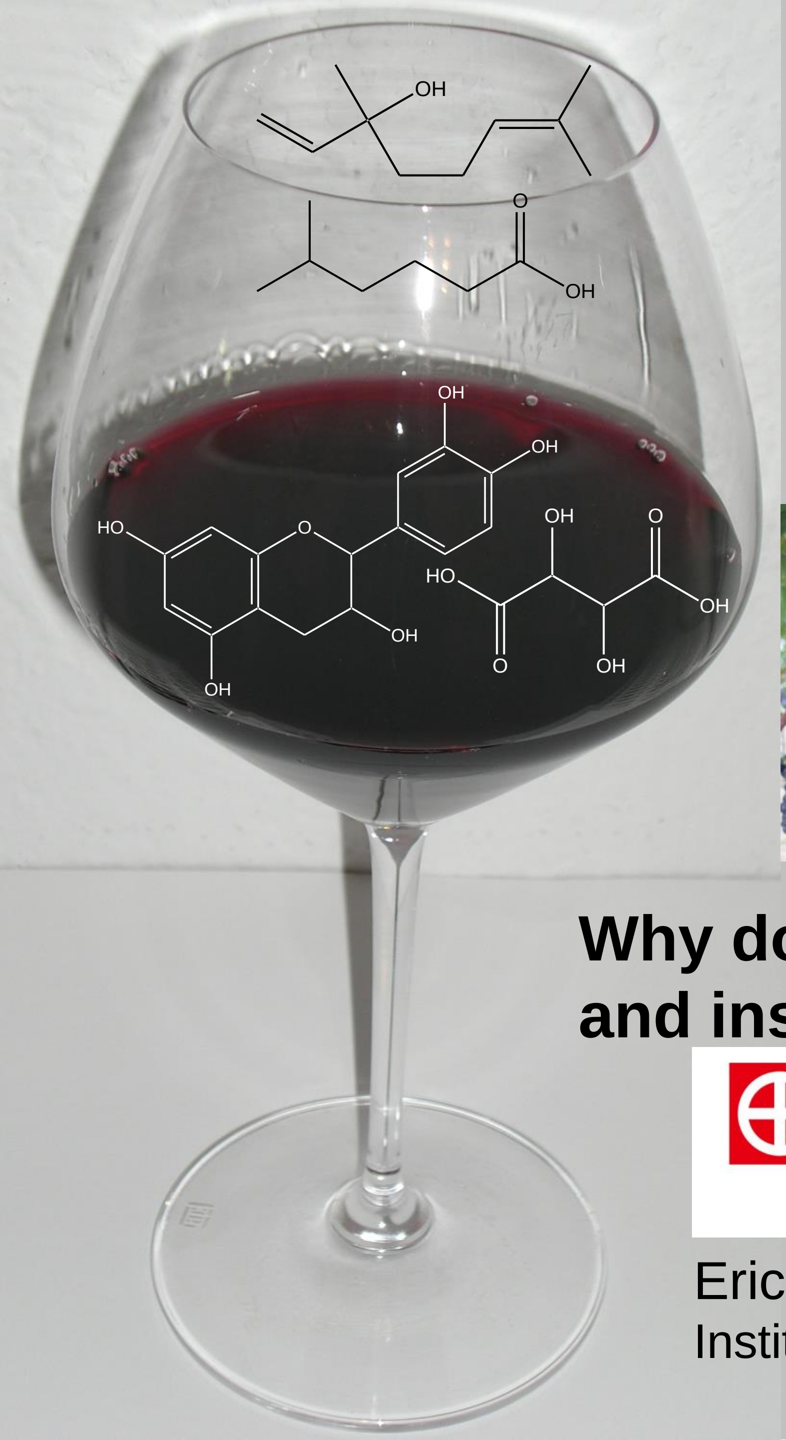
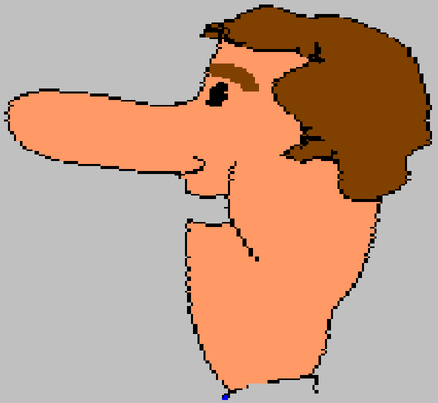




Why do we need different GC methods and instruments to analyse wine?

SHIMADZU
Excellence in Science

TU Graz
Graz University of Technology

Erich Leitner
Institute of Analytical Chemistry and Food Chemistry

Overview

The importance of aroma compound in wine

Aroma Fingerprint

Optimization Strategies
- Enhancing chromatographic resolution GCxGC-MS
- Enhancing the sensitivity GC-MS/MS

Food Chemistry and Sensory Analysis

Barbara Siegmund

Related quality

Training courses

Toxicologic and sensory relevant compounds

Method development

Product development and optimization

Erich Leitner

SENSORY ANALYSIS

Quality of Food Products and Food Contact Materials

INSTRUMENTAL ANALYSIS

Product Characterisation

Instruments I use

Several GC Systems with the following detectors:

FID; BID; NPD; FPD; ECD also with dual detection)

Several GC-MS Systems (SQ (EI, PCI, NCI), TQ)

MDGC

GCxGC-MS

GC-Olfactometry

(in total 14 GC based systems)

UPHLC-TQ-MS

Sample preparation (10 CTC in routine applications):

Arrows, SPME, ITEX, SPDE, HS, P&T, LINEX……

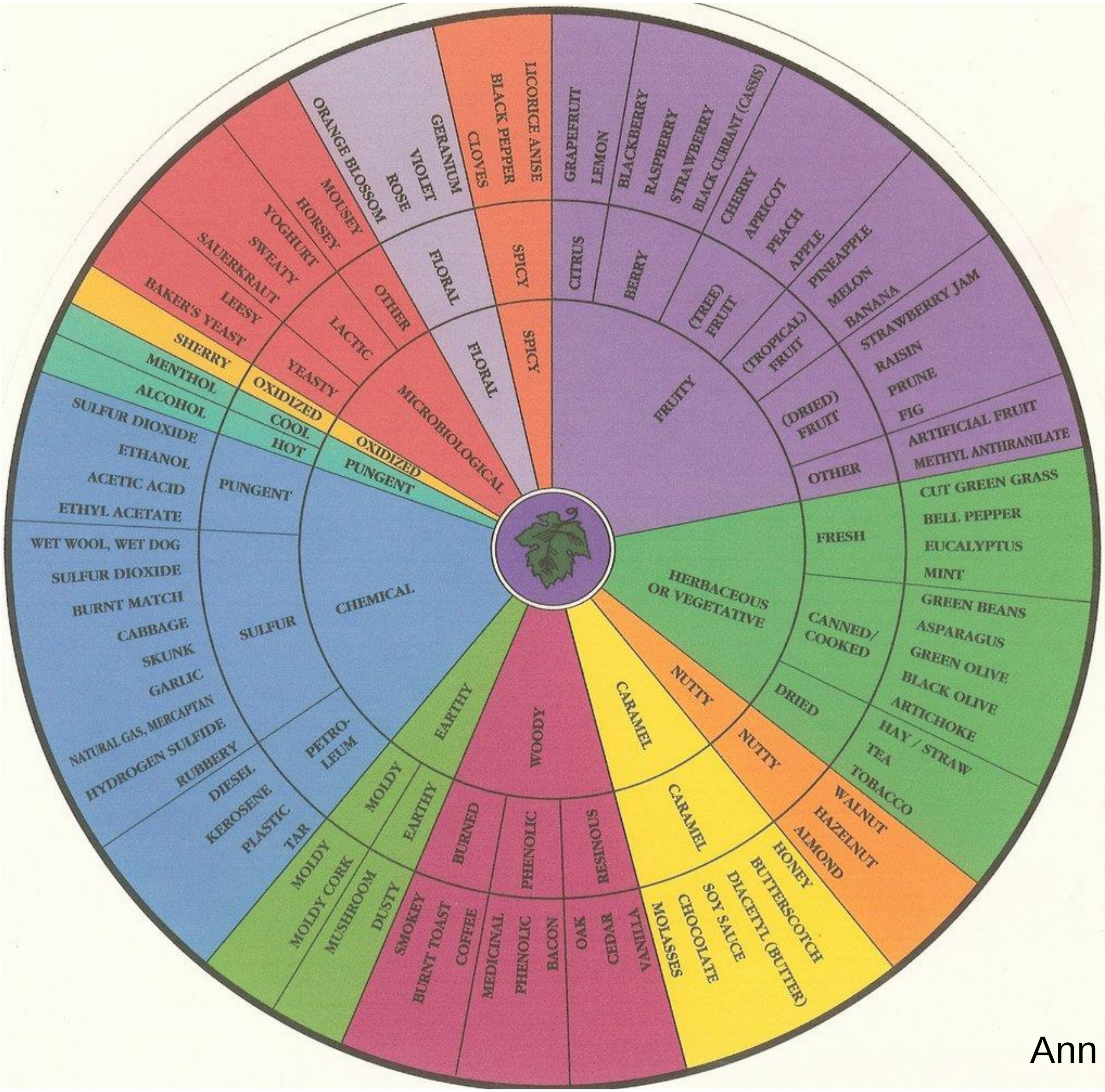
Aroma Compounds

– **varietal compounds**, either free forms or those obtained from precursors present in grapes
Rotundone, Isobutylmethoxypyrazine, Monoterpenes e.g. Linalool

– compounds formed during **winemaking** that are essentially fermentative compounds

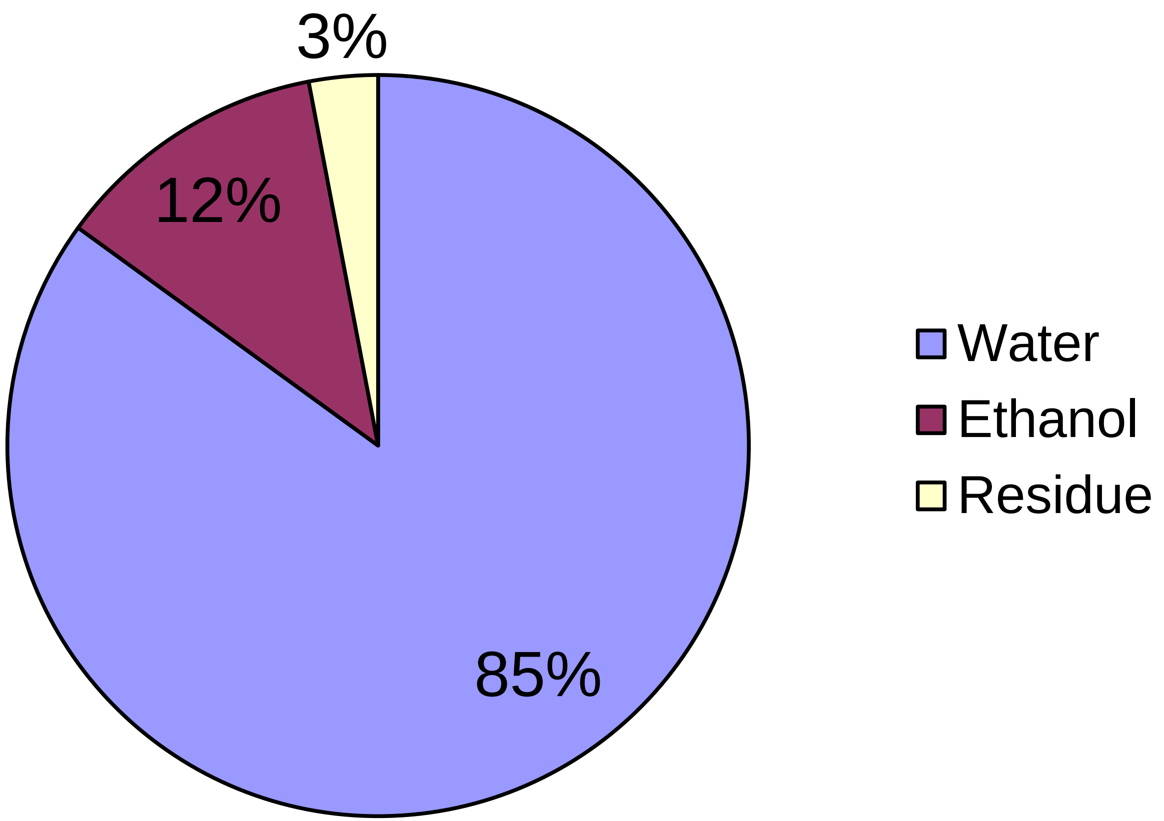
– compounds formed during the **maturation** of wines

The Aroma Wheel

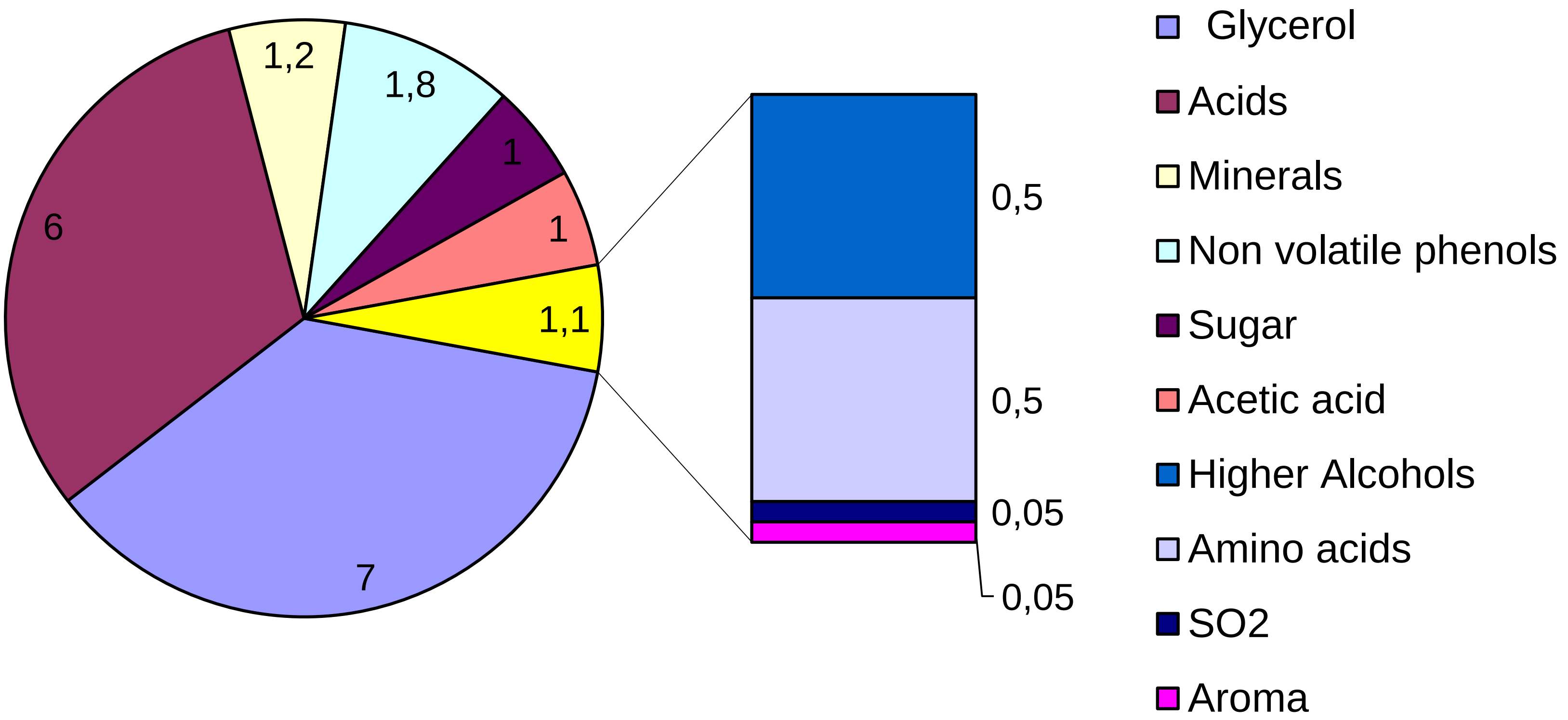


Ann Noble UC Davis

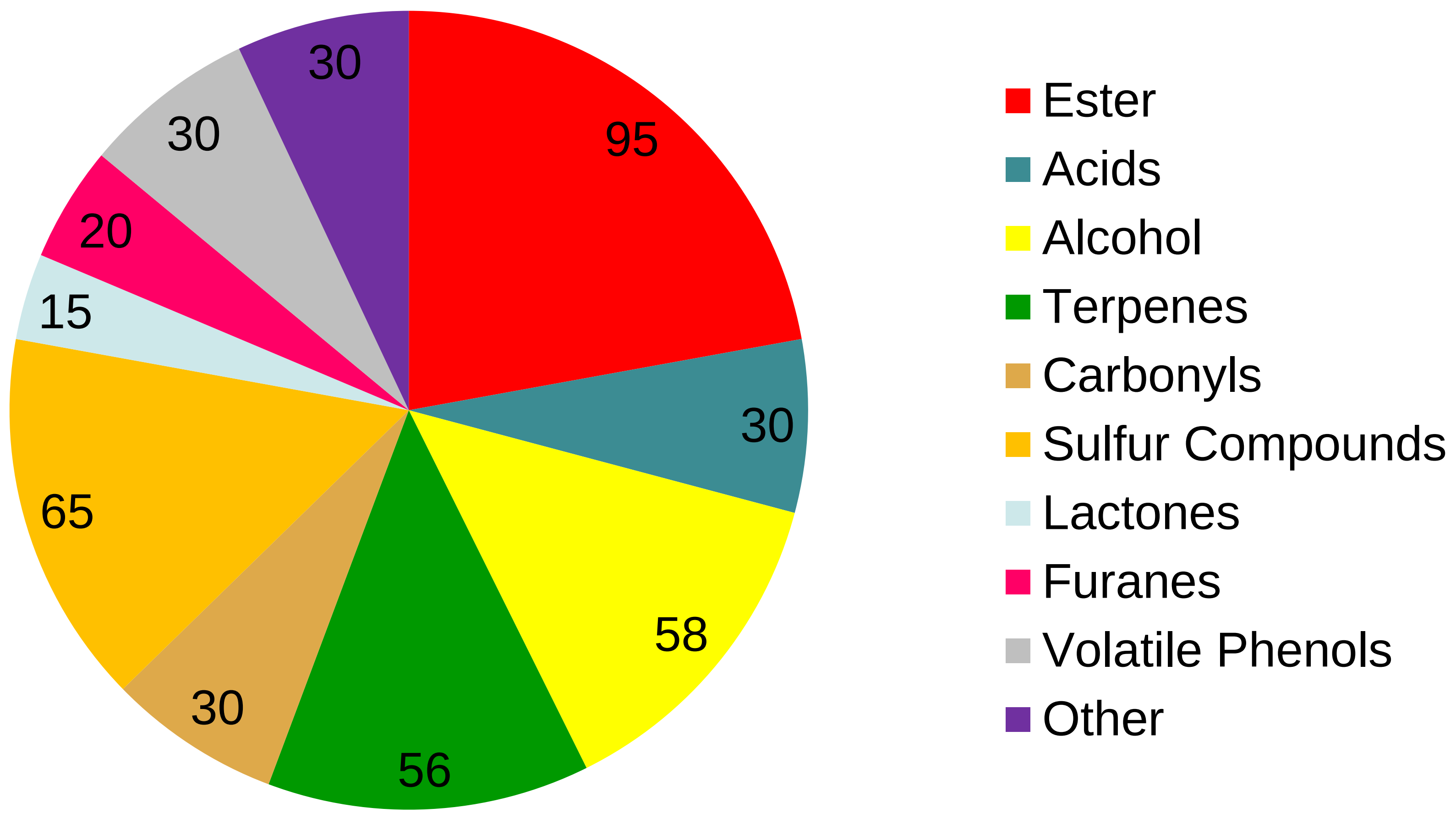
Average Composition of Wine [%]



Minor Constituents [g/L]



The Aroma Compound Wheel



The Concentration Ranges

Compounds	Concentrations [µg/L]
Esters	10-5000
Acids	20-4000
Alcohols	20-1000
Terpenes	0.01-800
Carbonyls	0.1-3000
Sulfur Compounds	0.01-300
Lactones	10-1500
Furanes	20-700
Volatile Phenols	30-3500
Other	0.0008-80

Odour of Mixtures

„For mixtures containing more than four components, the odorants were found to lose their individuality and produce a new odor percept conveying a unique odor quality not elicited by the single components.“

3-40 individual components give the product specific odor

Less than 3% of foodborne volatiles constitute to the chemical odorant space

Th. Hoffmann et al,
Natures Chemical Signatures in Human Olfaction: A Foodborne Perspective for Future Biotechnology
Angew. Chem. Int. Ed. 2014, 53, 7124 – 7143

Odour Activity Value OAV

Expresses the ratio of the odour intensity/activity to its threshold

The larger the absolute number of the OAV the larger is the impact of this compound for the total aroma

Values ≤ 1 do not have an impact on the overall aroma

$$OAV = \frac{c_x}{a_x}$$

c_x Concentration of substance x in a matrix

a_x Sensory threshold of substance x in a matrix

Ref: Rothe M., Thomas B.,1963

Compound	ST [µg/L]	Scheurebe	Gewürztraminer	OAV	OAV
Ethylisobutyrate	15	480	150	32	10
Diacetyl	100	180	150	2	2
3-Methylbutylacetate	30	1450	2900	48	97
3-Methylbutanol	30000	109000	127800	4	4
Ethylhexanoate	5	280	490	56	98
cis-Rose oxide	0.2	3.0	21	15	**105**
4-Mercapto-4-methylpentane-2-on	**0.0006**	**0.40**	**<0.001**	**667**	**<1**
Ethyloctanoate	2	270	630	135	315
Linalool	15	307	175	20	12
(E)-β-Damascenone	0.05	0.98	0.84	20	17
Geraniol	30	38	221	1	7
Wine lactone	0.01	0.1	0.1	10	10
Acetaldehyde	500	1970	1860	4	4
Ethylacetate	7500	22500	63500	3	8

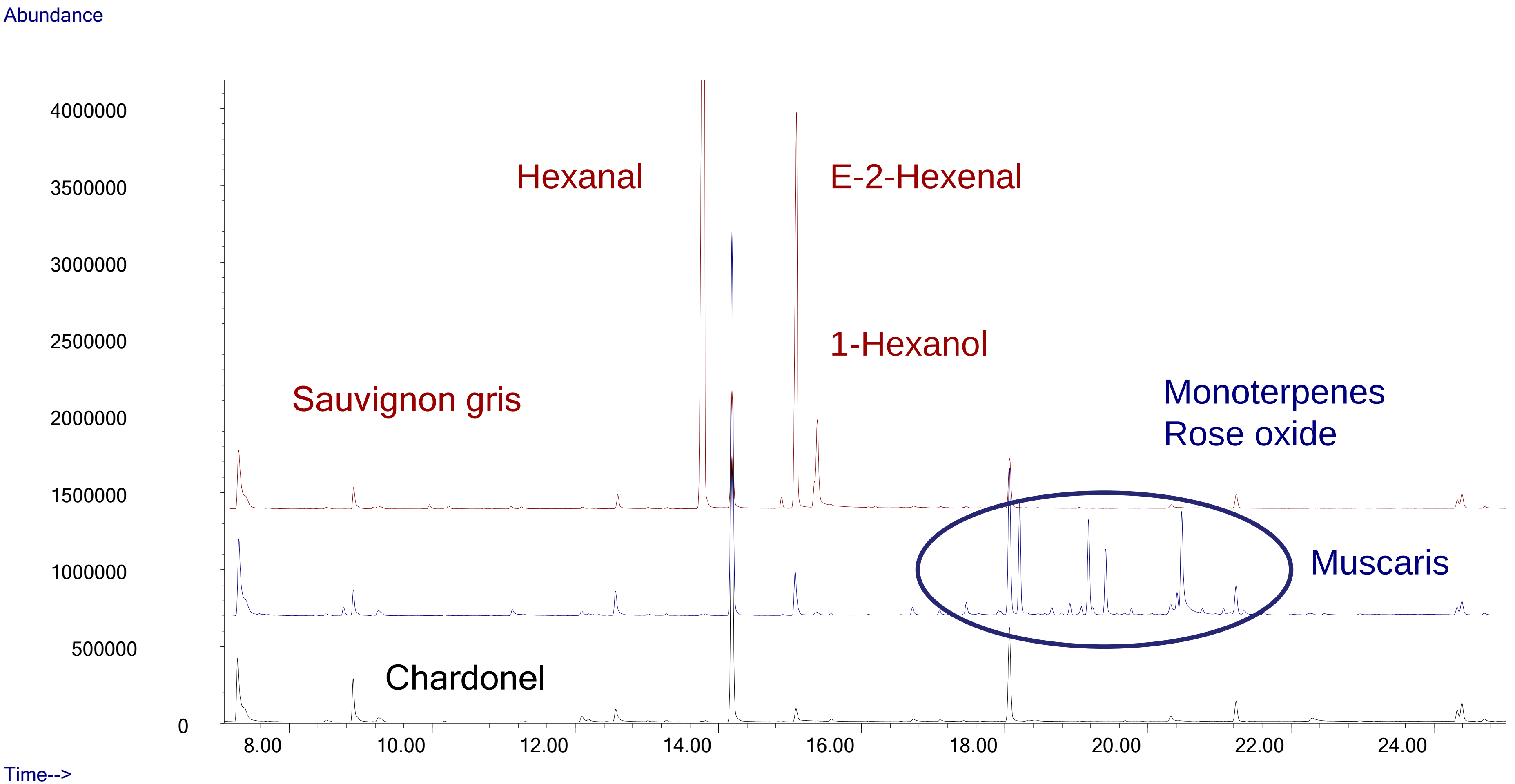
H. Guth, J. Agric. Food Chem. ***1997, 45, 3022-3026***

Aroma Compounds in Wine and Threshold

Compound	Aroma descriptor	Concentration in wine [µg/L]	Sensory threshold [µg/L]
Diethyl sulfide	Cooked vegetable	4-32	0.93
Z Oak lactone	Coconut, flowery	n.d.-589	67
4-Ethylphenol	Medicinal, horse sweat	n.d.-6500	140
Ethyl butanoate	Floral, fruity	10-180	20
Ethyl hexanoate	Green apple	30-3400	50
Acetic acid [mg/L]	Vinegar	110-1150	280
Ethyl acetate [mg/L]	Nail polish, fruity	22.5-400	7.5

Bartkowsky E.J., Pretorius I.S., in Biology of Microorganisms on Grapes, in Must and in Wine, Springer Verlag 2009, ISBN 978-3-540-85462-3

Different Grape Juice Varieties



Products of Yeast Primary Production (Alcoholic Fermentation)

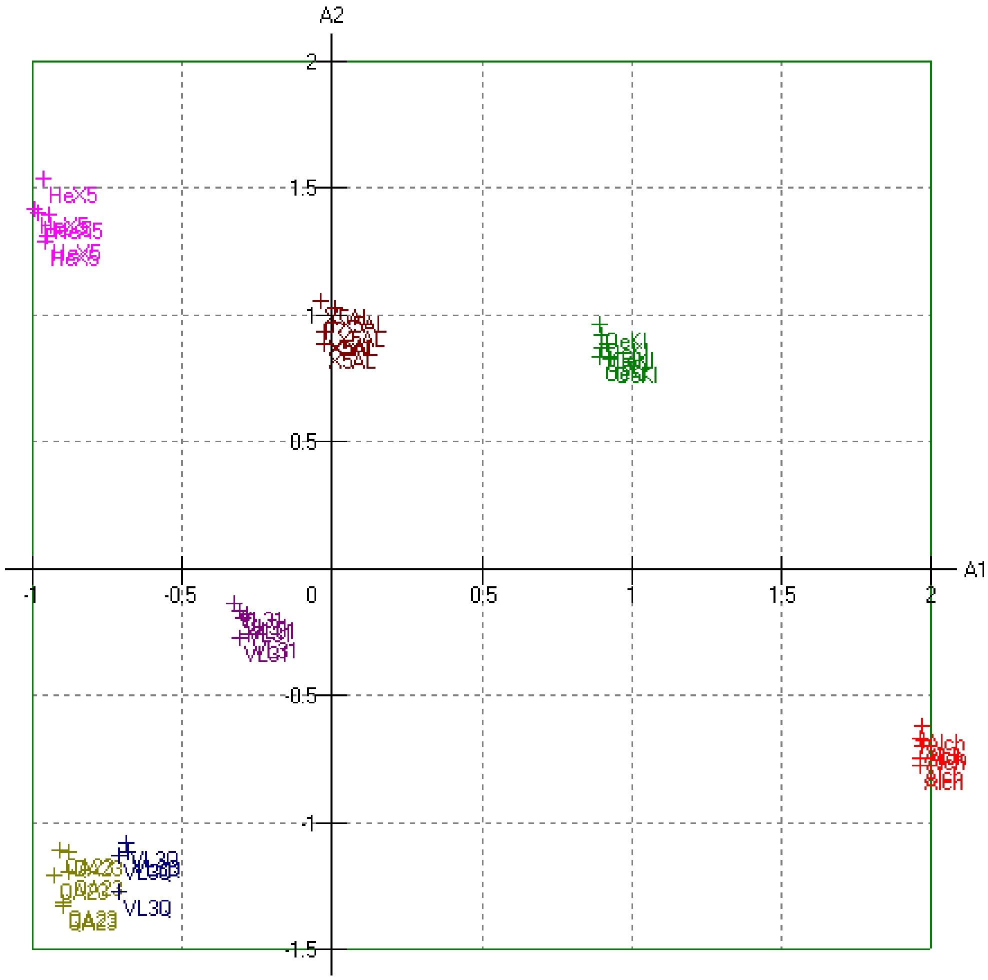
In wine-making conditions, the yeast *Saccharomyces cerevisiae* carries out alcoholic fermentation in almost anaerobic conditions

Grape must has a high fermentable sugar content (from 140 to 260 g/L, as a function of maturity), composed of similar amounts of glucose and fructose. It is also highly acidic (pH3.0–3.5).

Complete fermentation by *S. cerevisiae* under these conditions produces between 8 and 15% (v/v) ethanol, together with several other fermentation products like

- Glycerol
- higher alcohols
- sulfur compounds
- volatile phenols
- esters: linear ethyl fatty acids (lipid metabolism); fusel alcohol acetates

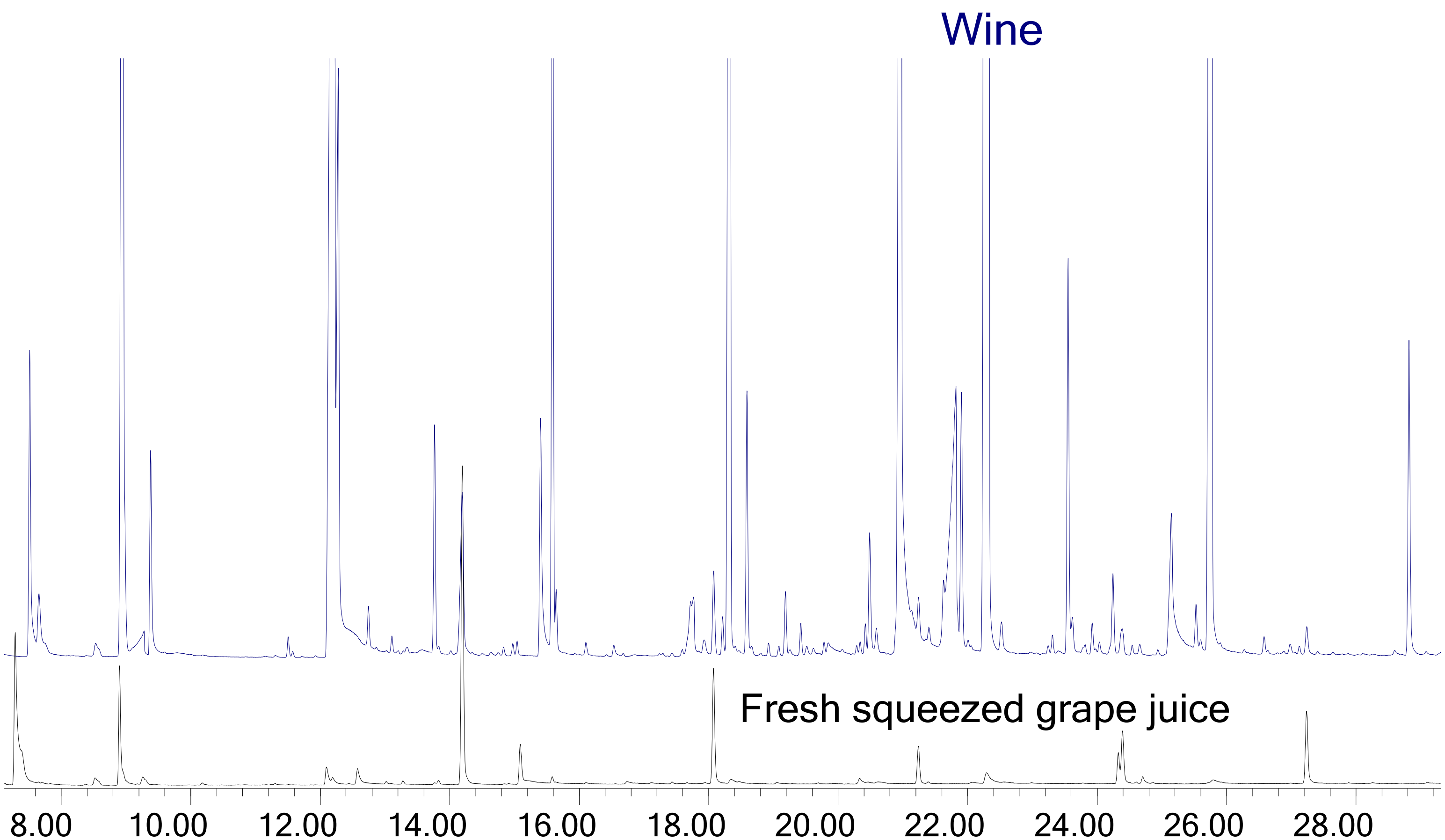
PCA Aroma Profiles



7 different single yeast strains used for fermentation of SB must

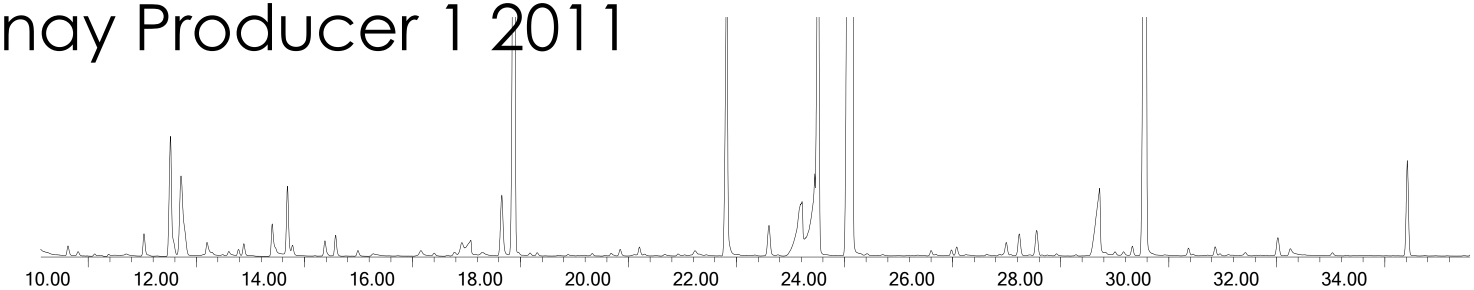
Selecting the yeast strain can „fine tune“ the sensory quality

Magic Fermentation:From Grape Juice to Wine

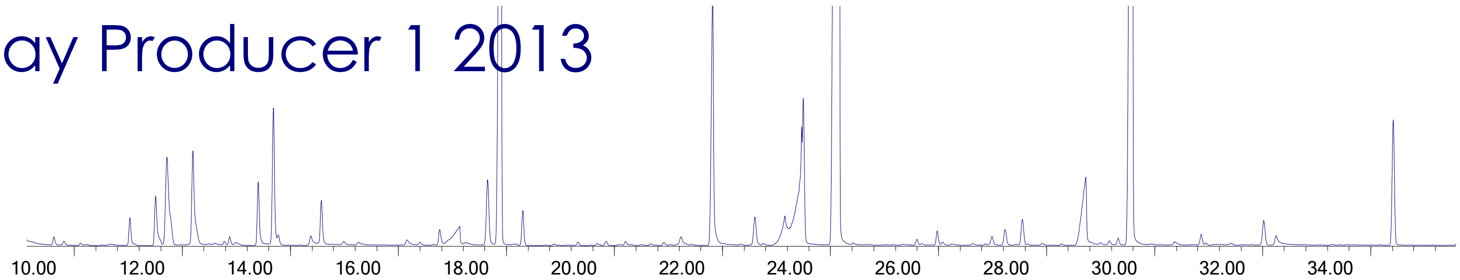


Looking for Differences: Aroma Profiling

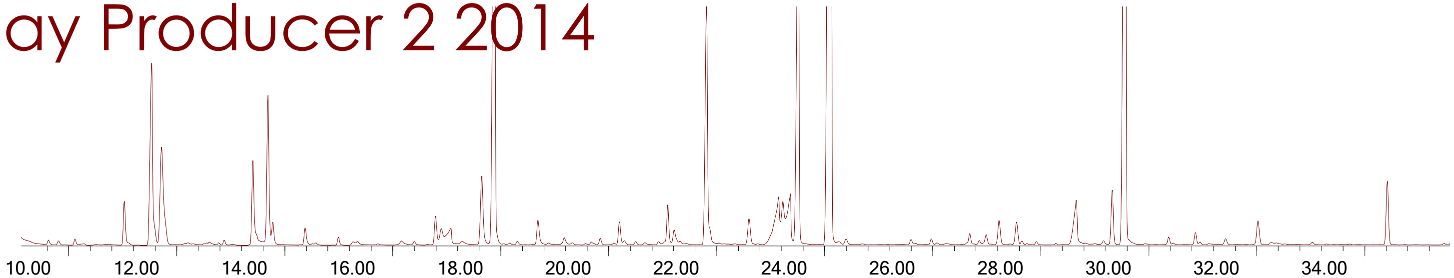
Chardonnay Producer 1 2011



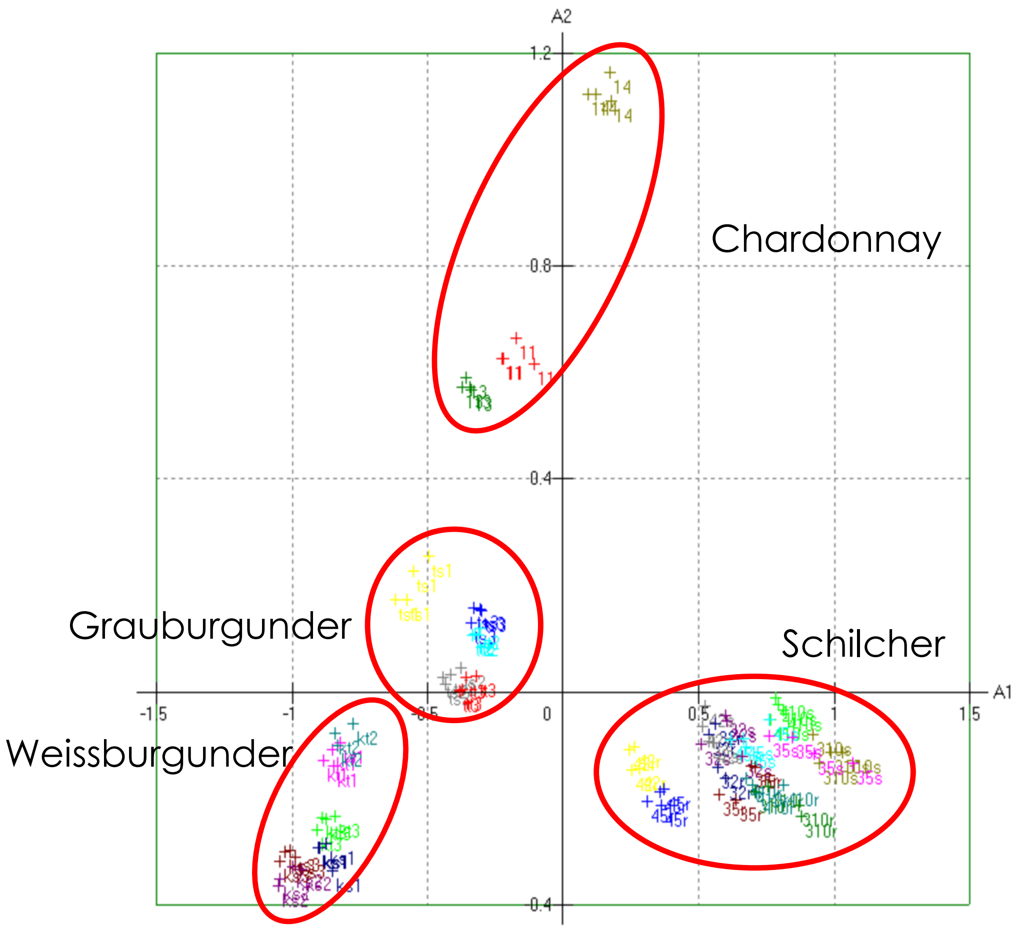
Chardonnay Producer 1 2013



Chardonnay Producer 2 2014



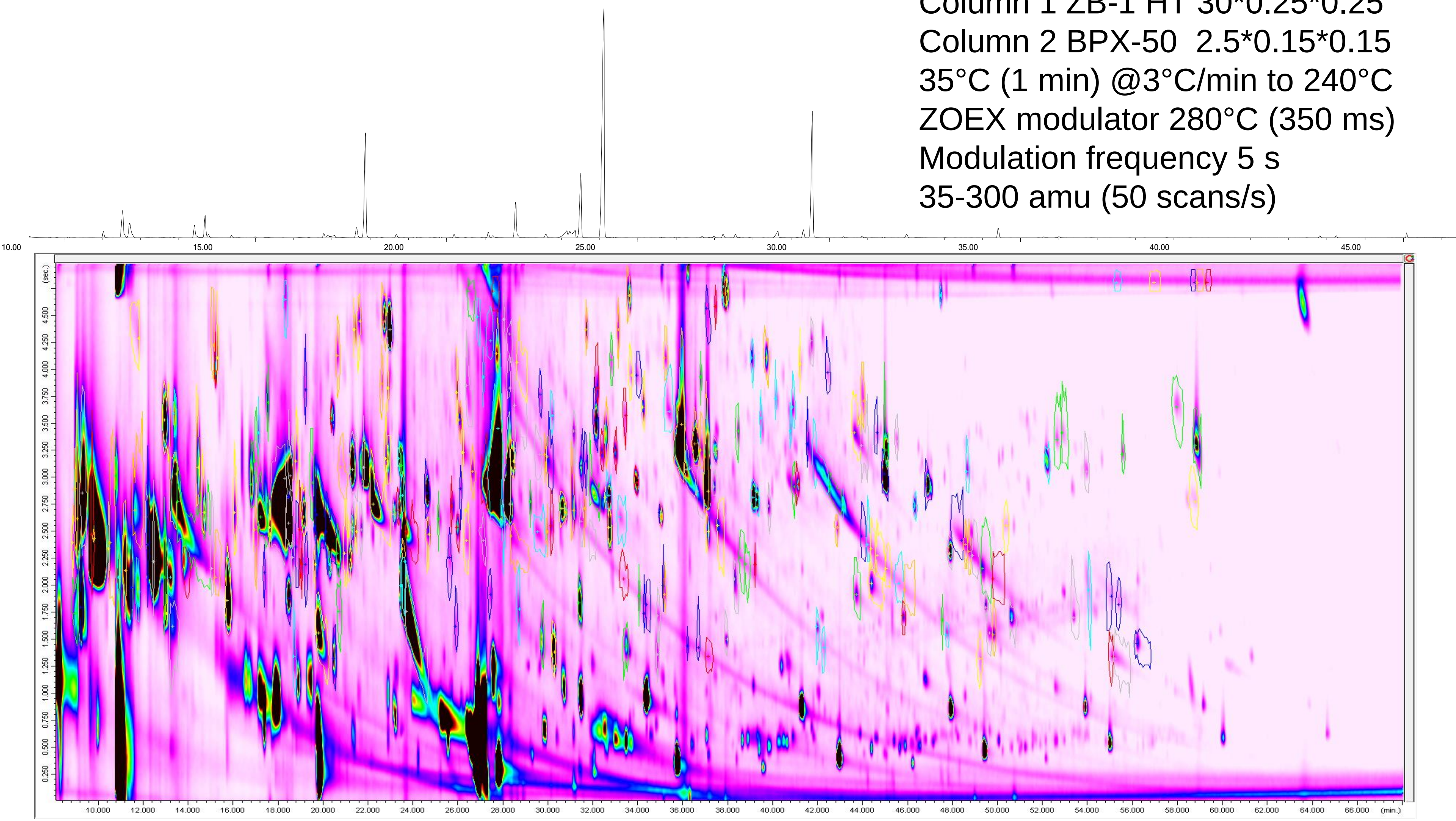
Statistical Analysis



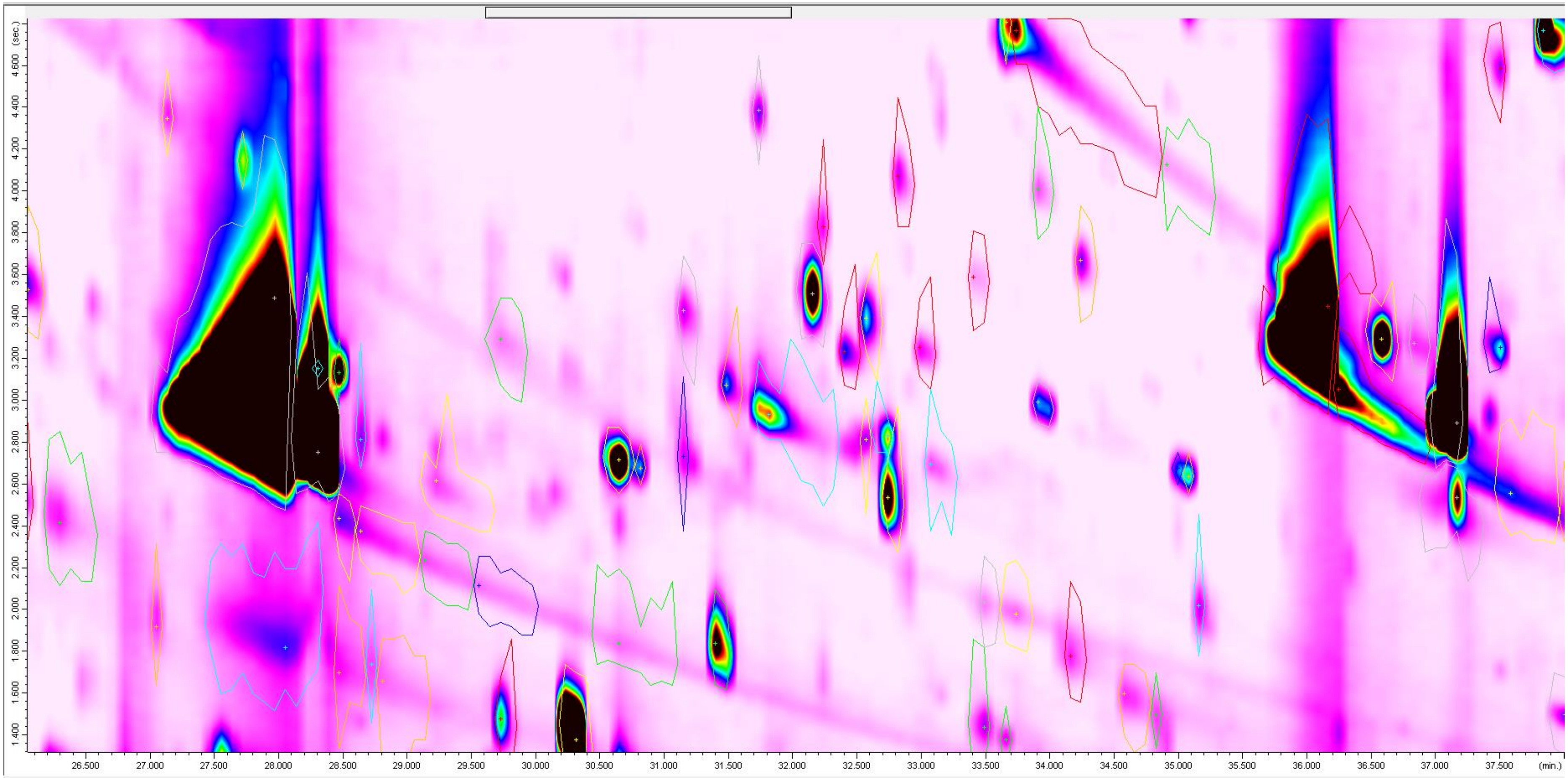
Statistical Analysis can be helpful in the differentiation of different grape varieties and/or vinification processes

Comprehensive GCxGC-MS

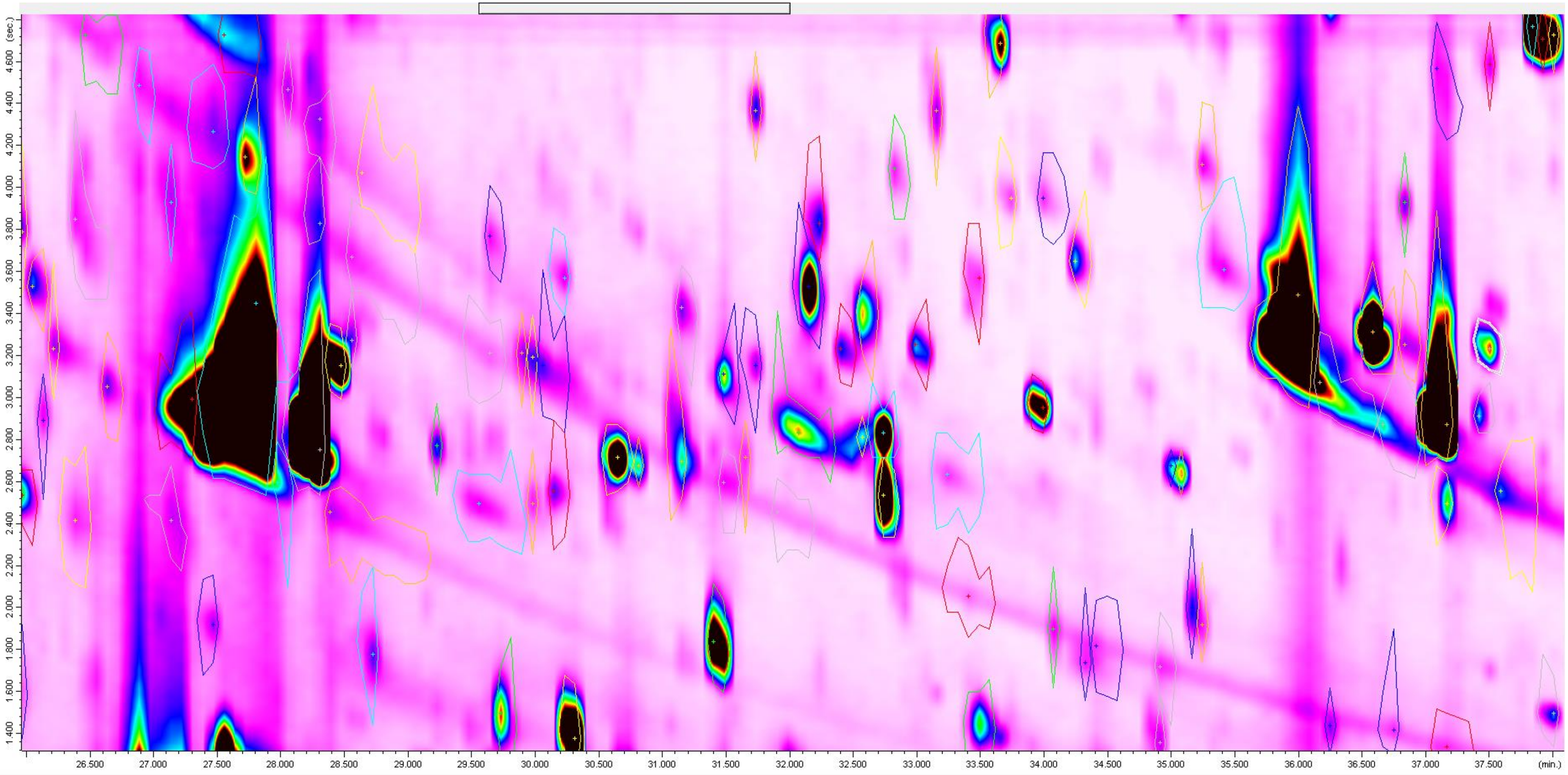
Shimadzu QP2010 plus
Column 1 ZB-1 HT 30*0.25*0.25
Column 2 BPX-50 2.5*0.15*0.15
35°C (1 min) @3°C/min to 240°C
ZOEX modulator 280°C (350 ms)
Modulation frequency 5 s
35-300 amu (50 scans/s)



Chardonnay Producer 1 2011



Chardonnay Producer 2 2014



The GC Methods

Tomorrow the following methods will be tested on different wines.
These wines will be also tested in the sensory application this afternoon)

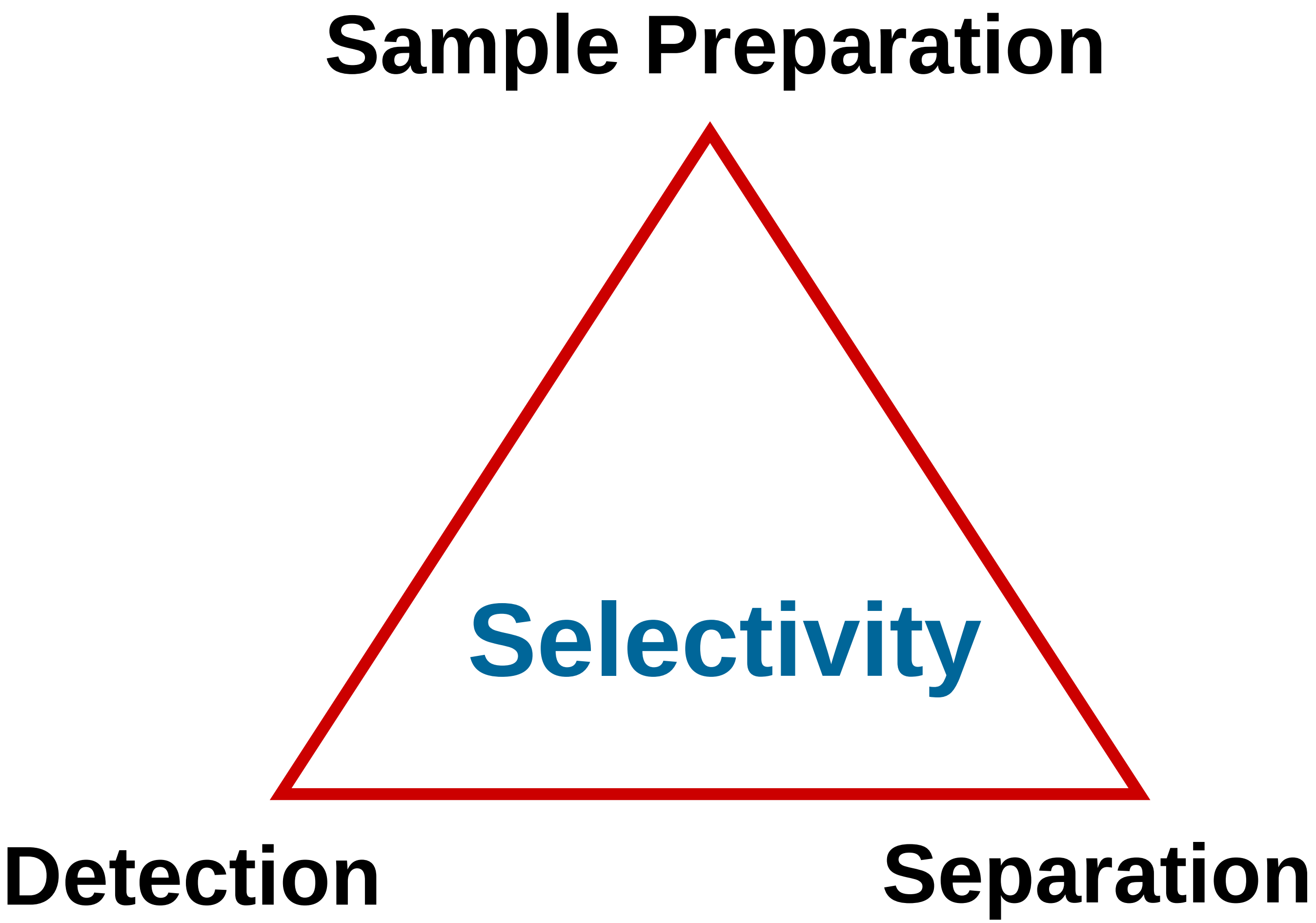
- Aroma profiles (GC-MS Scan)

- Detailed profiles (GCxGC-MS Scan)

- IBMP (GC-MS/MS) and some other „smelly“ compounds
- Linalool and degradation products (GC-MS/MS or GC-MS)

- „Norisoprenoids“ (Rose oxide, Vitispirane, TDN, β–Damascenone) GC-MS/MS

Fine Tuning your Analysis



Sample Preparation

Sample preparation for all methods is based on headspace techniques (SPME and/or Arrows)

WHY????

Just looking at the volatile fraction, so interfering and „harmful“ non-volatiles from the matrix (sugar, salts…) are not injected on the GC system

Increase in sensitivity

Drastically reduced sample preparation time and sample handling

Example Determination of a Target Compound at 1 ng/L

Conventional liquid-liquid extraction:

100 mL wine extracted three times with dichloromethane, combined and reduced to 0.1 mL (enrichment factor 1000!)

Final concentration 1 ng/mL = 1 pg by using 1 µL injection volume

Time consuming

Loss or cross contamination

SPME based methods:

1 mL in the head space vial = 1 pg on the fibre (theoretically)

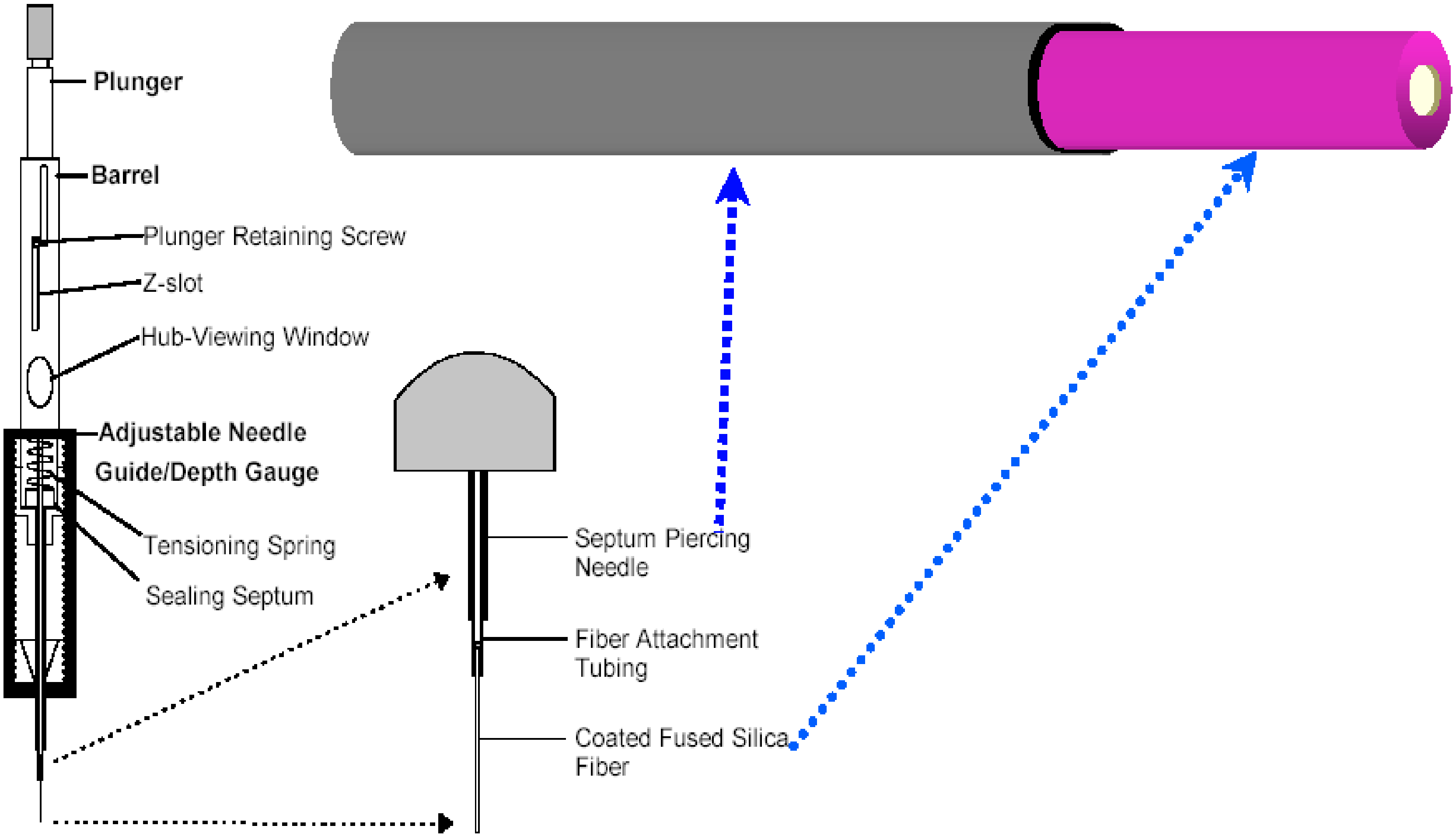
NO solvent, NO waste

NO non volatiles

NO cross contamination from the solvent

Fast and simple

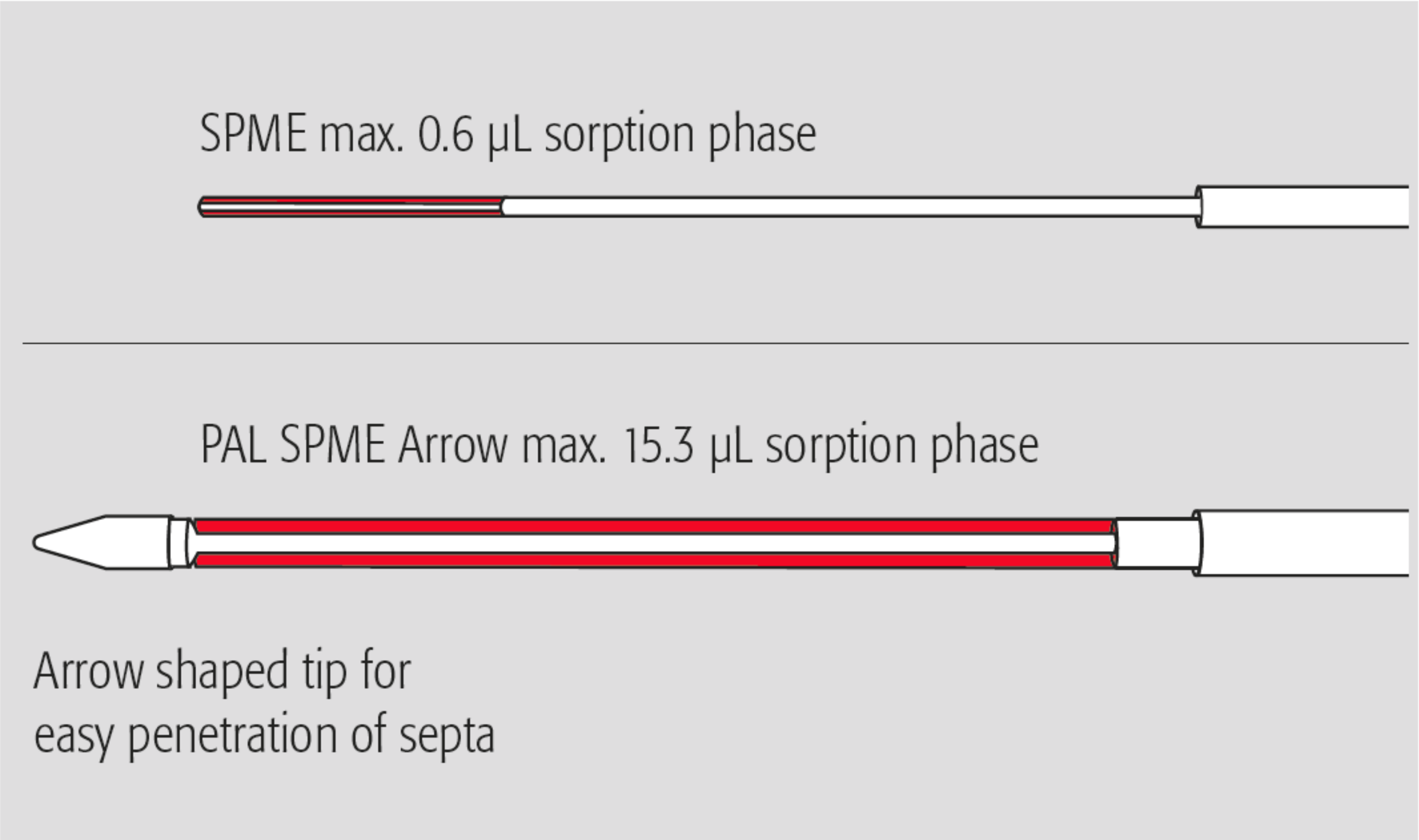
Solid Phase Microextraction



Arrows

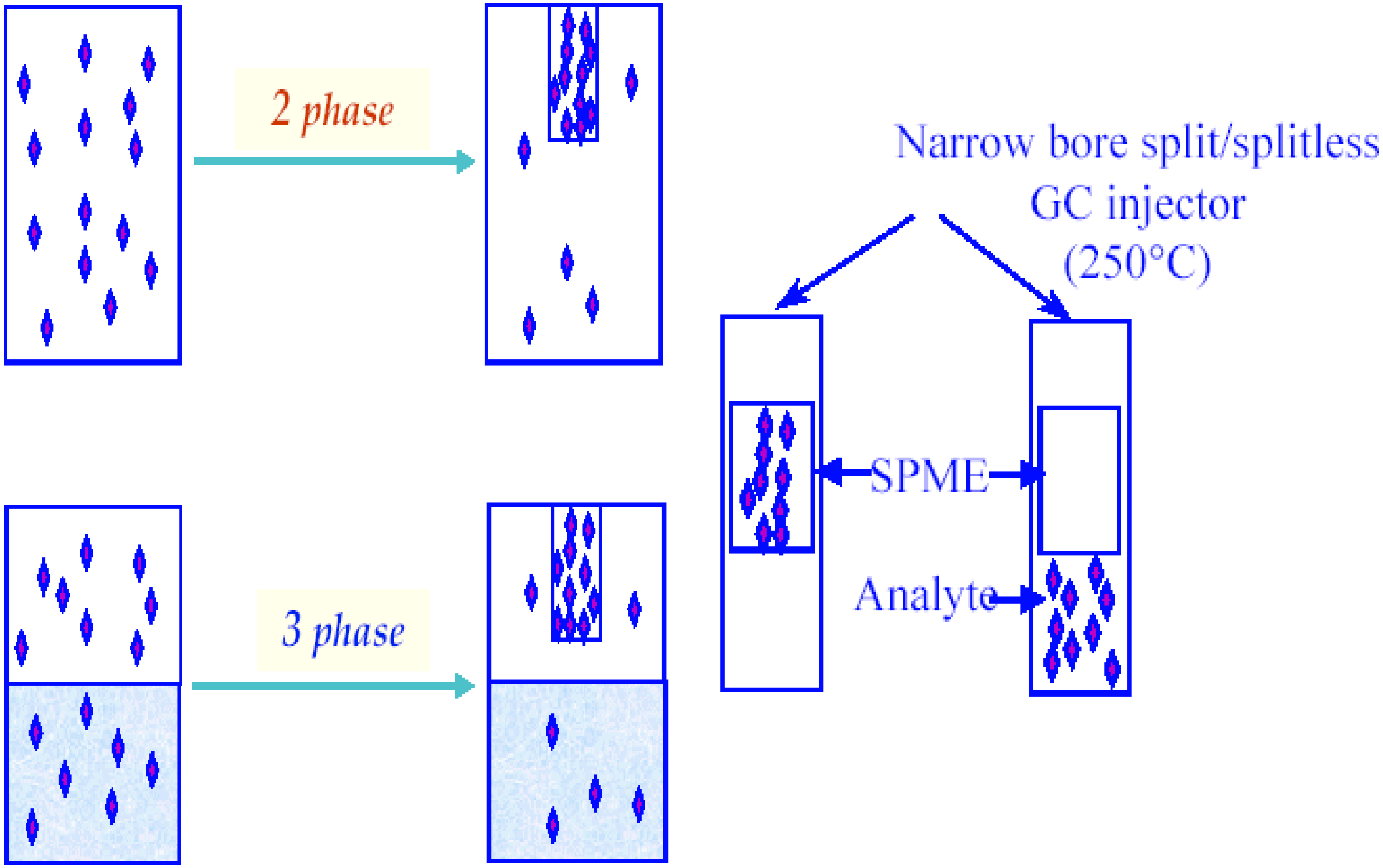
Advanced version of SPME. Available since 2016.

Requires modification and adaptation of autosampler



SPME-GC Sample Enrichment

+ polar comp.
- decreased lifetime



+ increased lifetime
+ only volatiles
+ NO system contamination
- decreased sensitivity

Sensory Thresholds of Relevant Aroma Compounds

Linalool	15 µg/L
TDN	2 µg/L
cis-Rose oxide	0.2 µg/L
b-Damascenone	0.05 µg/L
IBMP	0.001 µg/L

C13 Norisoprenoids

C13-norisoprenoids undergo acid-catalyzed reactions analogous to those observed with terpenoid compounds to form compounds that are not present in grapes or young wines.

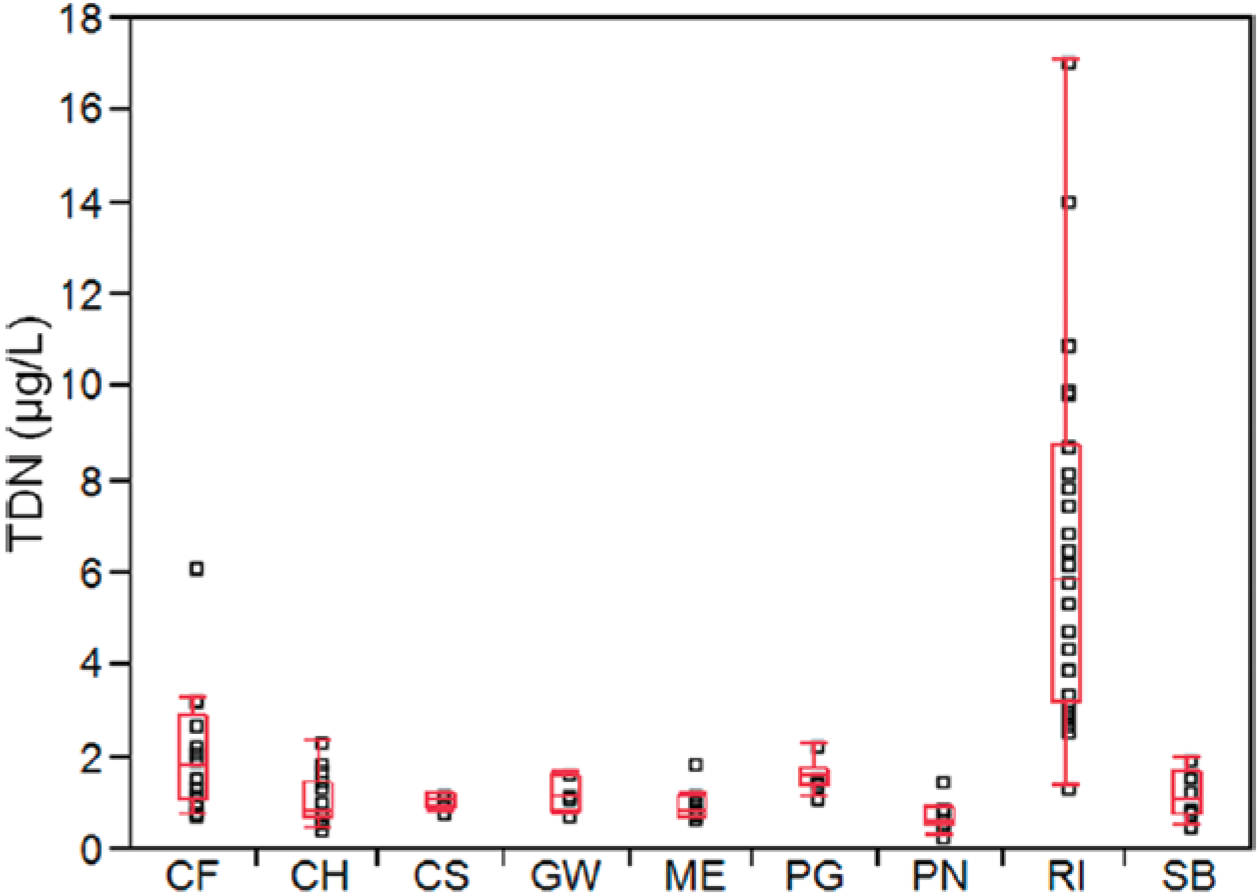
Important compounds:

isomers of vitispirane (eucalyptus odor)
theaspirane (tea odor)
damascenone (rose odor and exotic flower odor)
1,1,6-Trimethyldihydronaphthalene TDN (kerosene odor)

Norisoprenoid Method

Compound Name	Start Time (min)	End Time (min)	Acq. Mode	Event Time(sec)	Ch1 m/z	Ch1 CE	Ch2 m/z	Ch2 CE
Cis-Rosenoxid	9.20	11.20	MRM	0.200	139.00>69.0	20.00	139.00>139.	0.00
Vitispirane	14.20	15.30	Q3 SIM	0.030	136.00		177.00	
TDN	16.50	17.20	MRM	0.200	157.00>142.	15.00	157.00>141.	25.00
beta-Damascenon	17.21	18.00	MRM	0.200	121.00>105.	10.00	121.00>77.0	25.00
beta-Ionon	20.00	21.50	MRM	0.200	177.00>162.	15.00	177.00>147.	20.00

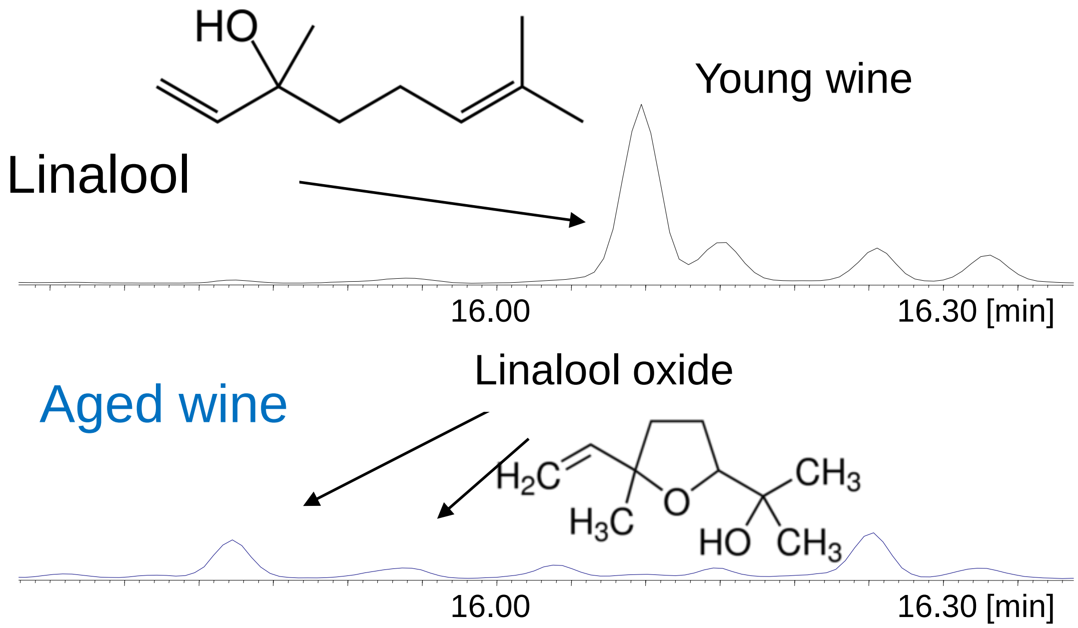
1,16-Trimethyldihydronaphthalene (TDN) in Different Wines



dx.doi.org/10.1021/jf205203b | *J. Agric. Food Chem.* 2012, 60, 2998–3004

Linalool Oxidation

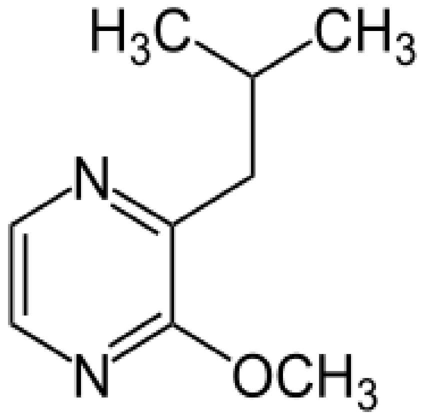
Sample Code	Linalool [µg/L]	OAV
1310	675±21	45
1376	552±12	37
1459	473±14	32
1381	464±9	31



Linalool sensory threshold (ST)	15 µg/L
cis-Linalool oxide ST	210 µg/L
trans-Linalool oxide ST	255 µg/L

Varietal Aroma

2-Isobutyl-3-methoxypyrazine



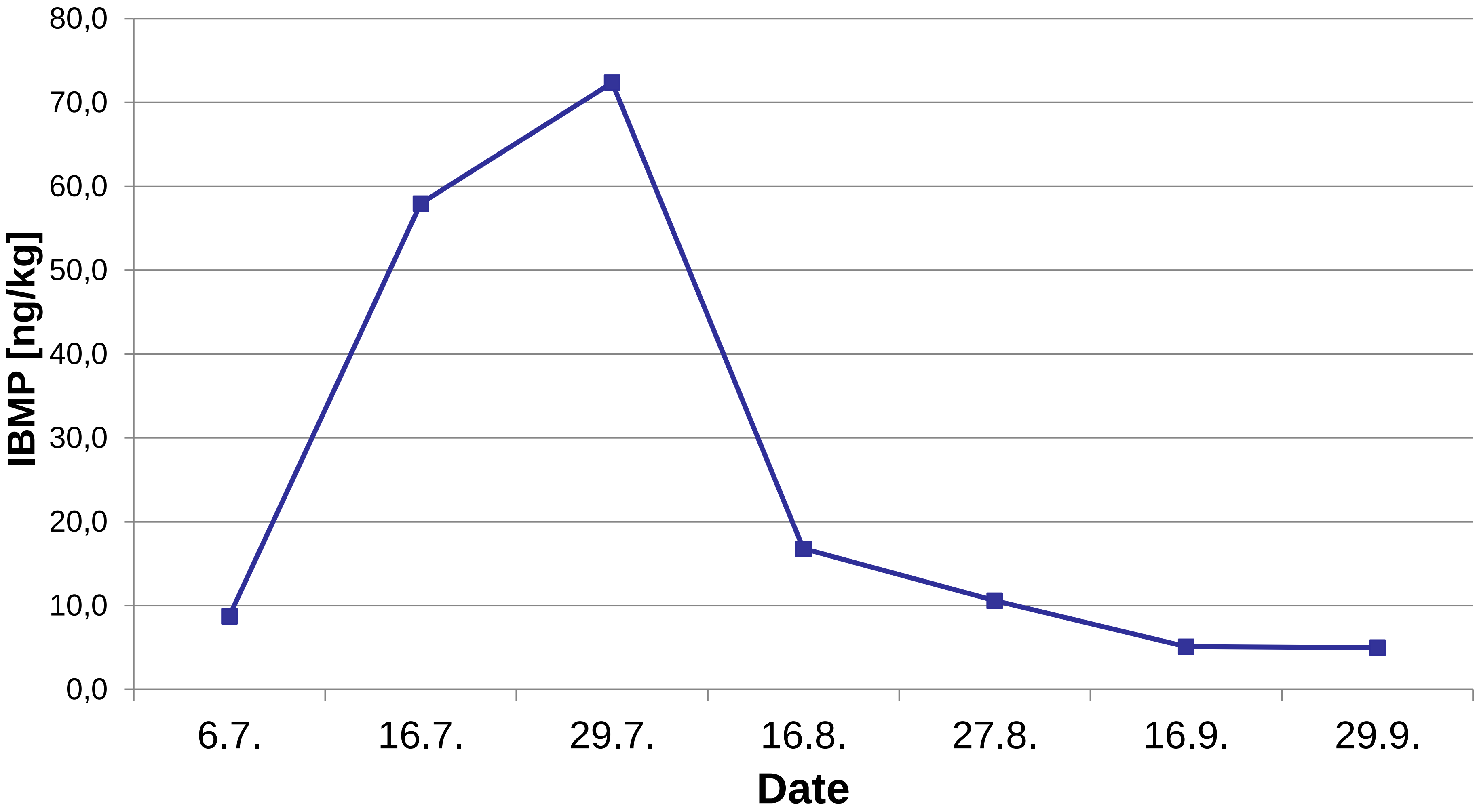
Initially identified in Cabernet Sauvignon grapes, also found in Cabernet Franc, Merlot and Sauvignon Blanc

Sensory threshold: 1-2 ng/L

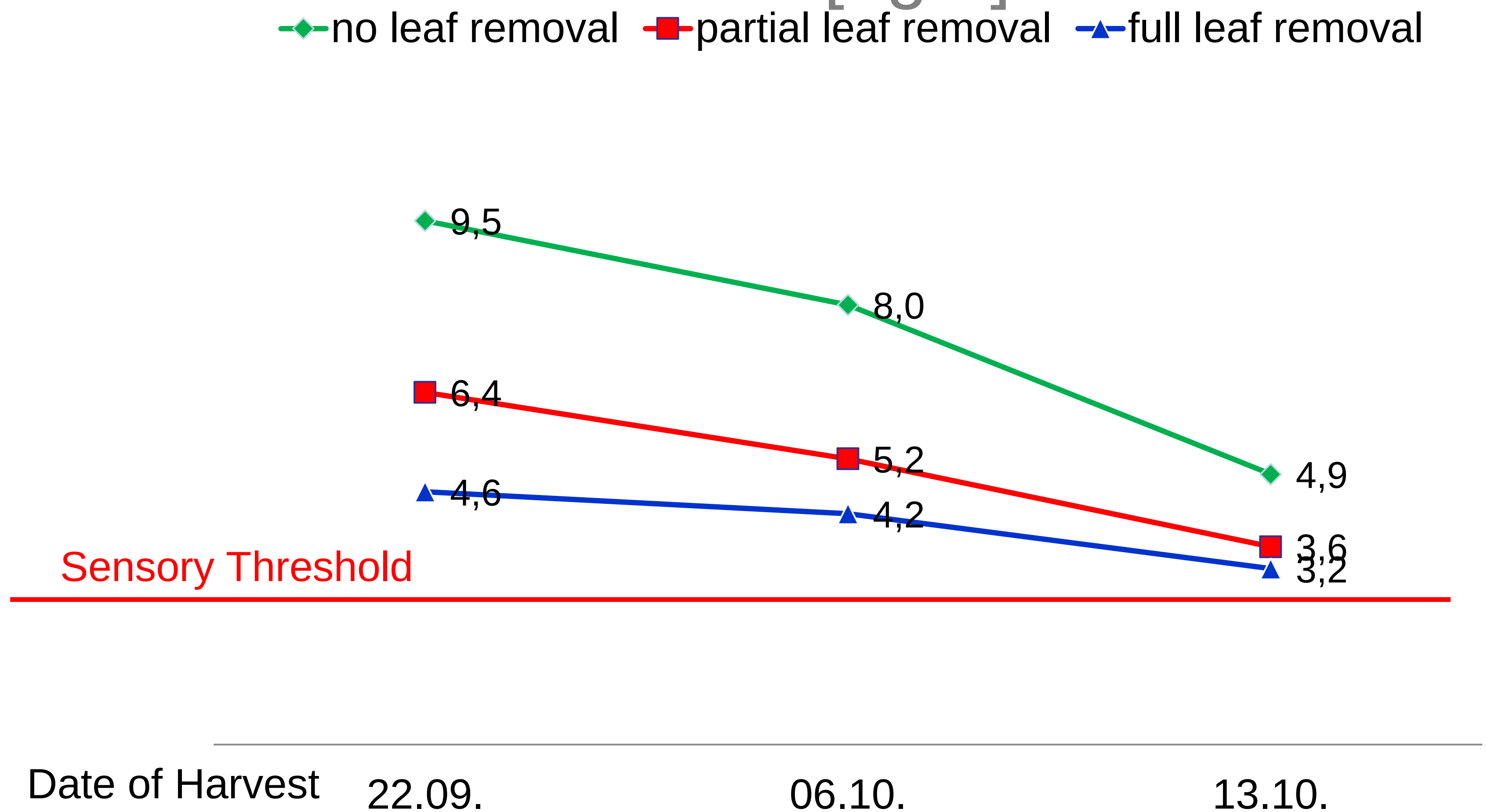
Odour descriptors: green bell pepper, green gooseberry, aparagus, vegetal

Survives the fermentation and is stable in the wine

Changes of IBMP during SB Grape Ripening



Isobutylmethoxypyrazine (IBMP) in the Grape Juice [ng/L]

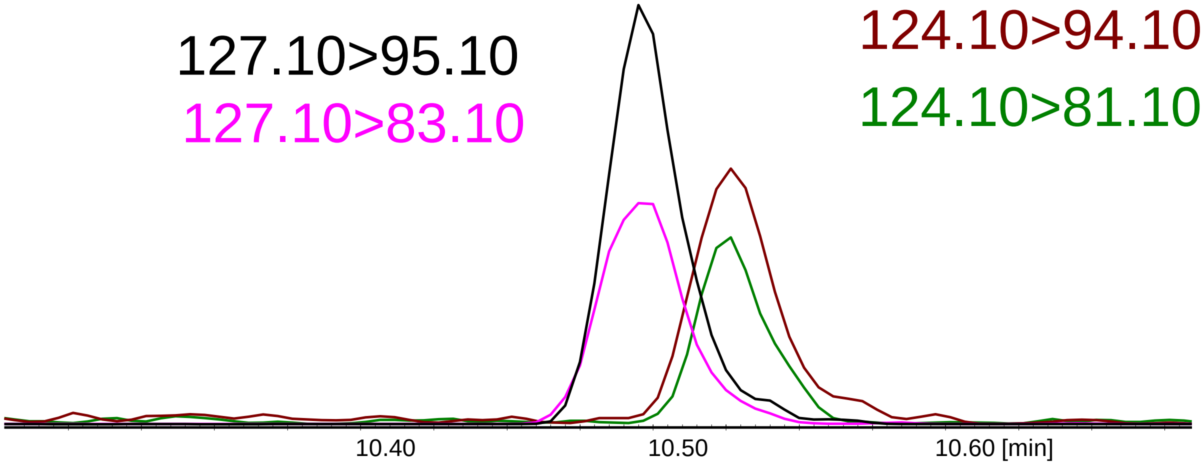


Leitner E., Renner W., 7th International Cool Climate Conference, Seattle WA, 2010

Quantification using MS/MS with Stable Isotope Dilution Analysis

IS IBMP (d3) 10 ng/L

IBMP 6.1 ng/L



Sample preparation: HS-SPME 1 mL, 50/30 µm DVB/Carboxen/PDMS 50°C, 30 min
Shimadzu TQ8040
Column: ZB-5 MS 30*0.25*0.25
Temperature programme: 40°C (1 min) @10°C to 290°C

Substance	Loop Time	Transition	CE	Transition	CE	Transition	CE	Q1 Res	Q2 Res
IBMP-d3	0.1	127.10>95.10	13	127.10>83.10	7	127.10>97.10	11	High	High
IBMP	0.1	124.10>94.10	11	124.10>81.10	7	124.10>79.10	23	High	High

The wines for the sensory and instrumental analysis

1. Sauvignon blanc
2. Gelber Muskateller
3. Mystery Wine (GCxGC and blind tasting)
4. Gewürztraminer
5. Riesling