1ST GREEN ANALYTICAL CHEMISTRY WORKSHOP
5/6 FEBRUARY, 2024 - NOVOTEL PARIS CHARENTON www.tgacworkshop-paris.com

THE GREEN ANALYTICAL CHEMISTRY WORKSHOP

Of mice and wine
An approach using SBSE-GC-MS

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Mousy off-flavour: a wine defect

?

becoming more frequent in recent years

linked to
- pH increase
- decrease in the use of sulphur dioxide
- use of native microbiota

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linked to
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- decrease in the use of sulphur dioxide
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Sensory descriptors

rodent urine
(dirty mouse cage)

grilled foods
popcorn, basmati rice, rice cakes, crackers, bread crust

dried sausage skin

Mousy off-flavour: molecular markers

?

3 *N*-heterocyclic molecules

2-acetyl-1-pyrroline (APY) *(Herderich et al., 1995)*

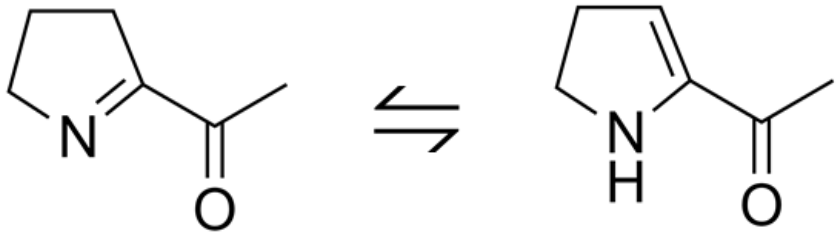
2-acetyltetrahydropyridine (ATHP) *(Strauss & Heresztyn, 1984)*

2-ethyltetrahydropyridine (ETHP) *(Craig & Heresztyn,1984)*

Mousy off-flavour compounds: tautomerism and protonation

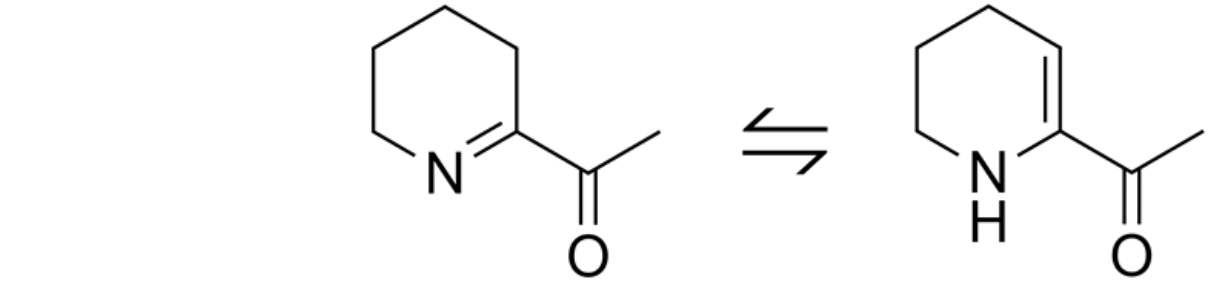
APY

2-acetylpyrroline



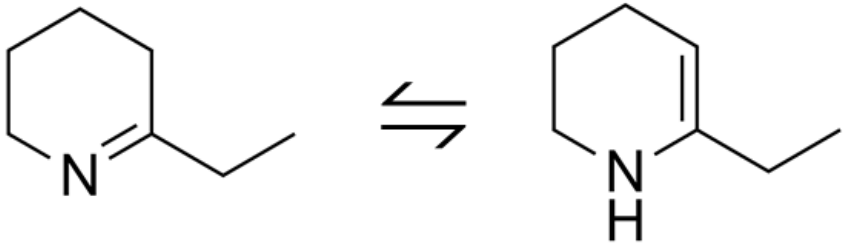
ATHP

2-acetyltetrahydropyridine



ETHP

2-ethyltetrahydropyridine



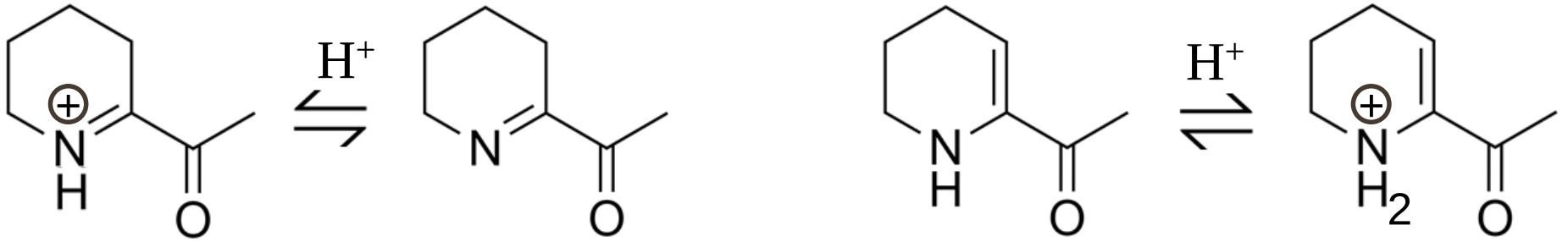
Mousy off-flavour compounds: tautomerism and protonation

APY

2-acetylpyrroline

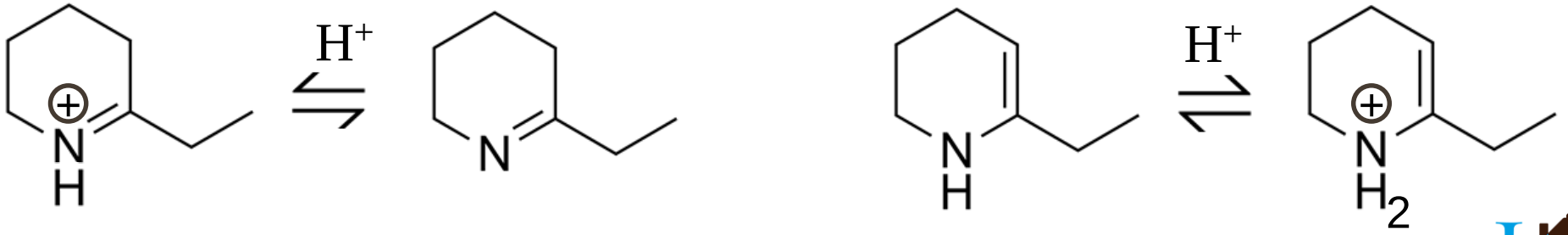
ATHP

2-acetyltetrahydropyridine



ETHP

2-ethyltetrahydropyridine



Mousy off-flavour: a wine defect already mentioned in 1956

Archiv für Mikrobiologie, Bd. 24, S. 266—280 (1956)

(Station agronomique et oenologique de Bordeaux)

Sur les Brettanomyces isolés de Raisins et de Vins

par
E. PEYNAUD et S. DOMERCQ

?

Caractères organoleptiques

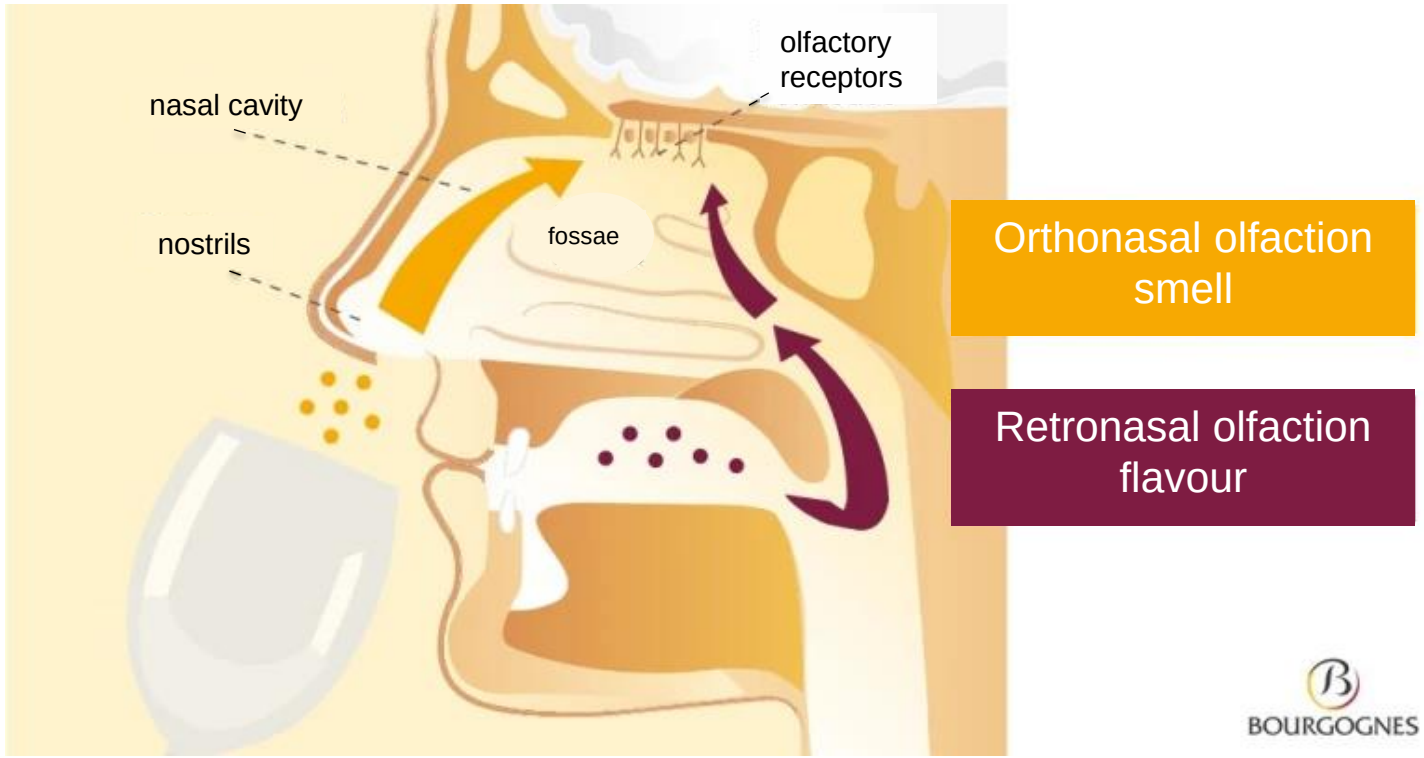
Les cultures de *Brettanomyces* sur moût de raisin présentent à l'odeur des caractères spéciaux assez typiques, que nous avons essayé d'analyser dans le tableau 7. Nous distinguons une odeur sure et aigre, désagréable, différente de l'odeur pénétrante d'acétate d'éthyle et plus complexe. La plupart des cultures ont une odeur fruitée, aldéhydique, rappelant la pomme; certaines possèdent un caractère particulier, butyrique par exemple. Mais toutes ont une odeur plus ou moins intense et toujours infecte, qui rappelle l'acétamide. Cette odeur, assez difficile à définir par un autre terme qu'odeur «de souris», se perçoit à la fin de la dégustation et semble être due à des produits peu volatils. Elle est exaltée surtout, et c'est de cette façon qu'on la distingue le plus aisément, lorsqu'on se mouille les doigts avec le liquide et qu'on les sent après un certain temps d'évaporation.

Mousy off-flavour: olfactory perception

?

- more strongly perceived in the mouth at the end of tasting

  ⇨ retronasal route



- alteration better detected when pH > pH_{wine}

  → oral pH: 5.76 to 7.96 *(Larsen, Jensen, Madsen, & Pearce, 1999)*

  → skin pH: 4.7 on average *(Lambers, Piessens, Bloem, Pronk, & Finkel, 2006)*

Mousy off-flavour: olfactory perception

orthonasal evaluation preferred ⇨ reduce interindividual variations due to retronasal evaluation

3 protocols tested
1. alkalinized paper strips with a 0.1 M sodium hydroxide solution
2. wine pH adjustment with sodium bicarbonate to pH = 5
3. wine pH adjustment with sodium bicarbonate to pH = 7

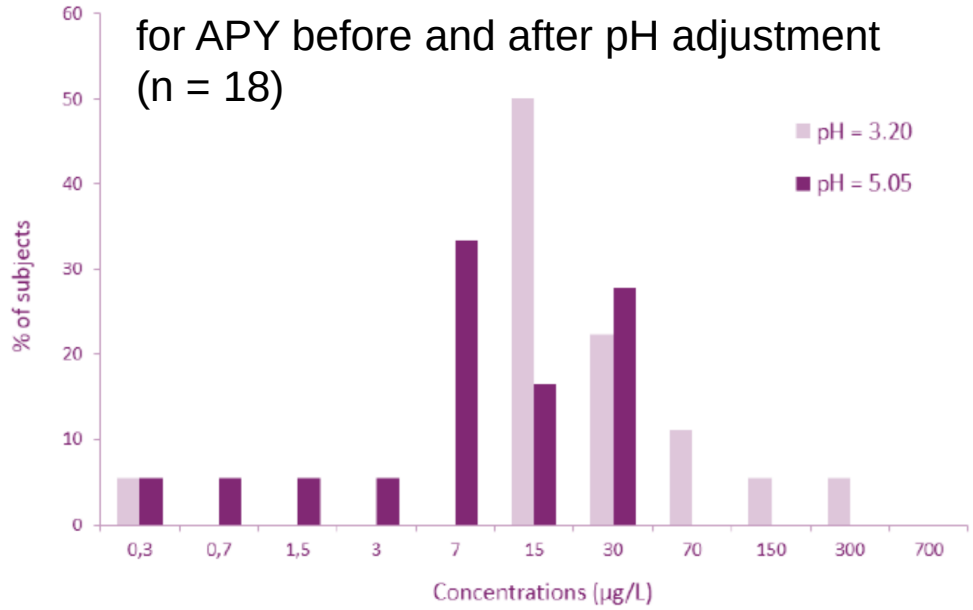
adjusting pH to around 5

increased the consensus among tasters

ensured clear discrimination among samples, according to contamination level

?

Individual detection threshold distribution for APY before and after pH adjustment (n = 18)



Tempère, S. *et al.*, (2019). OENO One, 53(2), 95-105

Mousy off-flavour: olfactory perception

?

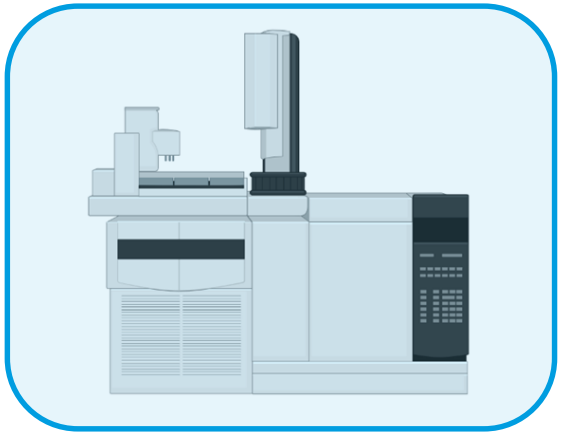
	Detection thresholds in water (orthonasal evaluation)	Reported concentrations in spoiled wine
APY	0.1 µg.L^{-1} *(Buttery et al., 1983)*	7.8 µg.L^{-1} *(Snowdon et al., 2006)*
ATHP	1.6 µg.L^{-1} *(Teranishi et al., 1975)*	0.7 to 106 µg.L^{-1} *(Costello, 1998)*
ETHP	140.5 µg.L^{-1} *(Tempère et al., 2019)*	< 150 µg.L^{-1} *(Grbin et al., 1996)*

Mousy off-flavour: an analytical challenge

APY in aromatic rice

→ HS-SPME-GC-MS
Hopfer *et al.* 2016, Bryant *et al.* 2011

→ HSSE-GC-MS
Grimm *et al.* 2011



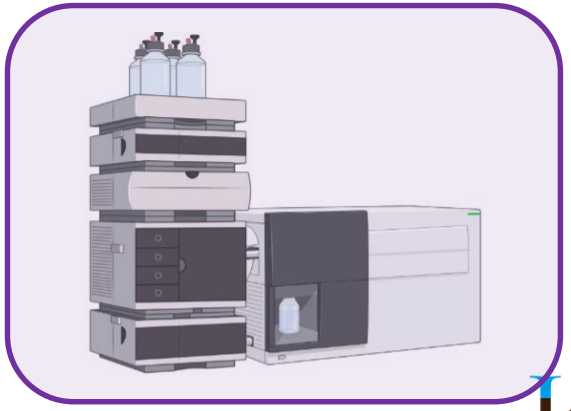
APY in wine

→ LLE-GC-MS
Herderich *et al.* 1995

ATHP in wine

→ LC-APCI-MS/MS
Hayasaka, 2019

LODs → 0.07 µg.L^{-1} (red wine) 0.08 µg.L^{-1} (white wine)
LOQs → 0.21 µg.L^{-1} (red wine) 0.23 µg.L^{-1} (white wine)



Stir Bar Sorptive Extraction in the lab

10 mL sample needed

Multi off-flavour profile in wine





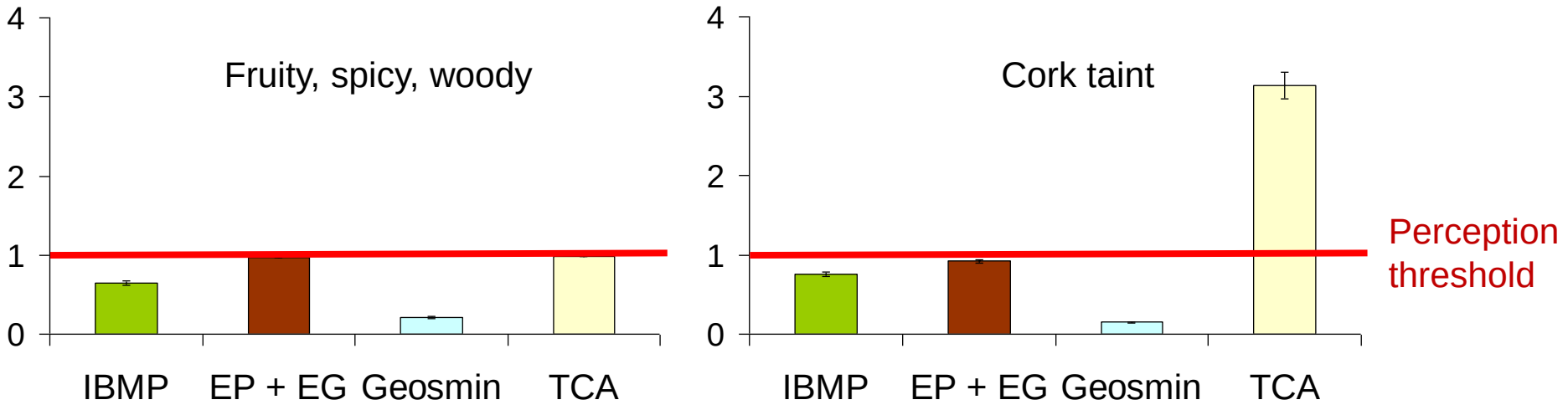
Abbreviation	Name	Smell	Perception threshold
IBMP	2-Isobutyl-3-methoxypyrazine	Vegetal, green pepper	**15 ng/L**
EP	4-Ethylphenol	Horse stable, leather	**430 µg/L**
EG	4-Ethylguaiacol	Spicy, pharmaceutical	**33 µg/L**
Geo	Geosmin	Muddy, musty earthy soil	**50 ng/L**
TCA	2,4,6-Trichloroanisole	Cork	**3 ng/L**
TeCA	2,3,4,6-Tetrachloroanisole	Dust	**35 ng/L**
TBA	2,4,6-Tribromoanisole	Mouldy	**3 ng/L**
PCA	2,3,4,5,6-Pentachloroanisole	Dust	**100 ng/L**

Franc, C., David, F., de Revel, G. Journal of Chromatography A (2009) 1216(15), pp. 3318-3327

Stir Bar Sorptive Extraction in the lab

Multi off-flavour profile in wine

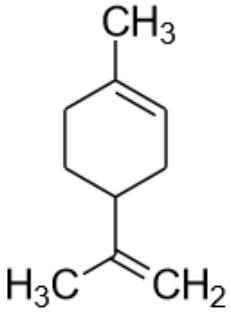
Comparison of 2 bottles of the same 1999 wine, one rejected by the panel of tasters



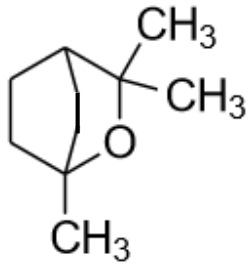
Franc, C., David, F., de Revel, G. Journal of Chromatography A (2009) 1216(15), pp. 3318-3327

Stir Bar Sorptive Extraction in the lab

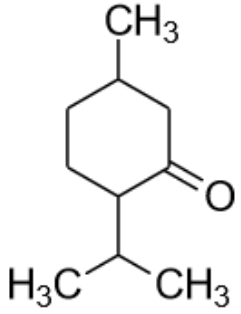
Aroma : mint, freshness
Originating from limonene



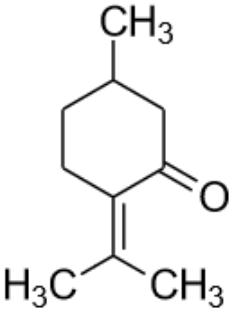
limonene



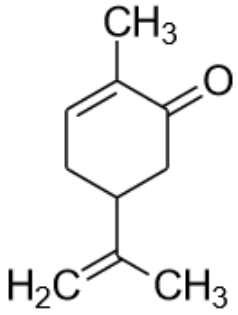
1,8-cineole



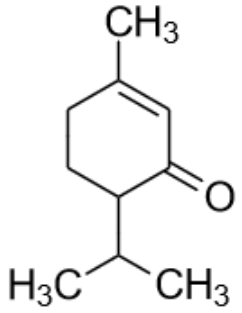
menthone



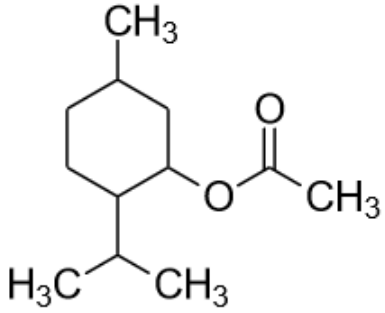
pulegone



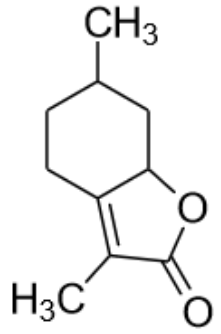
carvone



piperitone



menthyl acetate
and
neomenthyl acetate



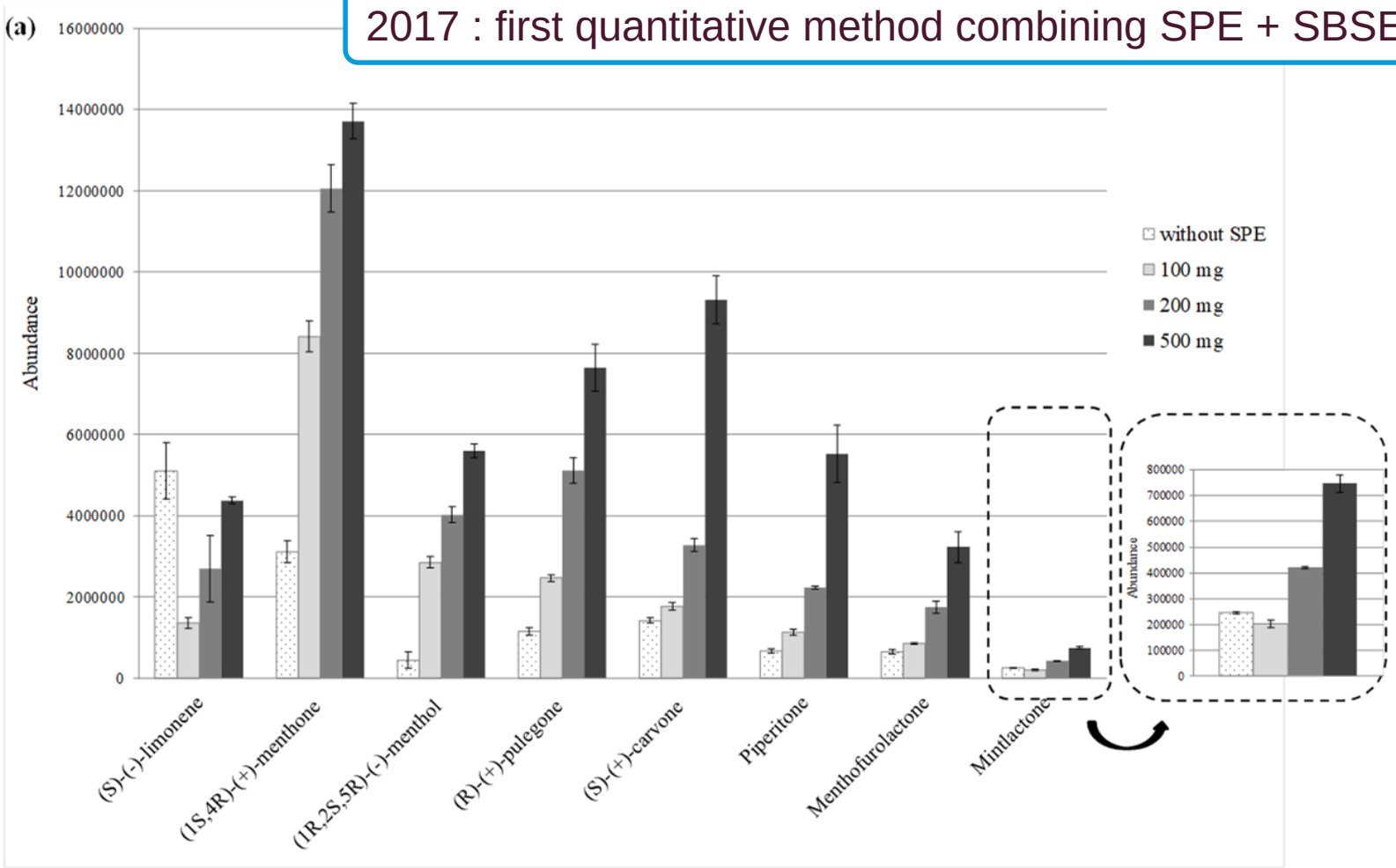
mintlactone

Stir Bar Sorptive Extraction in the lab

Aroma : mint, freshness
Originating from limonene

100 mL sample needed

2017 : first quantitative method combining SPE + SBSE



Picard, M., Franc, C., de Revel, G., Marchand, S. Analytica Chimica Acta (2018) 1001, pp. 168-178

Stir Bar Sorptive Extraction for mousiness in wine

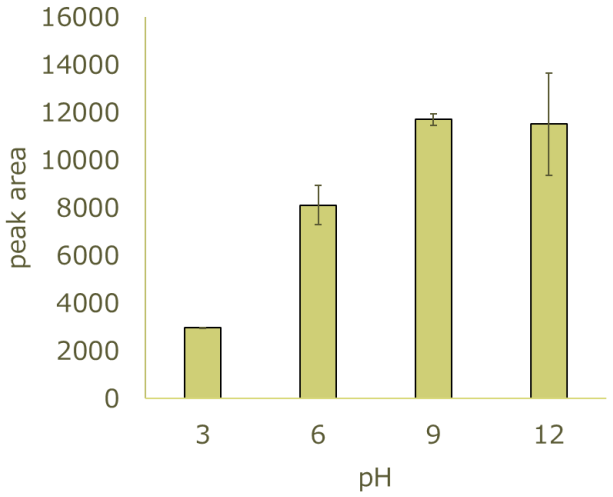
SBSE optimisation

- pH optimisation

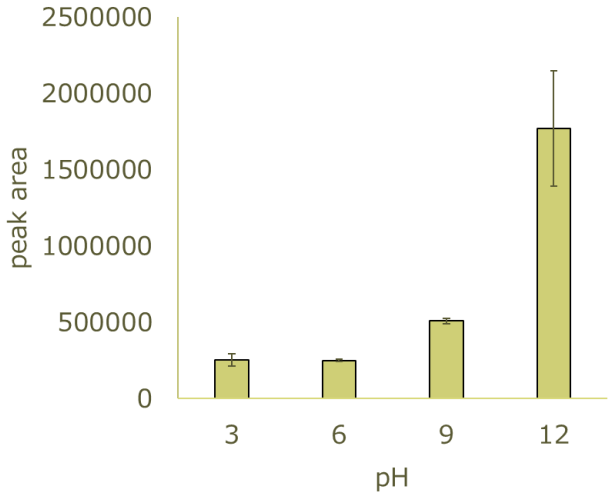
Polar compounds are barely extracted by Twisters®
Ionizable species have to be in their neutral forms
Basic sites are protonated below their pKa

Evolution of extraction efficiency with sample pH in white wine

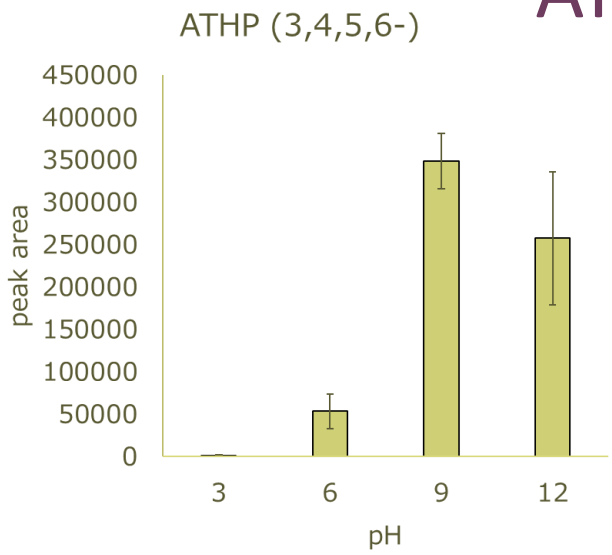
APY

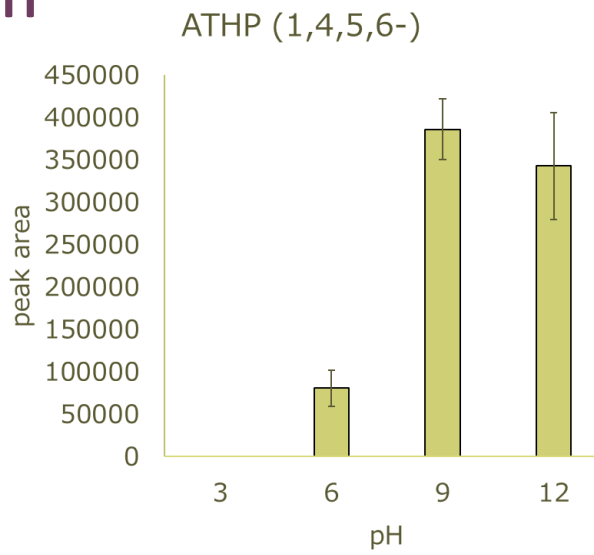


ETHP



ATHP





Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. Food Chemistry (2023)

Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. IVES Technical Reviews vine & wine (2023)

Stir Bar Sorptive Extraction for mousiness in wine

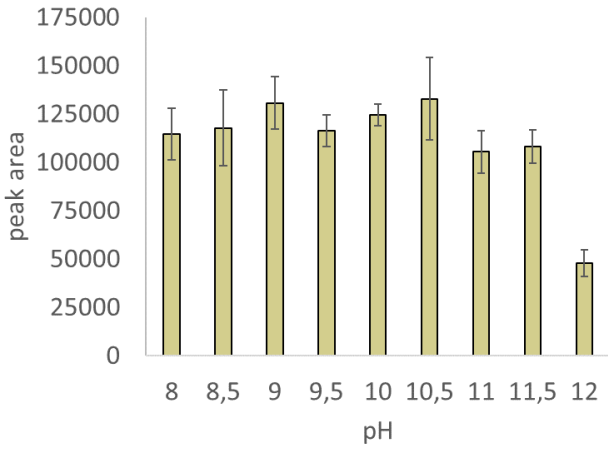
SBSE optimisation

- pH optimisation

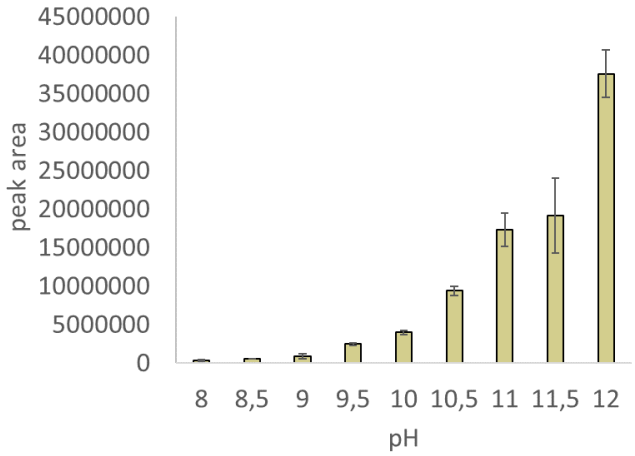
⇨ Best compromise pH = 10.5

Evolution of extraction efficiency with sample pH in white wine

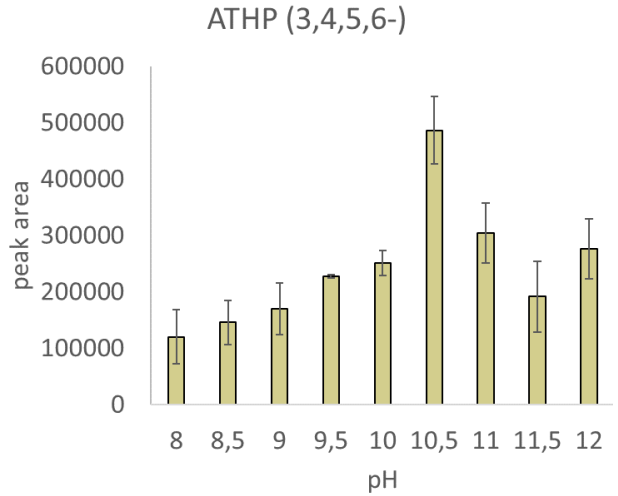
APY

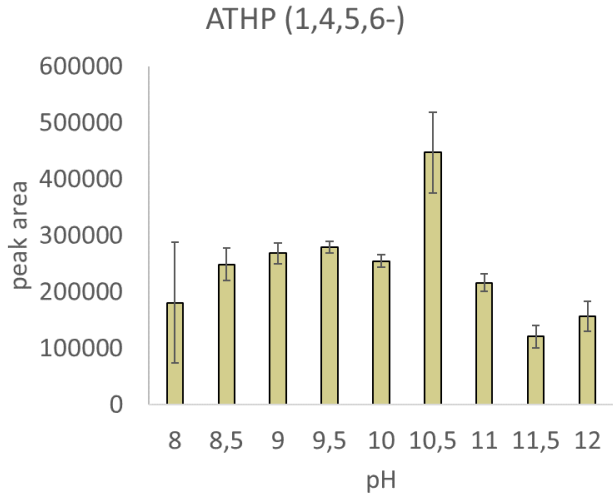


ETHP



ATHP





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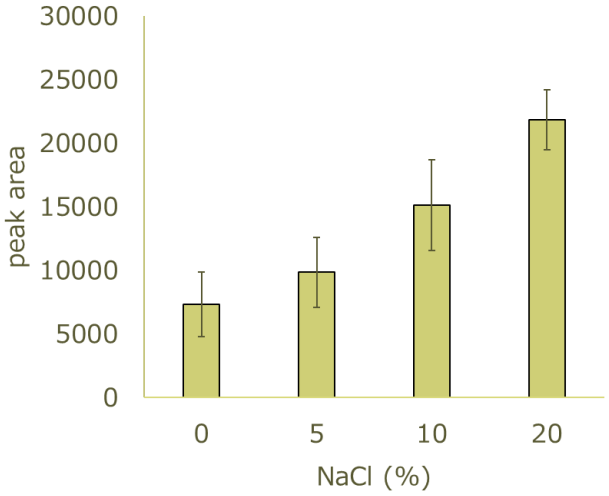
Stir Bar Sorptive Extraction for mousiness in wine

SBSE optimisation

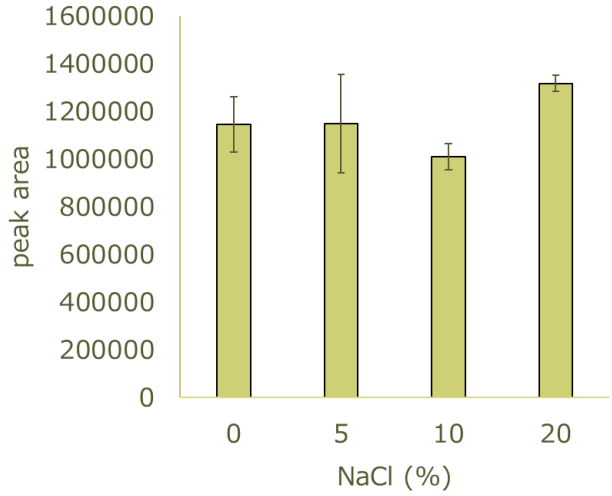
- salt addition

Evolution of extraction efficiency with % of NaCl in white wine sample

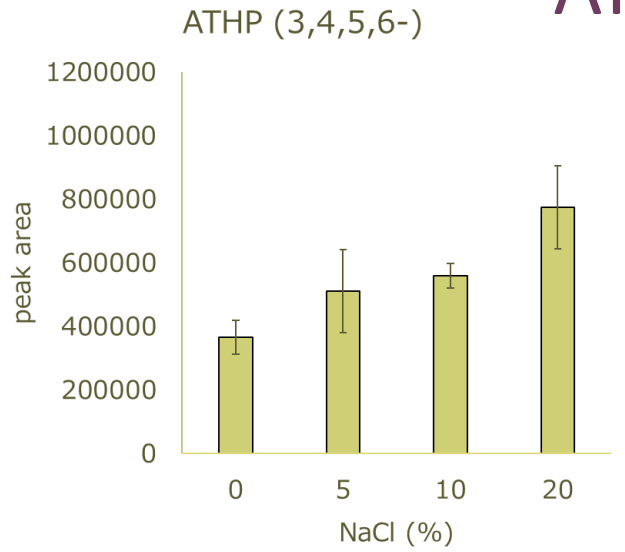
APY

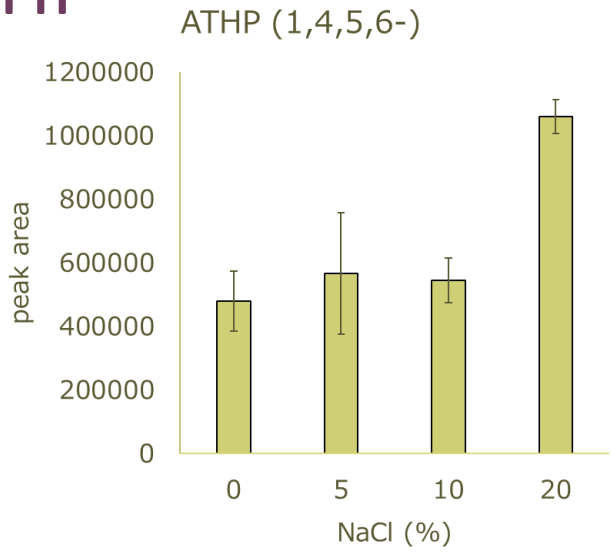


ETHP



ATHP





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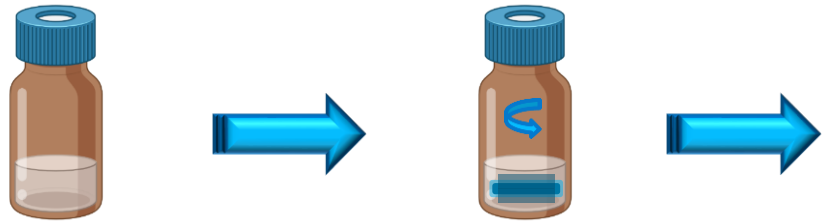
Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. IVES Technical Reviews vine & wine (2023)

Stir Bar Sorptive Extraction for mousiness in wine

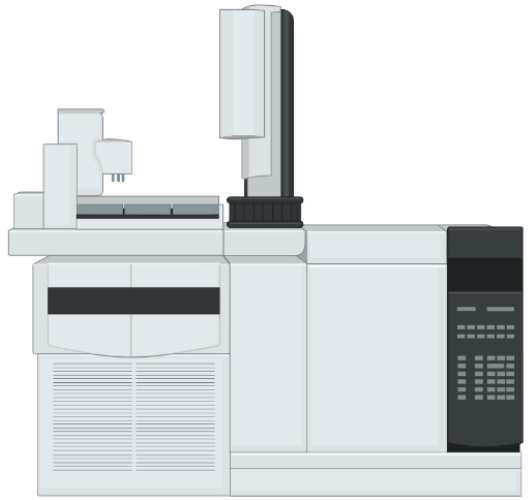
SBSE optimisation

- pH optimization
- salt addition
- sample volume
- Twister® size



10 mL of wine
2 g of NaCl
ISTD: IBMP-d_3
pH = 10.5 (1M NaOH)

Twister® PDMS
20 × 1 mm
600 rpm
1 h



GC separation and MS detection in SIM mode

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Stir Bar Sorptive Extraction for mousiness in wine

SBSE-GC-MS method performance

Red wine:

Compound	Linear range ($\mu g.L^{-1}$)	LOD ($\mu g.L^{-1}$)	LOQ ($\mu g.L^{-1}$)	Average recovery (th. conc., $\mu g.L^{-1}$) (%, n=10)	Intra-day precision RSD (%, n=10)	Average recovery (th. conc., $\mu g.L^{-1}$) (%, n=10)	Intra-day precision RSD (%, n=10)
APY	3.6 – 71.3	0.5	1.8	107 (10.0)	26	95 (49.9)	19
ATHP	5.3 – 105.0	1.5	4.9	119 (23.5)	19	83 (117.5)	25
ETHP	14.1 – 282.7	2.7	8.9	98 (21.2)	18	116 (106.0)	21

Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. Food Chemistry (2023)

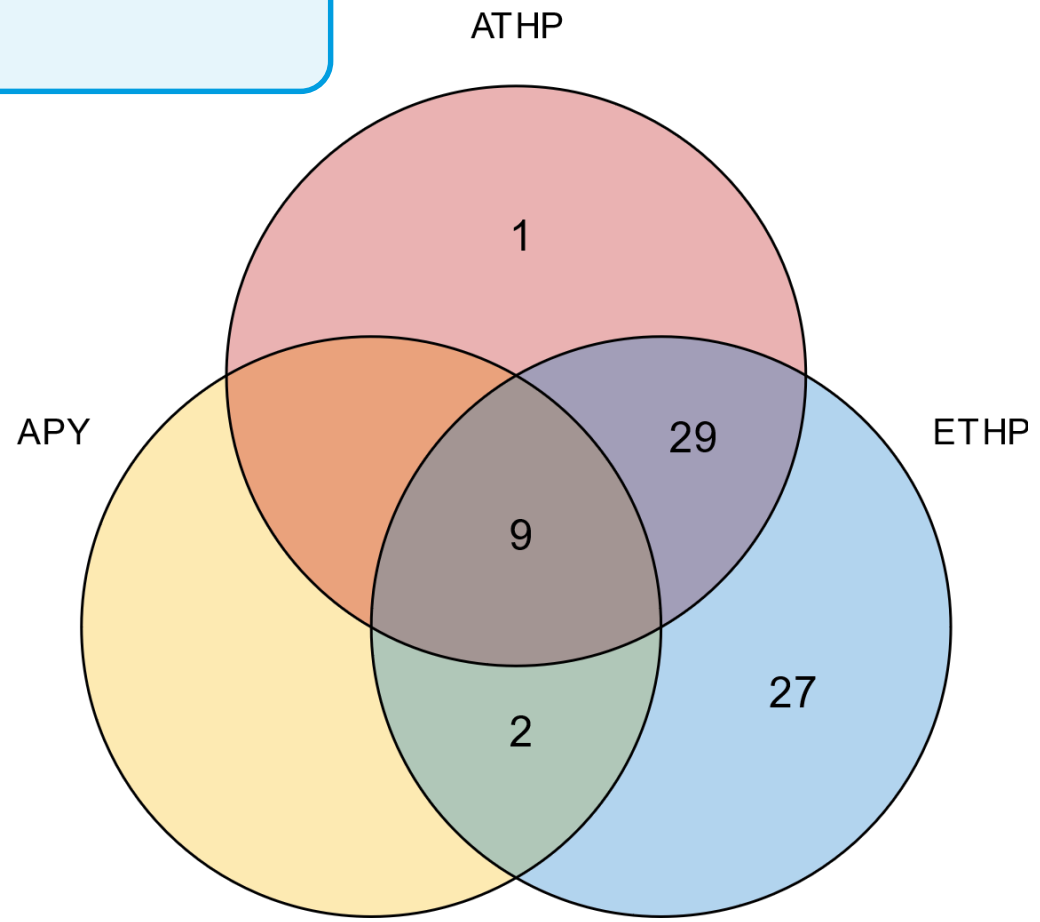
Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. IVES Technical Reviews vine & wine (2023)

Mousy off-flavour: wine analysis

68 wines without SO_2 addition or with limited addition of SO_2
6 control wines with classical use of SO_2

- No APY nor ATHP in control wines
- ETHP below LOQ in control wines

- ETHP in almost all wines with no or limited SO_2 addition (from 14.0 µg/L to 120.5 µg/L)
- ATHP in more than 57% of wines with no or limited SO_2 addition (from below LOQ to 54.5 µg/L)
- APY only in 16% of wines with no or limited SO_2 addition (from below LOQ to 7.7 µg/L)



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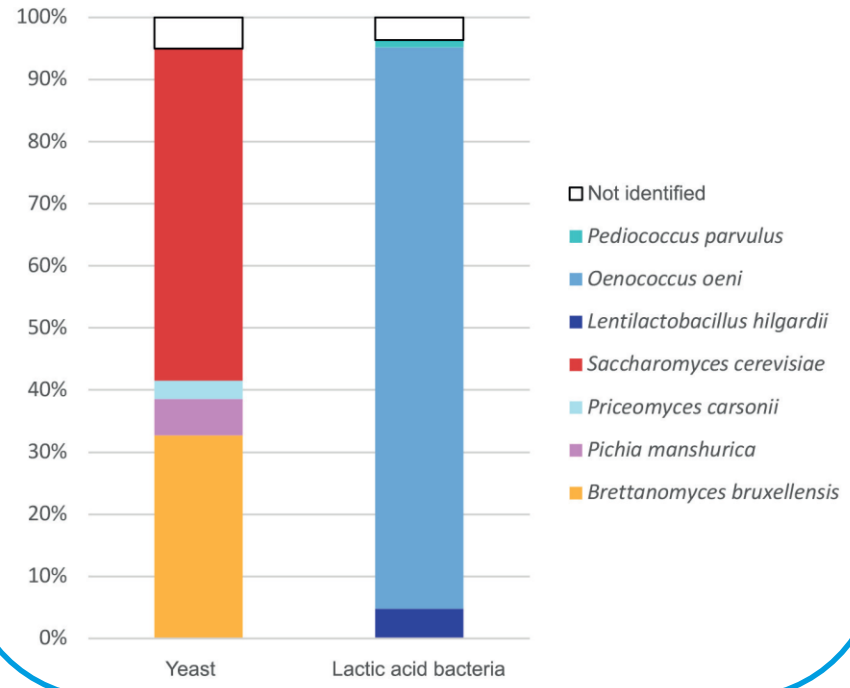
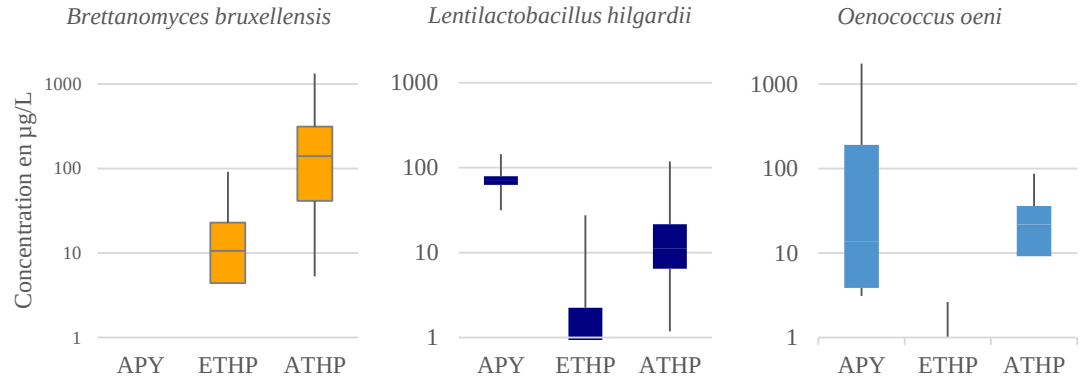
Kiyomichi, Franc, Moulis, Riquier, Ballestra, Marchand, Tempère, & de Revel. IVES Technical Reviews vine & wine (2023)

Mousy off-flavour: production by microorganisms

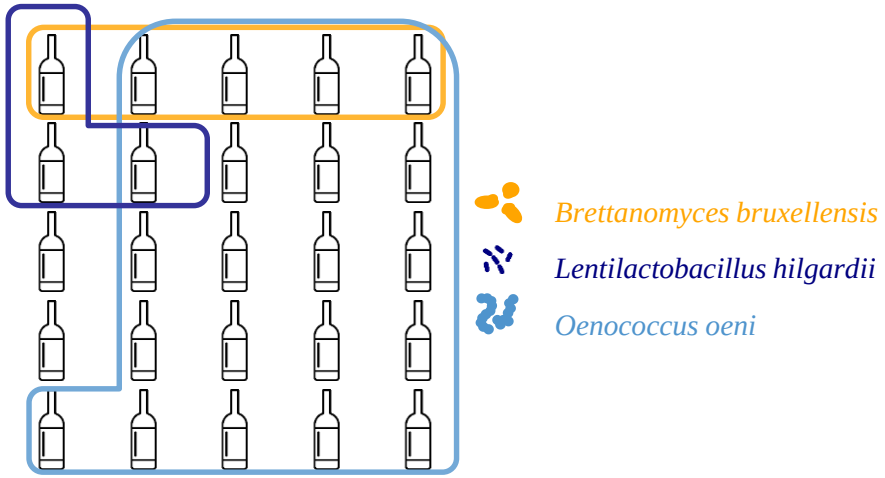
Microbial species identified among 353 isolates from 25 altered wines



Mousiness production capability in model medium



Prevalence of isolated species from 25 altered wines



Pierre Moulis, PhD thesis, UMR œnologie, Univ. Bordeaux & Hochschule Geisenheim Univ. (2023)

Moulis, Miot-Sertier, Cordazzo, Claisse, Franc, Riquier, Albertin, Marchand, de Revel, Rauhut, & Ballestra. OENO One (2023)

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Mousy off-flavour: what next?

?

Use of this analytical method to further study mousiness in wine:

- implicated microorganisms
- wine evolution
- redox status

Thank you for your attention

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