

TWISTAROMA

Screening des composés volatils dans les matrices alimentaires par SBSE–GC–MS

Damien STEYER

Founder of TWISTAROMA

TWISTAROMA

- ▶ Founded in 2011
- ▶ Located in Colmar



Colmar



Introduction



« Spicy »

«Fruity »

« Floral »

« Sweet »



« Fatty, Rancid »

Introduction

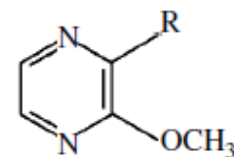
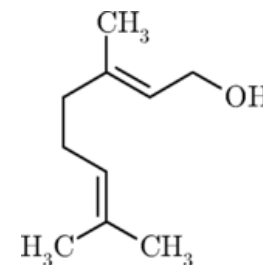
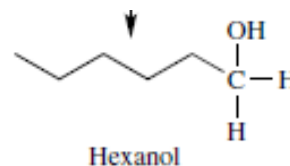
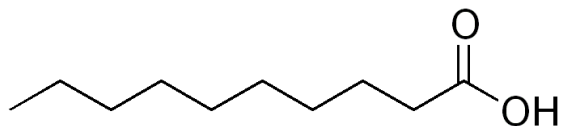
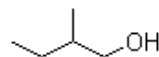
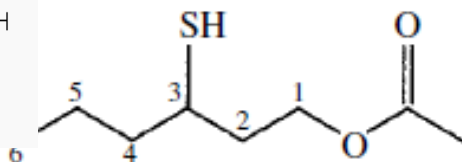
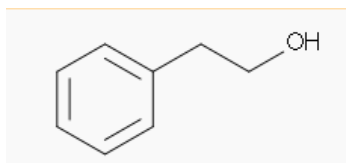


► Enology



Introduction

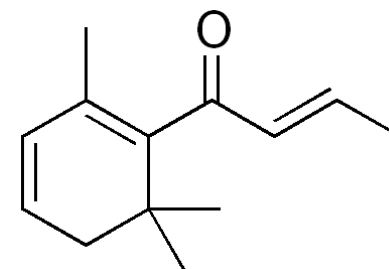
► Chemistry

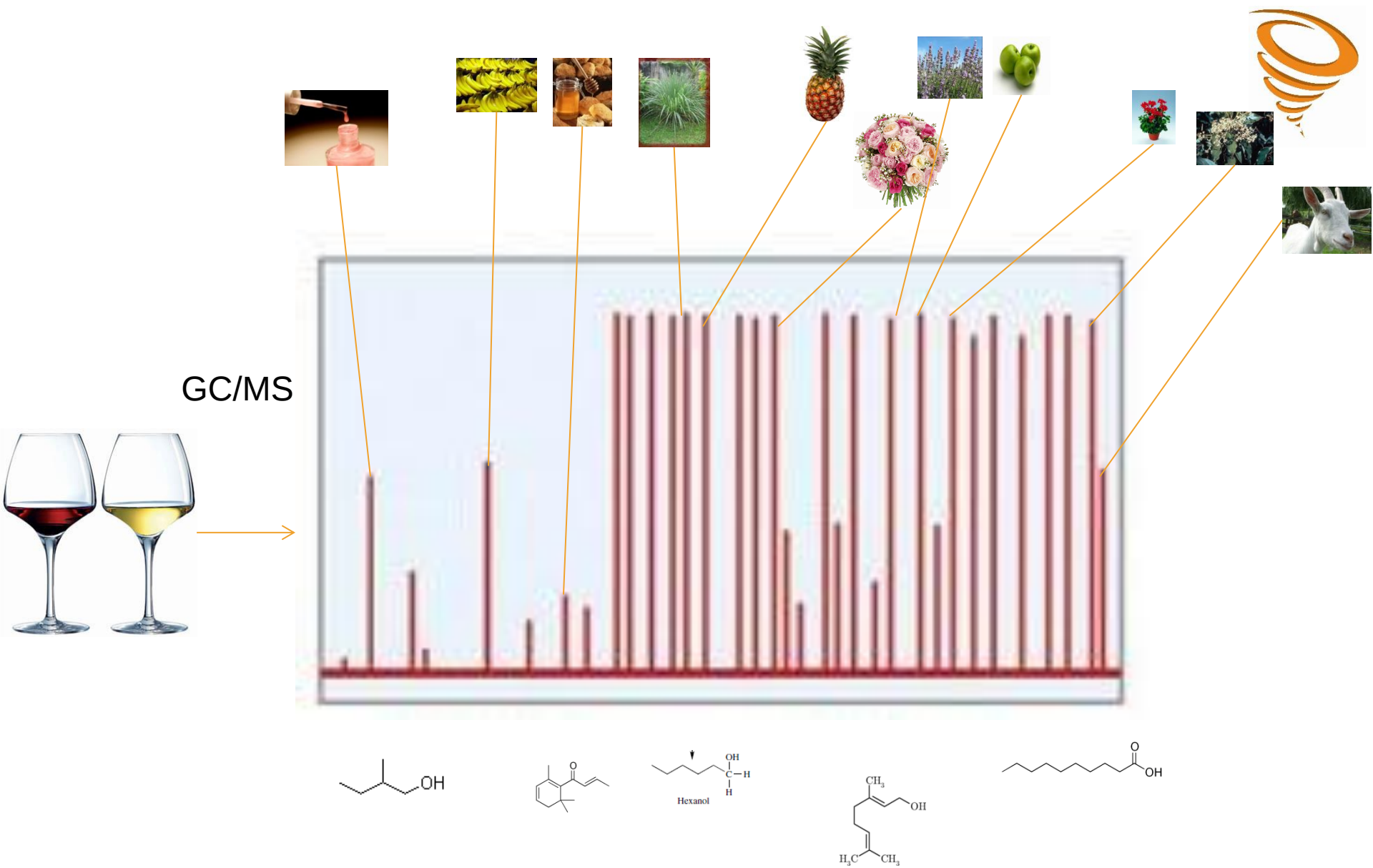


R: $\text{CH}_2\text{CH}(\text{CH}_3)_2$

R: $\text{CH}(\text{CH}_3)_2$

R: $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$

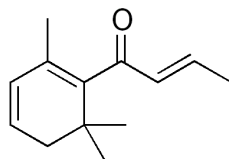




Grape compounds

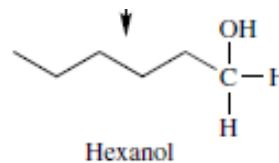


Grape volatile compounds



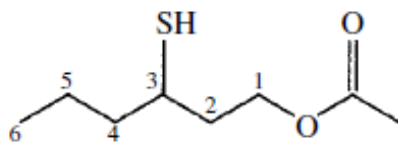
“baked apple”

Norisoprenoides



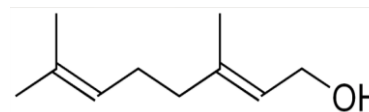
« Herbaceous »

C6 alcool



Volatiles thiols

« passion fruit »

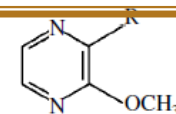


« Lychee »

Terpenols



Pyrazine



R: CH₂CH(CH₃)₂
R: CH(CH₃)₂
R: CH(CH₃)CH₂CH₃

« pepper »



Odor activity value (OAV)

$$\text{OAV} = \frac{\text{concentration}}{\text{Treshold}}$$

If $\text{OAV} > 1$ compound takes part of the global olfactory perception



In Alsace grape varieties are « Terpenic »



terpenols

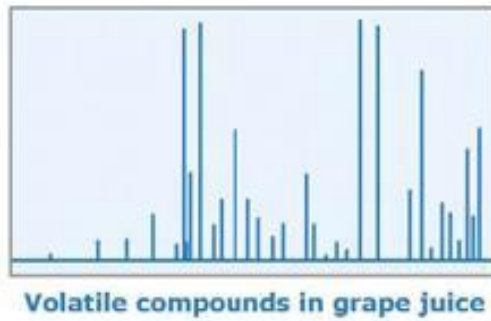
Compounds	OAV		perception
	Gewurztraminer	Muscat	
Géraniol	1,7¹⁻⁷	0,1 ^{1-3,6}	Rose, Lychee
Citronellol	ND ¹⁻¹²	0,66 ¹	Citronnella
Hotriénol	ND ^{1,2}	ND ¹	Linden (Tilleul)
A-terpinéol	0,2 ¹ -ND ²	0,1 ¹	Lily of the Valley
Nérol	0,3 ¹ -ND ²	0,1 ¹	Rose, Floral
Linalol	0,12 ¹⁻¹²	9¹⁻¹²	Lavender, grape



- 1) Ribéreau Gayon T1 ; 2) Guth et al; J. Agric. Food Chem. 1997, 45, 3027-3032
 3) Gamero et al; International Journal of Food Microbiology 145 (2011) 92-97



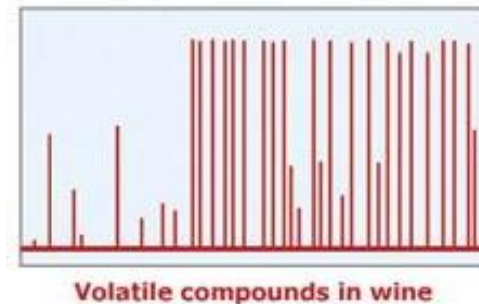
Volatile compounds



Volatile compounds in grape juice

≈ 40 volatile compounds

?

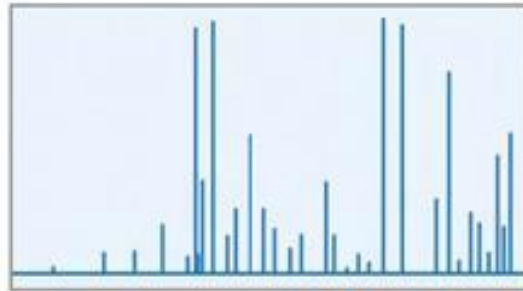


Volatile compounds in wine

More than 800 volatile compounds₁₁



Fermentation



Volatile compounds in grape juice



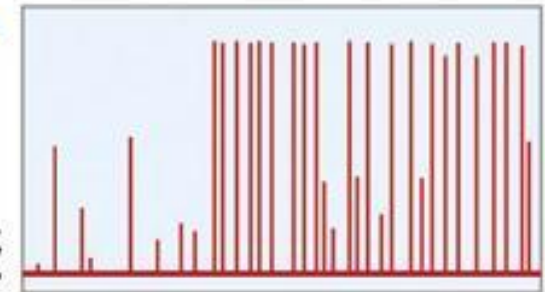
Saccharomyces cerevisiae



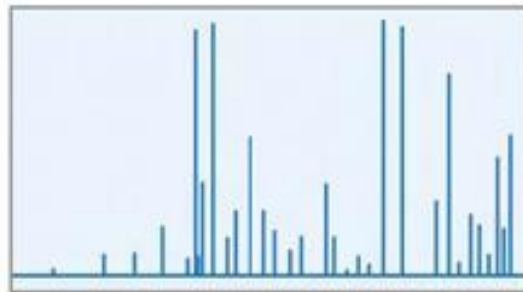
Oenococcus oeni



Which of these are the impact aroma and flavour compounds ?



Volatile compounds in wine



Volatile compounds in grape juice



Saccharomyces cerevisiae

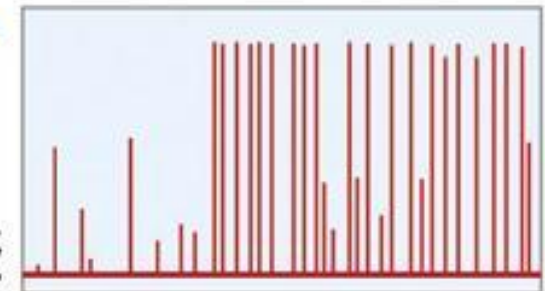


Fermentation

Oenococcus oeni



Which of these are the impact aroma and flavour compounds ?



Volatile compounds in wine



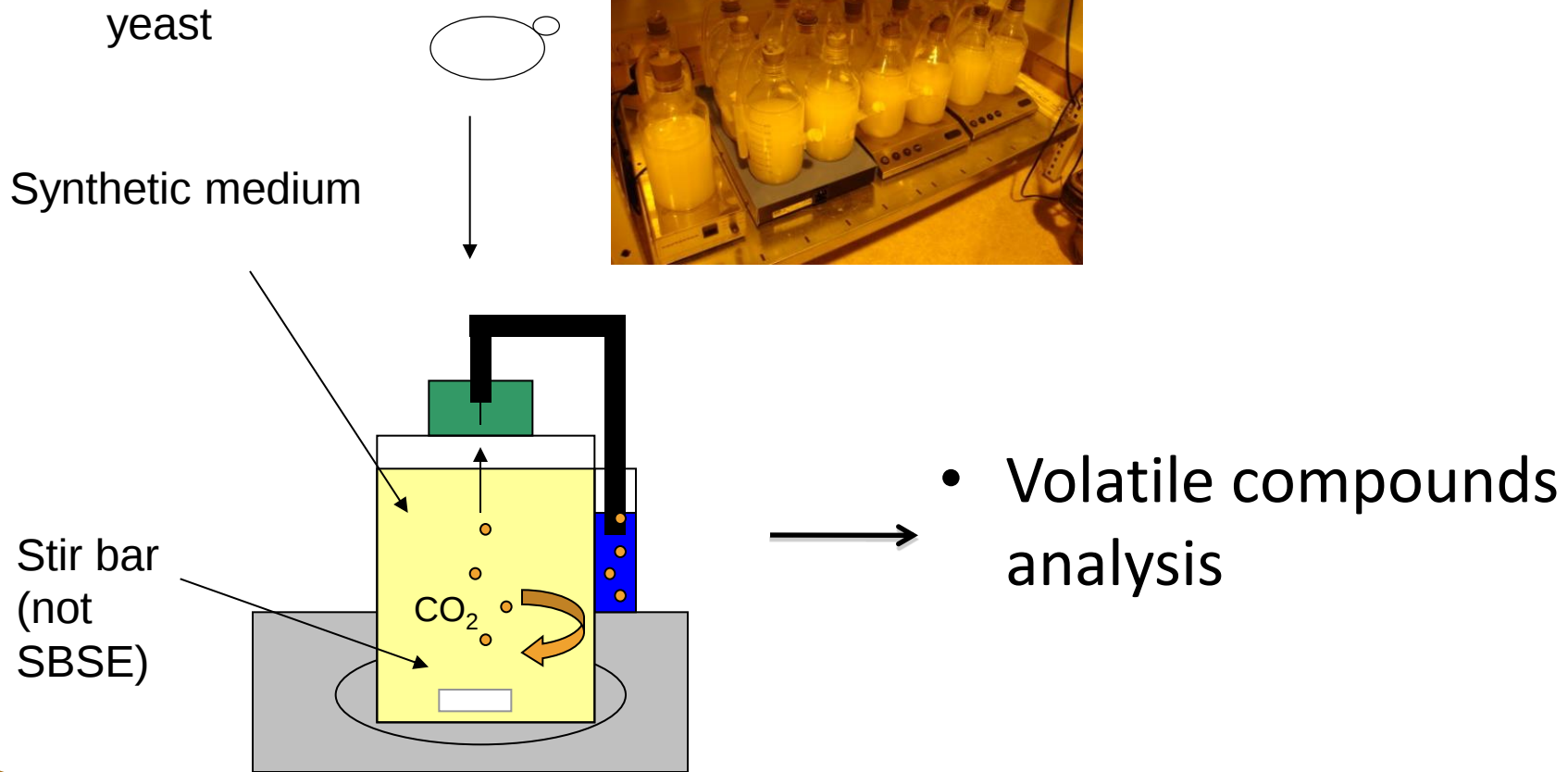


Yeast strains

En fonction de la région	
Alsace	
Levulia GE7	B10
Levuline ALS	EG8
Levuline D24	D24
Vitilevure 58W3	58W3
Bourgogne	
Lalvin CY3079	CY 3079
Levuline BRG	UP30Y5
Zymaflore VL2	VL2
Zymaflore X5	X5
Corse	
Equinox B1	B1
Côtes du Rhône	
Lalvin Rhône 2056	Rhône 2056
Lalvin Rhône 4600	Rhône 4600
Méditerranée	
ICV D47@	nc
ICV D254@	nc
ICV K1 marquée@	nc
Val de Loire	
Lalvin L905	L905
LB CXL	BCDL-CXL
En fonction des propriétés oenologiques particulières pour vins blancs et rosés	
Levures adaptées aux basses températures	
Anchor NT116	NT116
Anchor Vin13	Vin13
Fermol Cryaromae	PB 2007
IOC 18-2007	IOC 18-2007



How to study yeast strains ?



Bioconversion of geraniol by *S.cerevisiae*



Synthetic medium

[GERANIOL]=25mg/L



Fermented medium (≈wine)

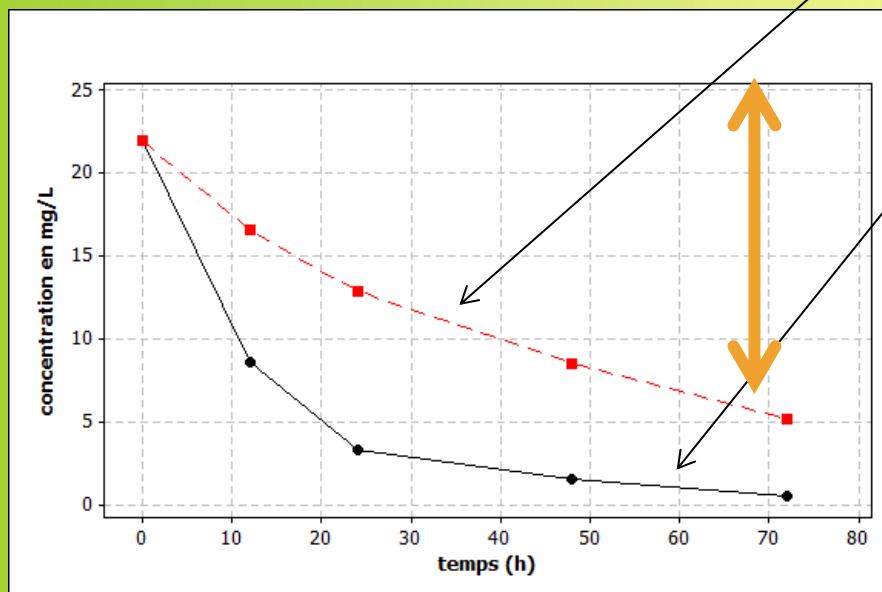
[CITRONELLOL]=1,85 mg/L

[LINALOL]=1,57 mg/L

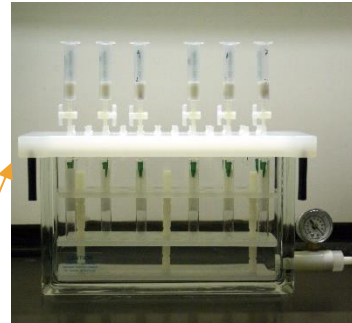
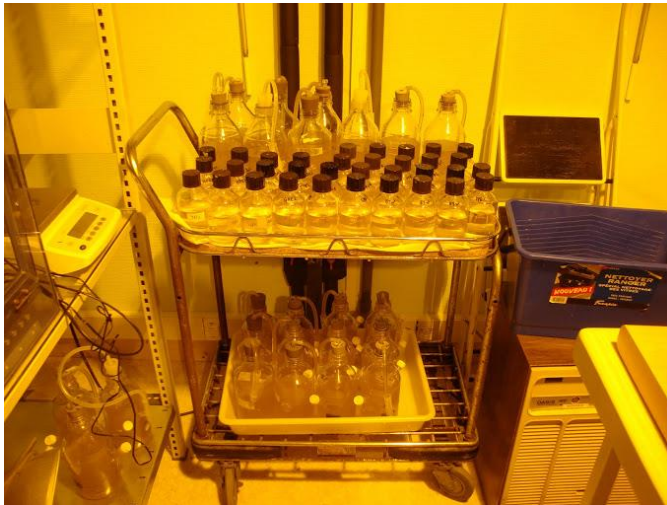
[α-TERPINEOL]=1,25mg/L

[GERANIOL]=0,5mg/L

**[missing]≈20mg/L
80% !**



Volatile compounds analysis in INRA Colmar before 2007 : extraction



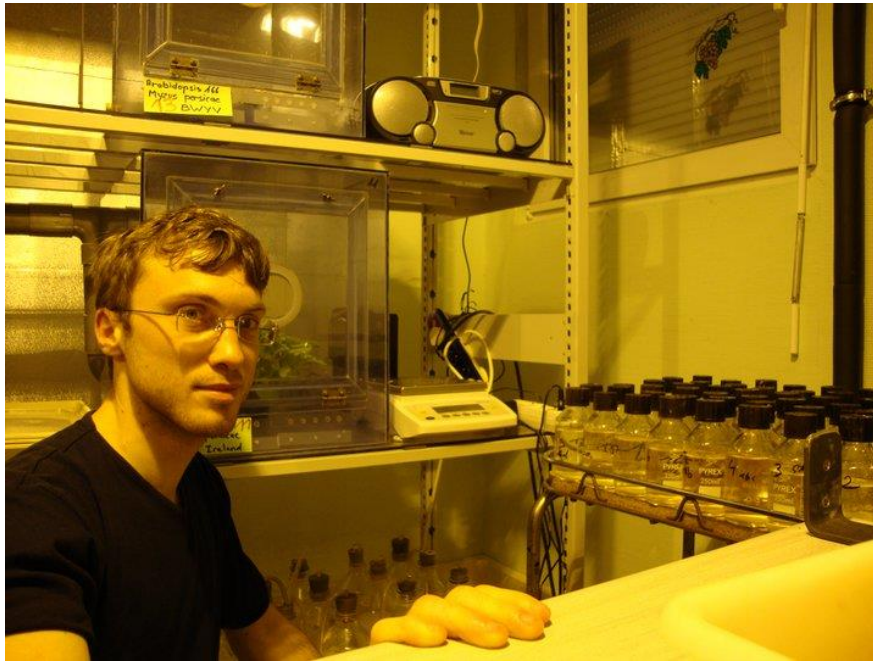
10-12 compounds

12 extractions : 4h

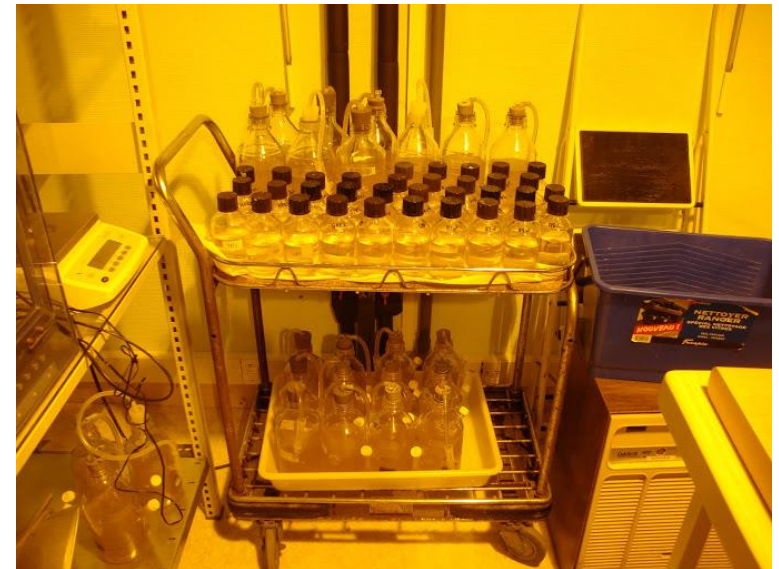
Volatile compounds analysis : separation/detection



SPE = impossible...



Experiment n°23 : 45 fermentations



Experiment n°27 : 60 fermentations

Volatile compounds analysis in INRA Colmar after 2007 : extraction



10-12 compounds

12 extractions : 4h



40 compounds

50 extractions : 45min

Calibration

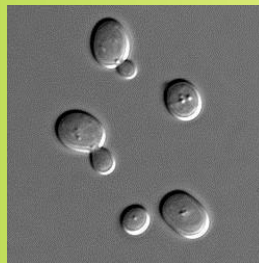


	Range (µg/L)	R ²	n	LOD (µg/L) (sb)	LOQ (µg/L) (sb)	RSD(%)
Esters						
Isoamylacétate	264–4 655	99,71%	15	119,2	361,1	5,28%
Ethyl C6	24–430	99,93%	15	5,2	15,7	2,48%
Ethyl C8	69–1215	99,84%	15	22,7	68,7	3,85%
Ethyl C10	226–3 990	97,31%	6	12,9	39,1	8,02%
Ethyl C12	24–415	95,90%	15	40,4	122,3	20,06%
Ethyl C14	43–760	86,87%	15	139,1	421,4	37,73%
Ethyl C16	13–230	80,62%	15	53,1	160,9	47,59%
Géranylacétate	31–550	99,66%	15	15,2	46,0	5,68%
Citronellylacétate	31–545	99,08%	15	24,7	75,0	9,36%
Phénylethylacétate	2 585–1 2927	99,18%	12	789,9	2393,7	5,79%
Acides gras						
Acide C6	255–4500	97,99%	15	303,7	920,3	13,91%
Acide C8	281–4950	99,55%	15	156,0	472,6	6,50%
Acide C10	320–5 650	98,47%	15	331,2	1003,7	12,09%
Alcools						
Décanol	24–425	99,07%	15	19,4	58,8	9,41%
Isobutanol	266–4 700	98,65%	15	258,7	784,0	11,35%
Isoamylalcool	8 869–14 9000	99,71%	15	3807,1	11536,6	5,22%
Hexanol	14–60	99,37%	9	3,9	11,8	3,85%
Phénylethanol	108,5–2 170	99,47%	15	0,1	0,4	9,39%
Monoterpénols						
α-phaterpinéol	32–565	99,91%	15	7,9	24,0	2,90%
Citronellol	22–385	99,51%	15	12,7	38,5	6,80%
Géranol	29–515	99,29%	15	10,5	32,1	8,20%
Linalol	27–485	99,79%	15	10,5	31,7	4,44%
Nérol	26–455	99,40%	15	16,6	50,4	7,53%
Sesquiterpénols						
Farnésol	9–40	96,82%	15	5,9	17,8	8,40%
Nérolidol	22–87	94,31%	15	17,6	53,4	14,89%
Other						
Beta-damascénone	0,814–4,280	99,60%	15	0,21	0,65	4,65%
Rose oxyde	0,230–3,640	99,36%	15	0,17	0,50	7,89%

Terpenyl acetates

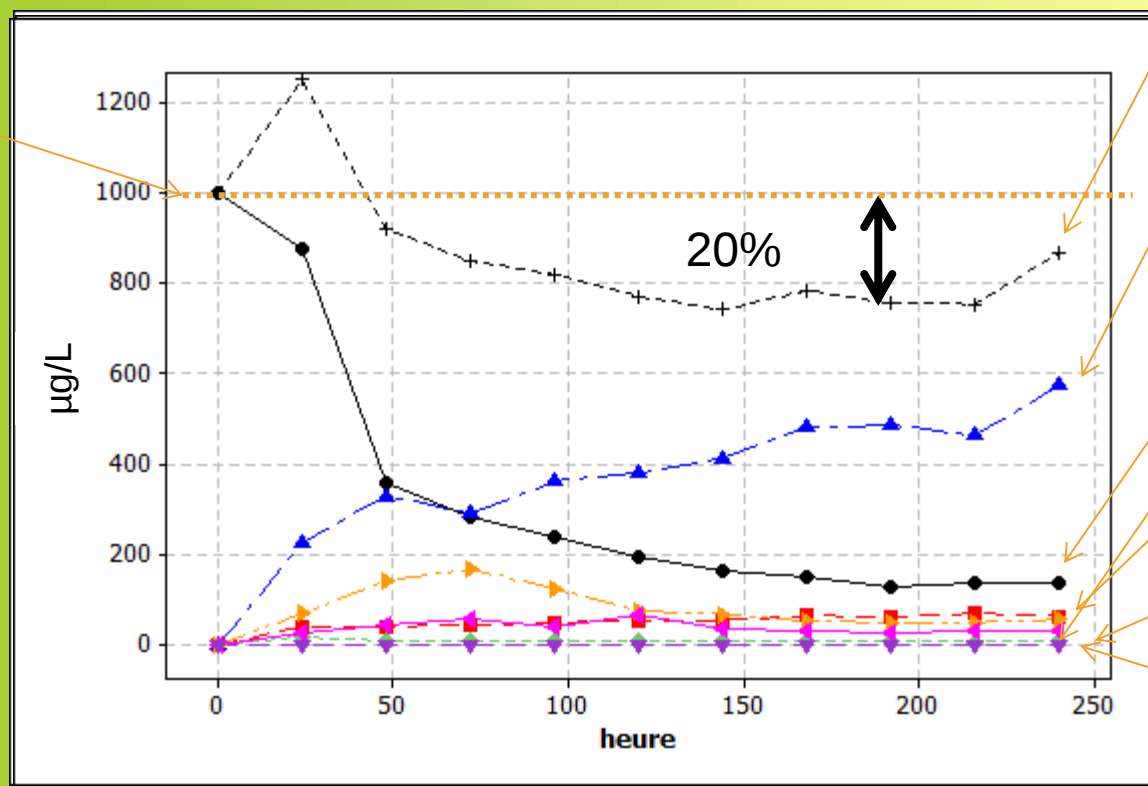


Synthetic must



wine

Geraniol



Sum

Citronellol

Linalol

Nerol

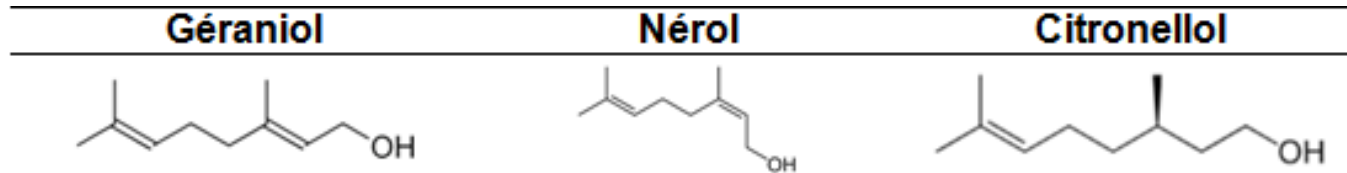
Geranylacetate

Citronellylacetate

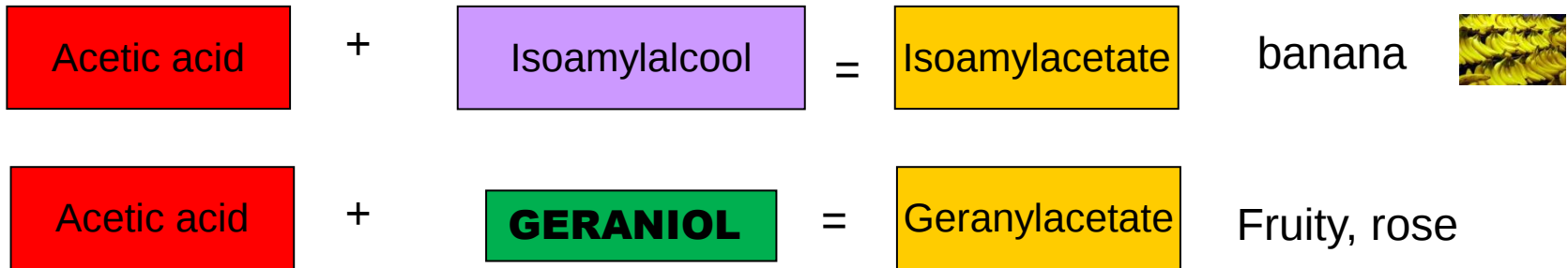
Nerylacetate



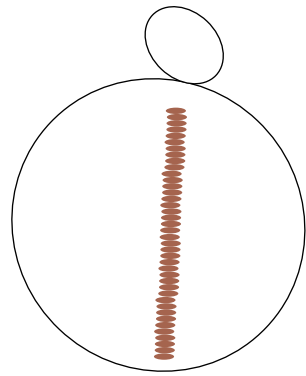
Terpenyl acetates



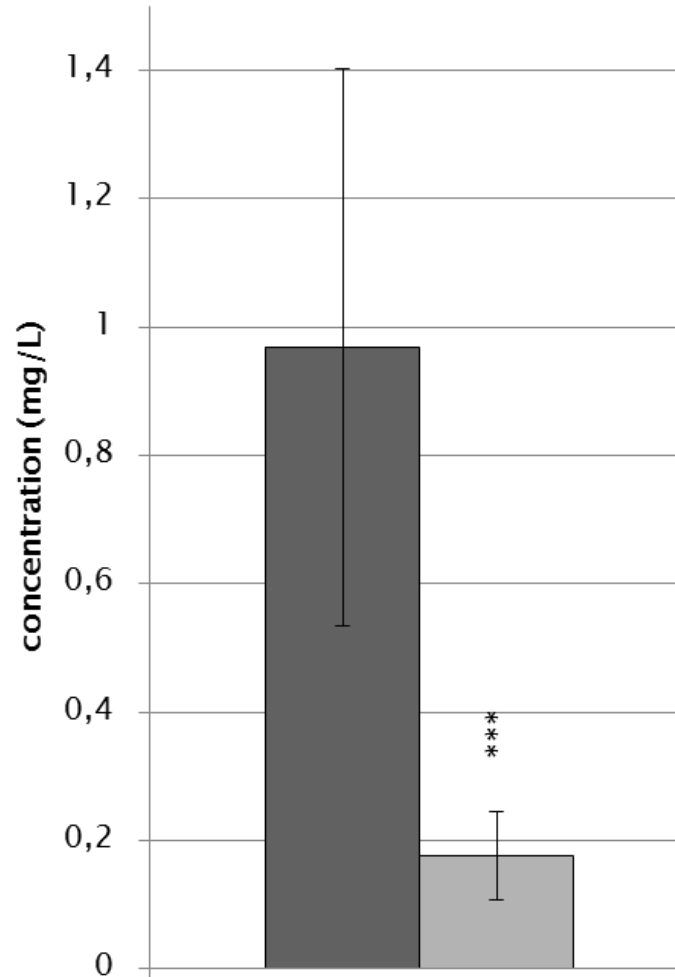
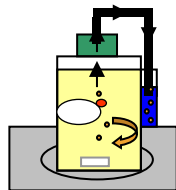
ATF1/ATF2



ATF1p is involved in esterification of terpenols



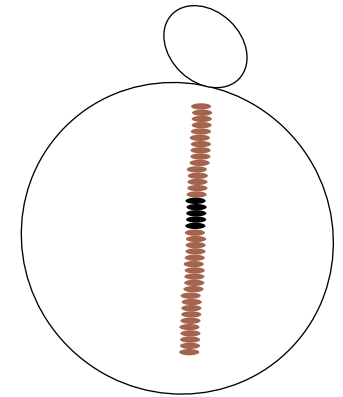
Wild type strain



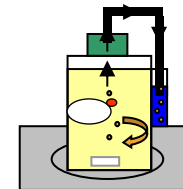
somme terpényles acétates

wt

$\Delta ATF1$

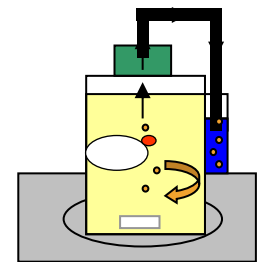
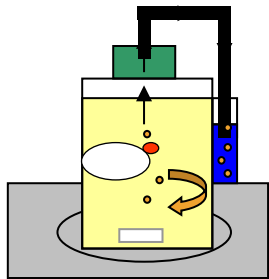
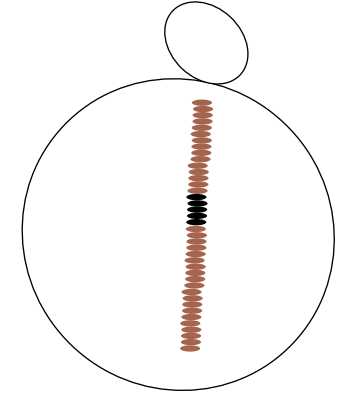
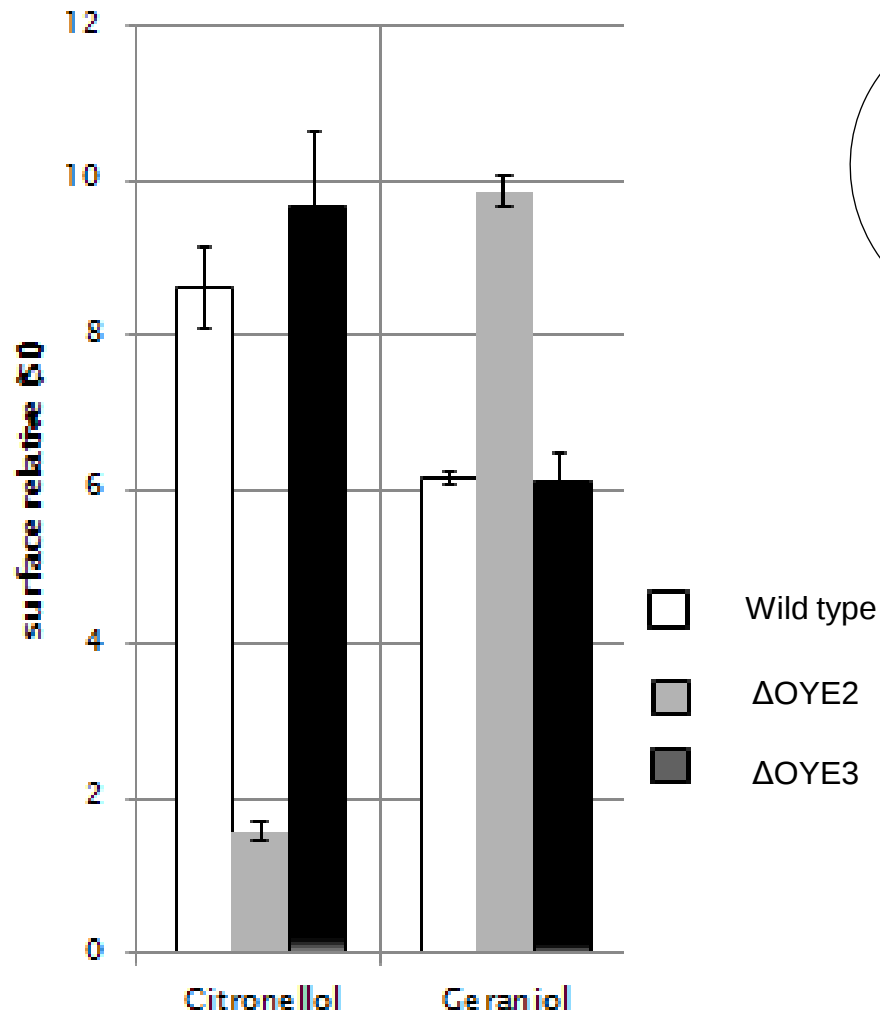
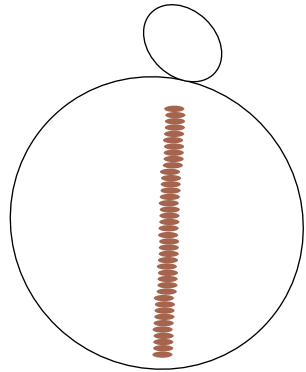


$\Delta ATF1$ strain





OYE2p is a geraniol reductase





Conclusion

- ▶ Yeast strain selection
 - Alsatian wines need yeast with low ester synthase activity and low OYE2 activity to keep free terpenols
- ▶ SBSE-LD is :
 - A powerful tool to study wine volatile compounds
 - High repeatability
 - Sensible and useful for untargeted volatile compounds analysis (more than 40 compounds are detected in wine).
 - Cheap : since 2007 : 4000 extractions with 50 stir bars

Next ?



Applications



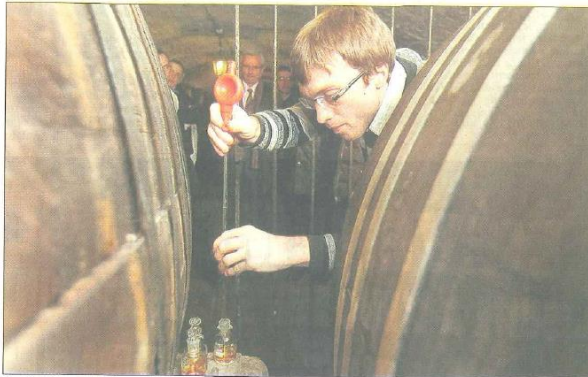
- ▶ Wine analysis from the « caves des hospices de Strasbourg » 1472 « vintage »

Viticulture Le plus vieux vin blanc du monde en tonneau mis à nu

L'analyse organoleptique d'un vin de 1472 issu de la cave des hospices de Strasbourg dévoile l'incroyable diversité de ses composés aromatiques.

La cave des hospices de Strasbourg attire des centaines de visiteurs chaque année, y compris des œnologues du monde entier fébriles à l'idée de déguster un vin de 1472... Il s'agit tout simplement du « plus vieux vin blanc en tonneau du monde » ! Ne demandez pas à Philippe Junger, responsable de la cave, de quoi sont constitués ces 300 litres de vin (30 hl historiquement) ouillés chaque année. Du cépage, de ses origines géographiques, on ne sait rien.

En revanche, on n'en sait désor-



Damien Steyer lors des prélèvements effectués en décembre dernier, à Strasbourg.

Photo J.-M. L.





Other matrix ?

Characterization of the terpenoids composition of beers made with the French hop varieties: Strisselspalt, Aramis, Triskel and Bouclier

Among the various compounds brought in to beer by hop, mono- and sesqui- terpenoids are the most studied and contribute to a certain extent to the hoppy aroma of beer. Previous studies have indicated that the concentration of these compounds in beer depends on the hop variety. The impact of new hop varieties created by the French hop growers association and their cooperative in comparison to the old variety Strisselspalt on the terpenoid content in beer has been analyzed by Stir-Bar Sorptive-Extraction-Gas-Chromatography (SBSE-GC-MS). Our results reveal that the new variety Aramis has a similar terpenoid profile to the Strisselspalt variety, both showing a similar content of sesquiterpenoids. In comparison, the new variety Bouclier was rather low in sesquiterpenoid content whereas Triskel showed the highest concentration of monoterpenoids, especially linalool.

Descriptors: hop, variety, volatile compounds, terpenoids, aroma

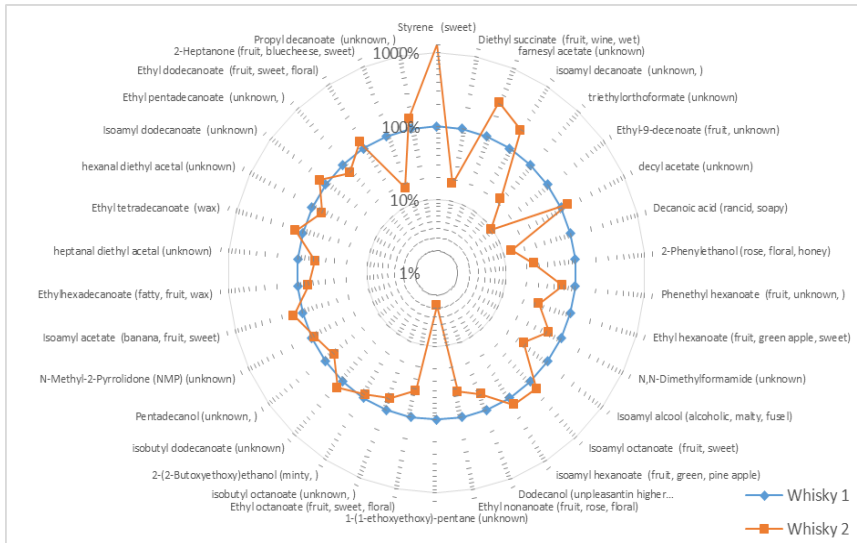
44 volatile compounds in beer

Yeast–yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts

Mohand Sadoudi ^a, Raphaëlle Tourdot-Maréchal ^a, Sandrine Rousseaux ^a, Damien Steyer ^d, Joan-Josep Gallardo-Chacón ^a, Jordi Ballester ^c, Stefania Vichi ^e, Rémi Guérin-Schneider ^b, Josep Caixach ^e, Hervé Alexandre ^{a,*}

41 volatile compounds in wine

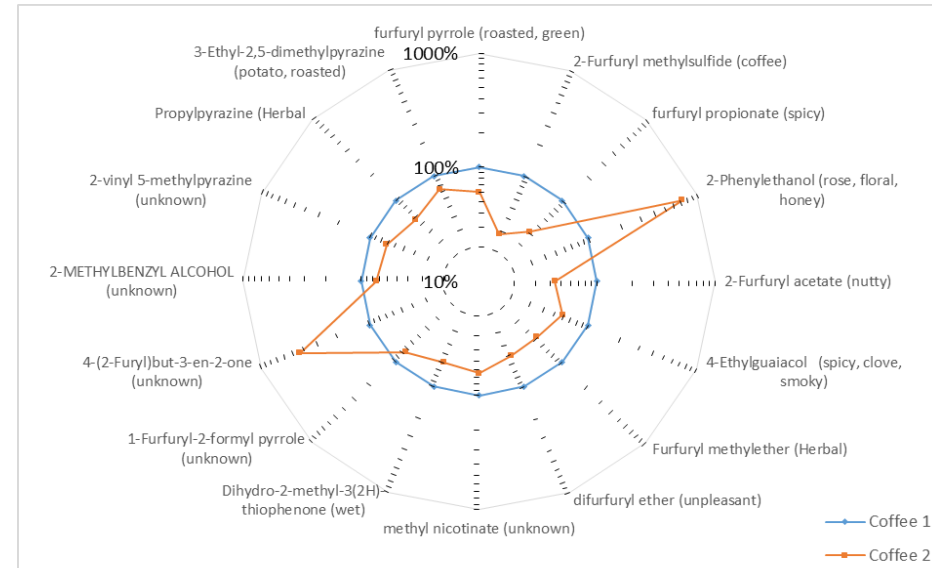
Comparison of matrix



Whisky : 57 compounds

Cider : 37 compounds

Apple : 47 compounds



Coffee : 59 compounds

Tea : 29 compounds

Vinegar : 27 compounds

Next



2007 INRA Colmar : Liquid
desorption



2015 TWISTAROMA :
Thermodesorption !!

First results < 100 compounds in
Champagne

Thank you for your attention

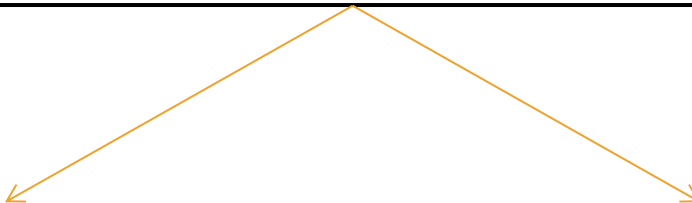
Damien Steyer
Céline Clayeux
Clémence Gros



Annexes

Cartographie des cépages alsaciens

Cave	Cépage							Total par cave
	Muscat	Pinot noir	Riesling	Gewurztraminer	Pinot gris	Pinot blanc	Sylvaner	
Turckheim	1	2	5	5	3	5	3	24
Cleebourg	1	1	1	2	3	1	1	10
Traenheim	1	1	2	2	1	1	1	9
INRA	1	1	1	1	1	1	1	7
Total par cépage	4	5	9	10	8	8	6	50



Les marqueurs
olfactifs

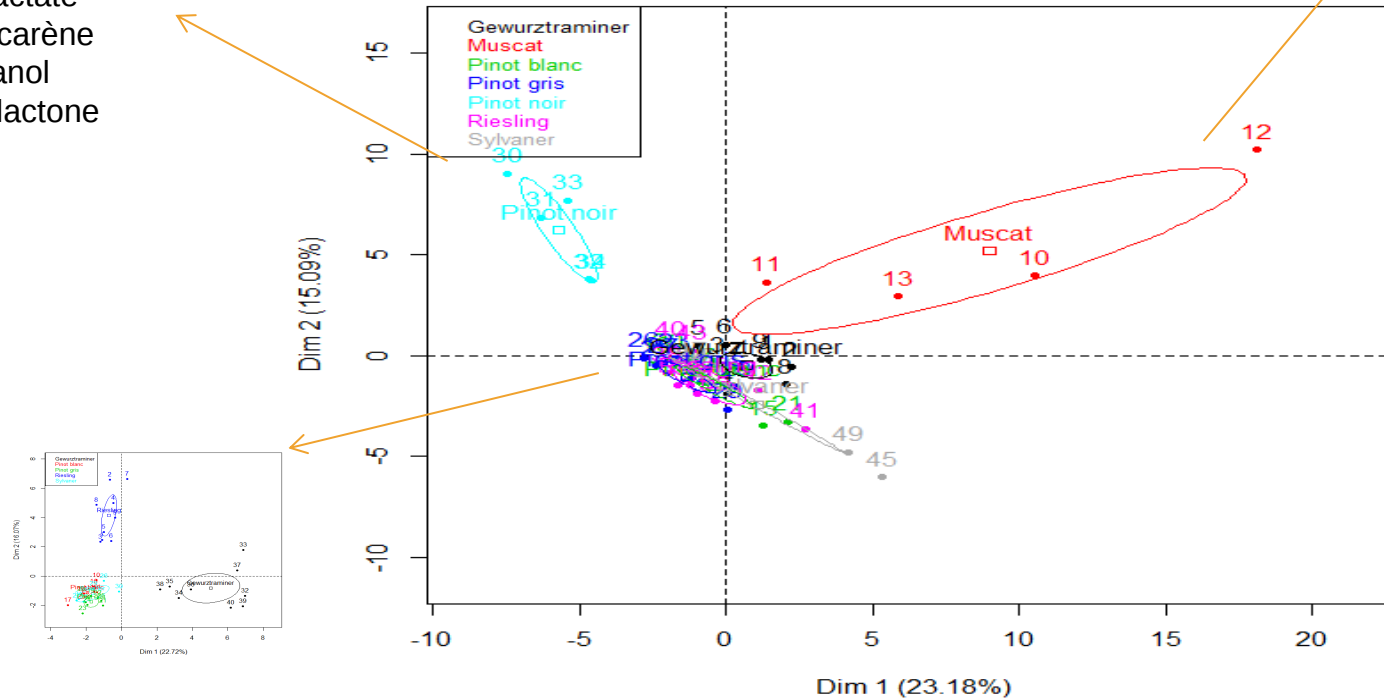
Les marqueurs
phénoliques

Les marqueurs olfactifs



benzyl alcool
methyl salicylate
ethyl lactate
elta 3-carène
2-nonanol
butyralactone

linalol oxide
neryl acétate
geranyl acétate
herboxide
ho-diendiol I
2-Ethyl-1-hexanol



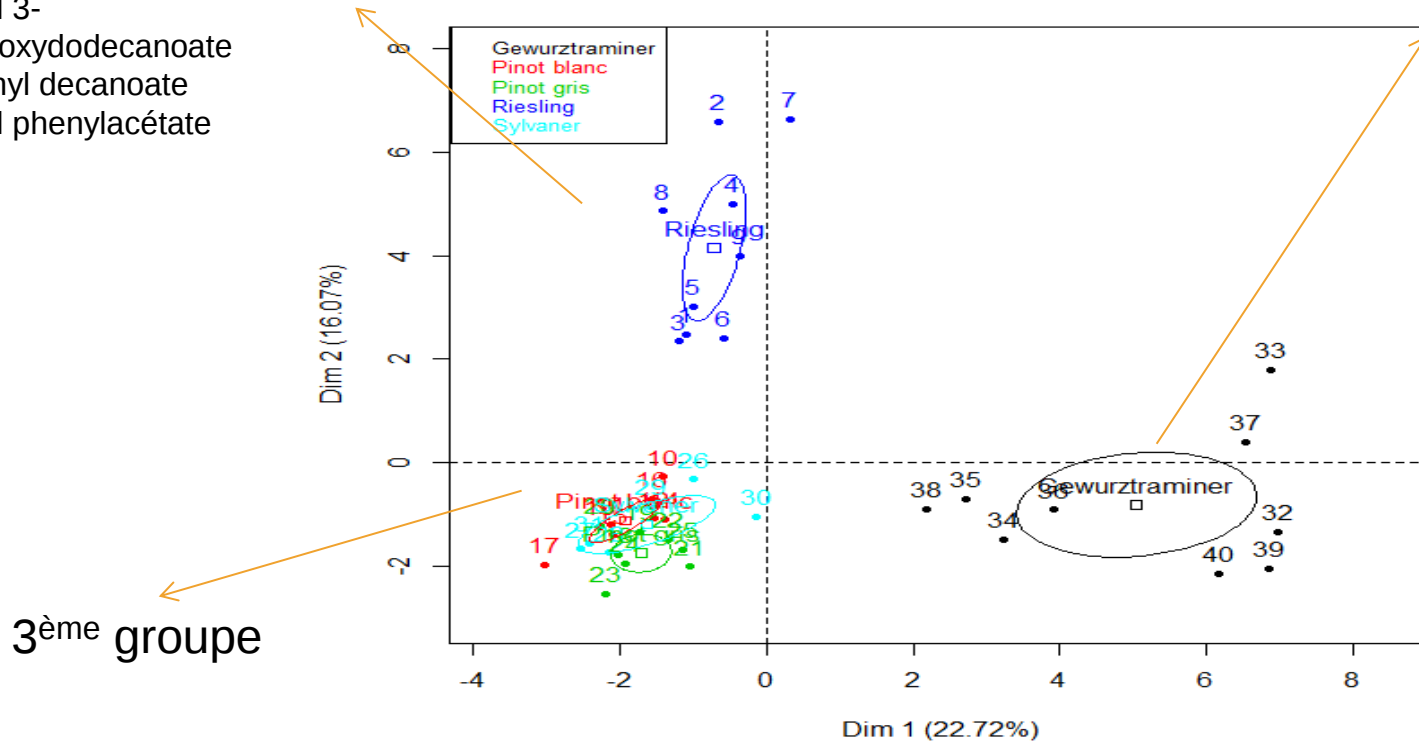
3^{ème} groupe

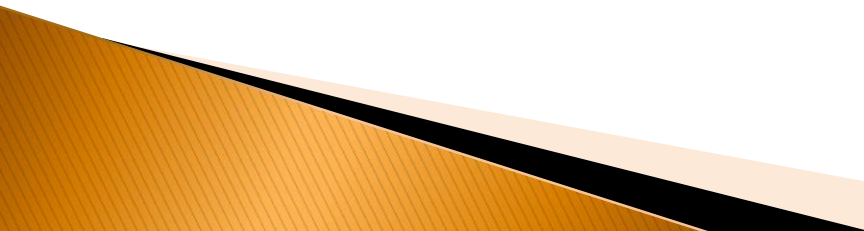
Les marqueurs olfactifs



TDN*
 Vitispirane*
 ethyl 3-
 hydroxydodecanoate
 methyl decanoate
 ethyl phenylacétate

trans-rose oxide*
 Citronellol*
 Geraniol*
 α terpinene
 2,6 dimethylnaphtalène
 nerol
 1-heptanol

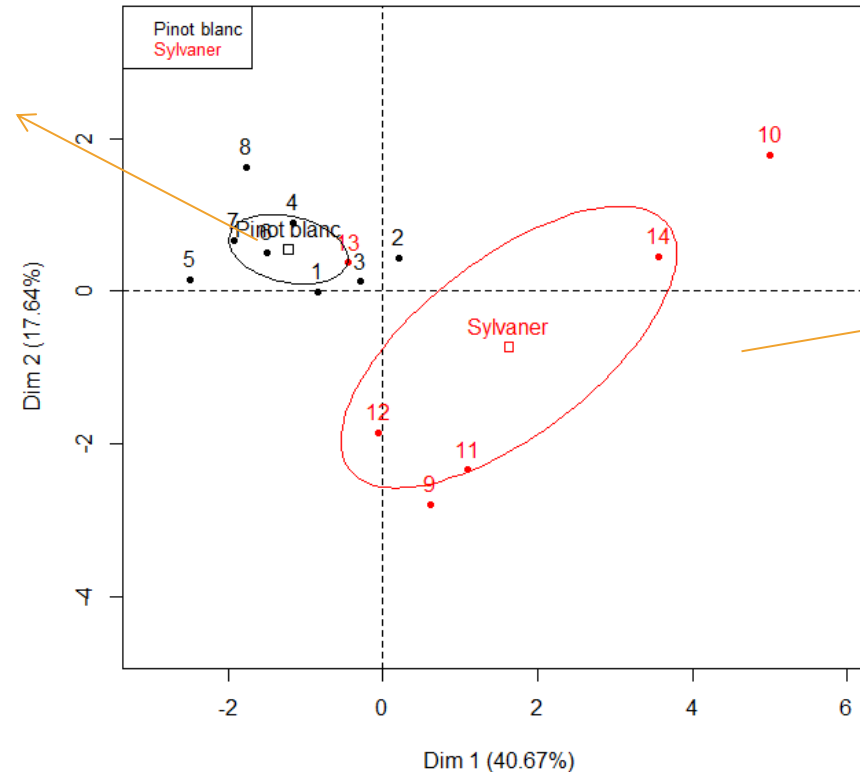




Les marqueurs olfactifs



composé inconnu
3-methyl-1-pentanol
1-nonanol



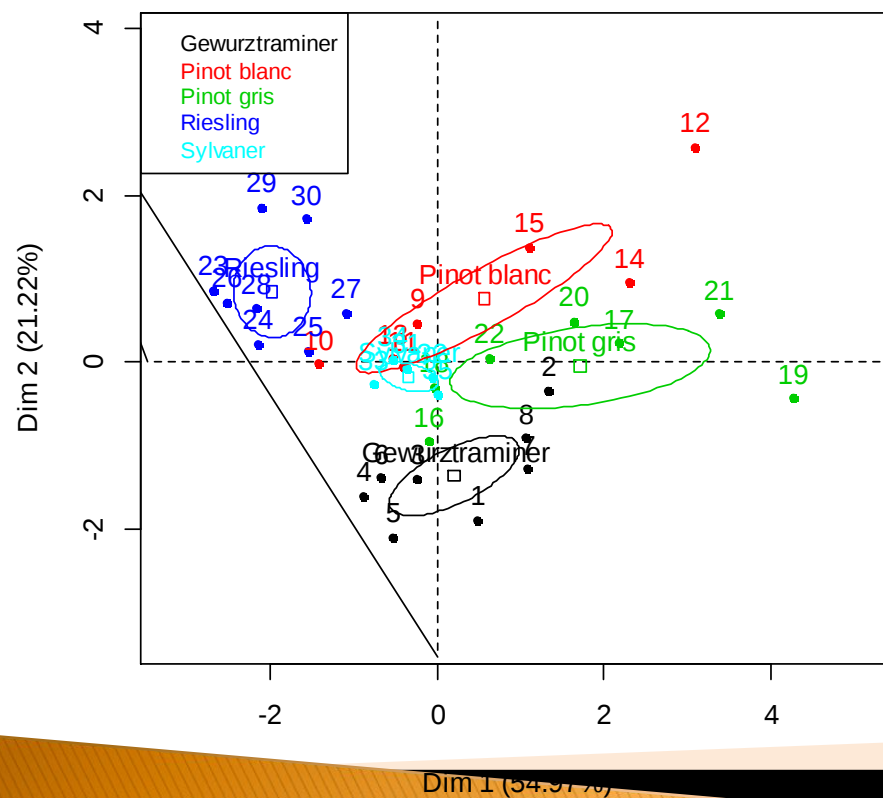
acide C6
acide C8
acide C10
isopentyl hexanoate
ethyl octanoate
ethyl hexanoate
ethyl decanoate
ethyl 3-hydroxyoctanoate
hexyl acétate

Les marqueurs olfactifs



- Distinction des cépages en fonction des composés volatils
- Identification de composés volatils caractéristiques d'un cépage

Les marqueurs phénoliques



Pas d'effets cépages