate	smoky stimulate	3.0	1911
benzothiazole	woody stimulate	3.0	1973
geranic acid	fruity	3.0	2360
2-octanol	earthy aromatic	2.0	1540
citronellol	green fruity	2.0	1787
ethyl vanilla	??	2.0	2656

Analysis of flavor and flavor precursors in wine grapes

Preliminary study of grape flavor proved that we can analyze many free aroma and aroma precursors in grapes (Table 6). We identified phenylethanol, geraniol, nerol, β-ionone and many other important alcohols and volatile acids. Many of them are important aroma compounds in wines or as flavor precursors. Majority of flavor compounds in wine grapes are expected to be exist as glycosidic precursors. Analysis of glycosidic flavor precursors in grapes is underway.

Table 6. Important Aroma Compounds in Ripe Pinot noir Grape

Compound	Descriptor	RI	Intensity
phenylethyl alcohol	rosy	1948	very strong
ethyl acetate	pungent sweet fruity stimulate	877	strong
n-hexanal	green vegetable	1101	strong
1-butanol	green herb	1157	strong
isoamyl alcohol	herb pungent	1221	strong
trans-2-hexenal	green grape	1245	strong
1-hexanol	cooked fruity, green	1371	strong
acetic acid	sour	1467	strong
geraniol	floral, green fruity	1871	strong
benzyl alcohol	floral	1912	strong
ethanal	stimulate chemical	713	moderate
2-butanol	floral	1066	moderate
propanol	sweet floral	1074	moderate
cis-3-hexenyl butyrate	fruity,	1118	moderate
3-methyl-2-butanol	sweet	1131	moderate
2-pentanol	green	1131	moderate

2-heptanol	green herb	1332	moderate
trans-3-hexen-1-ol	fresh grass	1381	moderate
cis-3-hexenol	heated grass, green	1403	moderate
Nonanal	sweet rubber	1416	moderate
2-hexen-1-ol	green grape	1426	moderate
butanoic acid	sweaty	1649	moderate
2-methyl-butyric acid	sweaty	1689	moderate
Nerol	vegetable, sweet fruity	1822	moderate
hexanoic acid	sweaty	1865	moderate
trans-2-hexenoic acid	sweaty	1990	moderate
benzenepropanol	fruity strawberry	2076	moderate
furanones?	sweet	2101	moderate
2-propanol	sweet fruity	937	weak
2-pentenone	sweet fruity	1051	weak
3-hydroxy-2-butanone	sweet caramel fruity	1313	weak
2-hepten-1-ol	sweet	1529	weak
octanol	herb tea	1599	weak
3.7-dimethyl-6-octen-1-ol	fresh vegetable	1785	weak
B-Ionon	fruity	1959	weak

Summary

Oregon Pinot noir wine flavor is a delicate balance of many flavor attributes including acid (contributed by hexanoic, 3-methylbutanoic, 2-methylpropanoic, acetic, benzeneacetic and butanoic acids), wine alcoholic (isoamyl alcohol and isobutyl alcohol), floral (benzeneethanol and benzylalcohol), fruity (ethyl caproate, isoamyl acetate, ethyl isobutyrate, ethyl phenylacetate, ethyl 3-phenylpropanoate, ethyl isovalerate, phenylethyl acetate, ethyl butyrate), green fruity (1-hexanol, cis-3-hexenol), vegetal (methionol), honey, sweet(_-damascenone), varietal(vanillin, eugenol, ethyl anthranilate, benzothiazole, r-nonalactone, ethyl 2-hydroxy-3-phenylpropanoate, trans-linalool oxide, trans-geraniol) and possible other attributes. Flavor compounds responsible for those sensory attributes were identified in this study. Many of these compounds were also identified in wine grapes.

Reference:

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(2) Miranda-Lopez, R.; Libbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of Pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). *J. Food Sci.* **1992**, *57*, 985-993, 1019.

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