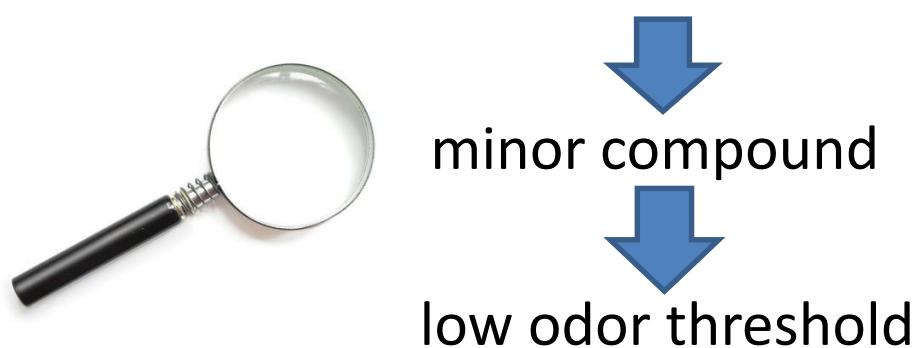


Advanced chromatographic and MS experiences in olive oils volatile compounds analysis

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- VOO aroma is largely attributed to its major volatiles (LOX derived)
- Some VOO sensory attributes cannot be directly related to any compound
 - Interactions between volatiles?
 - Compounds that are not usually determined?



Volatile thiols

- VOO comes from a fruit, wide number of aromatic substances



- Potent odorants:



- Very low odor thresholds



sensory impact in VOO even at low concentrations

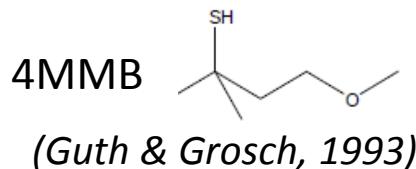
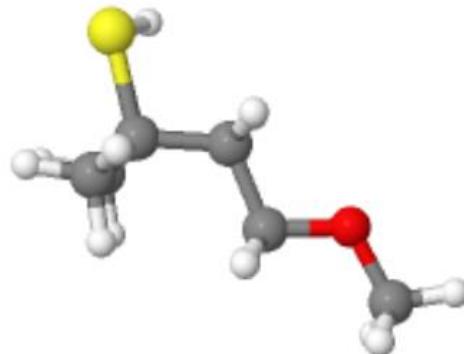
Alkylphenols

- Microbiological activity
- Microorganisms isolated in olives can produce alkylphenols
- Off-flavors:



- Relatively low odor thresholds

Volatile thiols



Alkylphenols



Occasionally reported in VOO
(Morales *et al.*, 2005; Jiménez *et al.*, 2006)

- Low concentration in VOO
- Specific analytical methods → high SENSITIVITY and SELECTIVITY
- Combination with sensory analysis



- **Objectives:**

- ✓ Develop simple, reliable methods to assess volatile thiols and alkylphenols in VOO
- ✓ Perform a screening of these compounds in VOO
- ✓ Determine their sensory impact on VOO



markers of organoleptic attributes or defects?



THIOLS

- Development of the analytical method

- ✓ Low concentrations (ng/kg)
- ✓ High reactivity of the SH group



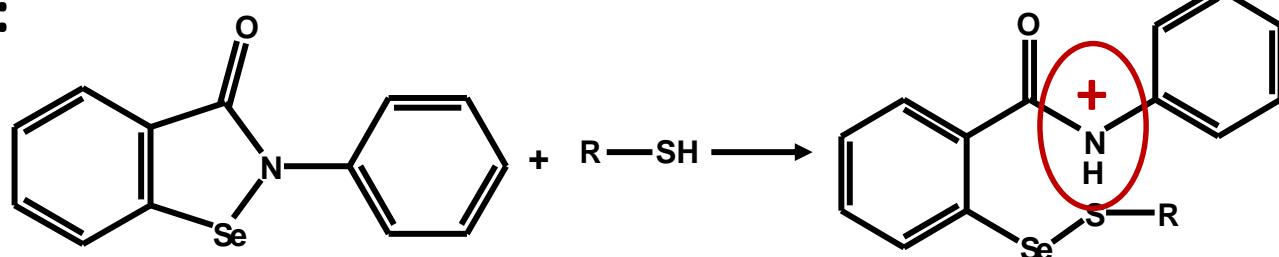
- ➡ Selective derivatization
- stabilization
- labelling
- ➡ Analysis: HRMS



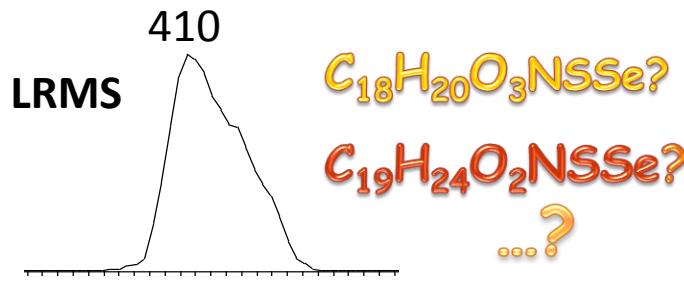
THIOLS

- Development of the analytical method

Derivatization:



ESI-HRMS analysis:



$C_{18}H_{20}O_3NSSe?$

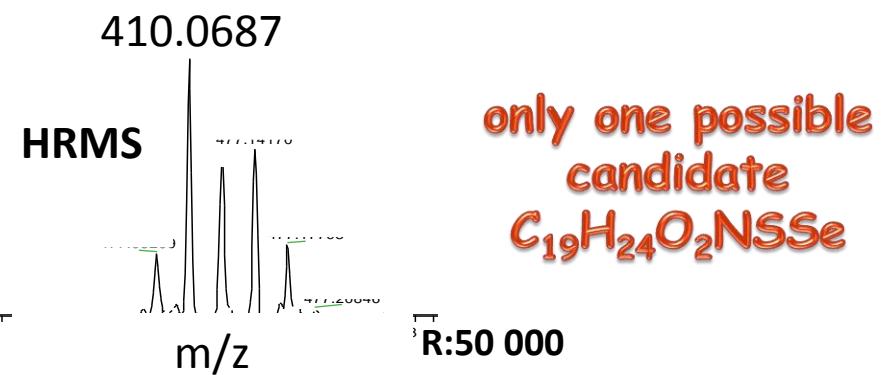
$C_{19}H_{24}O_2NSSe?$

...?

Not resolved peaks

Low accuracy

No possible formulae

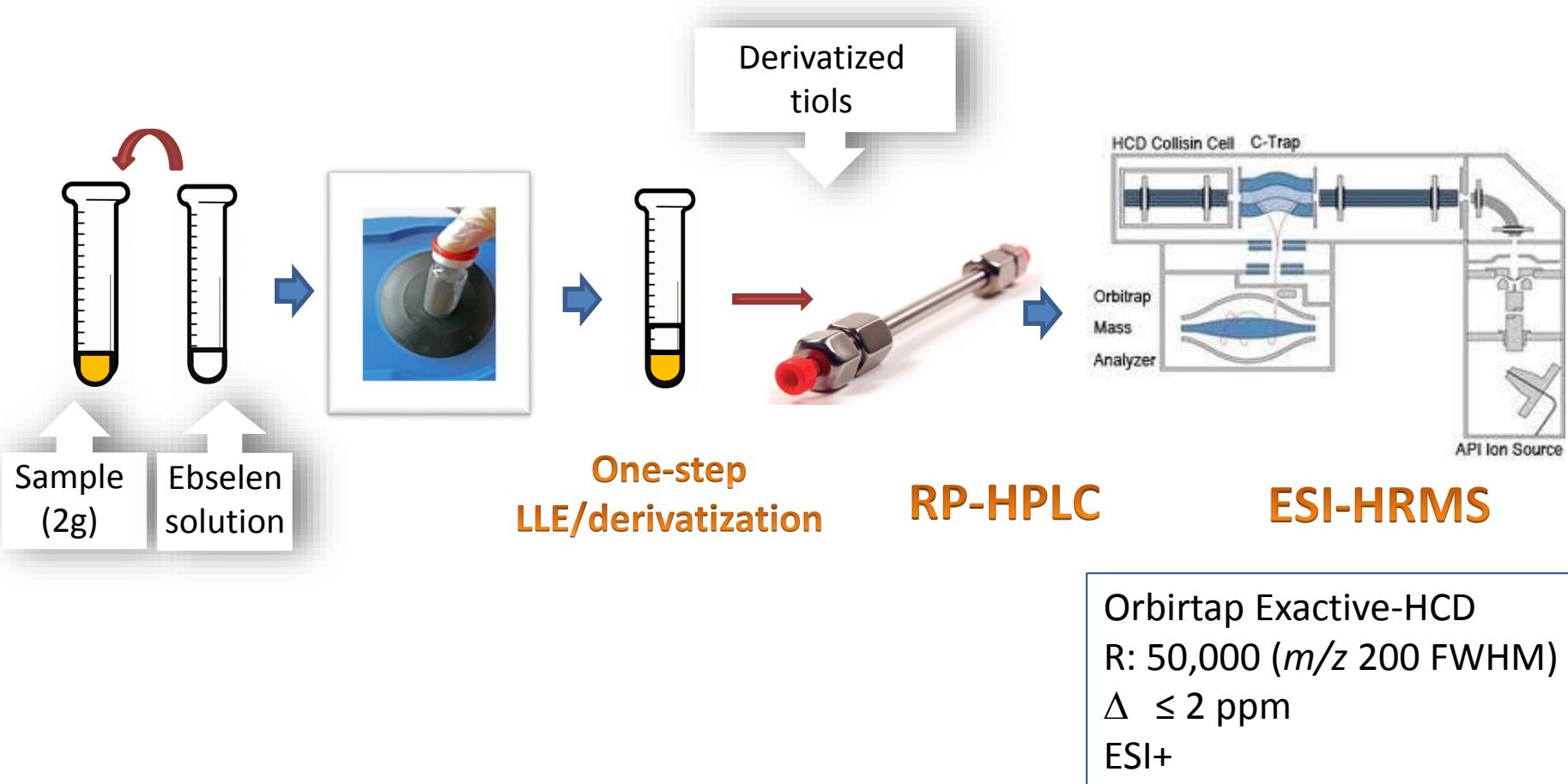


only one possible
candidate
 $C_{19}H_{24}O_2NSSe$

Separated Peaks
Better accuracy and precision
Formulae determined

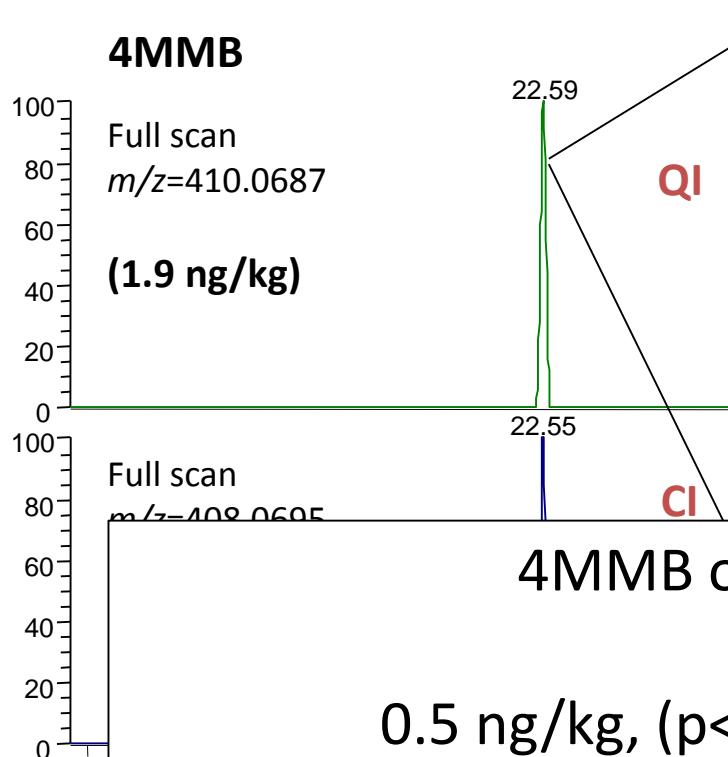
THIOLS

- Development of the analytical method



THIOLS

- Development of the analytical method



Quantification:

IS: methoxy- α -toluenethiol
RF by matrix-matched calibration curves (VOO)

Method performances (4MMB):

4MMB odor threshold in oil:

0.5 ng/kg, ($p<0.05$) > LOQ (0.05ng/kg)

THIOLS

- Screening of thiols in VOO and sensory impact

Thiols analysis

Derivatization-ESI-LC-HRMS

First VOO screening for 4MMB (25 oils – EVOO, VOO, LOO)

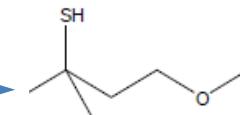
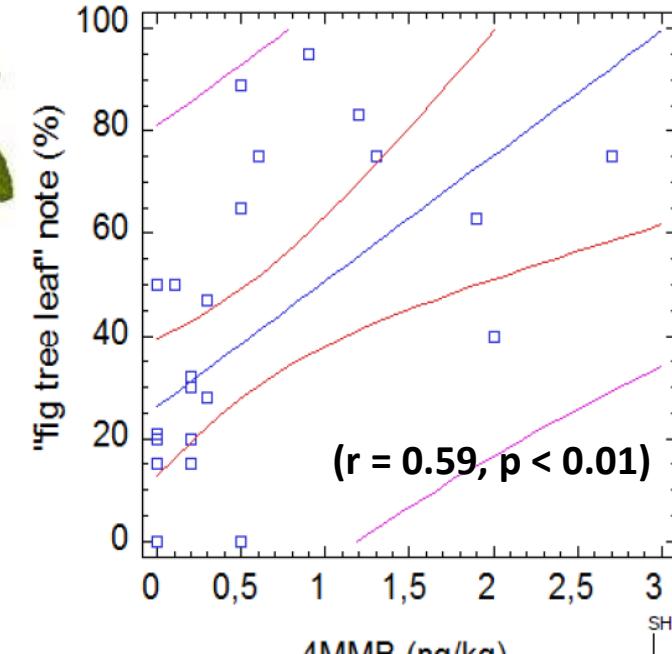
- ✓ 70% of the samples analyzed
- ✓ Concentrations 0.1-2.7 ng/kg
- ✓ Not related to olive oil quality
- ✓ **Not related to fruity note**
- ✓ **Not related to defects**
- ✓ **Correlated to “fig tree leaf note”**



Sensory analysis

Official panel

- EU 796/2002; EU 640/2008
- Open generic profile



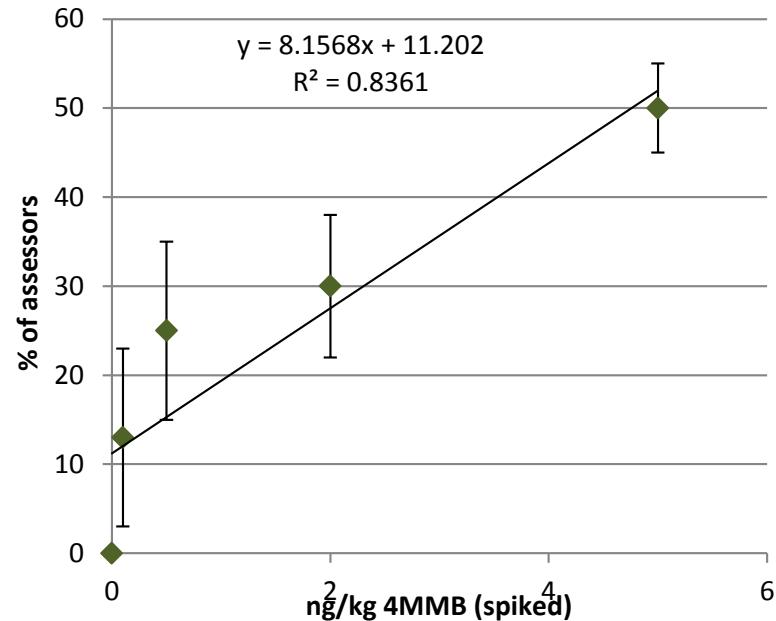
THIOLS

- Screening of thiols in VOO and sensory impact

Sensory analysis

Odor recognition assays

- Spiking two different oils (Arbequina and Picual) with 4MMB
- Assessors were asked to indicate the samples presenting the “fig tree leaf” note
- Odor recognition assays confirmed the correlation between 4MMB and the sensory note



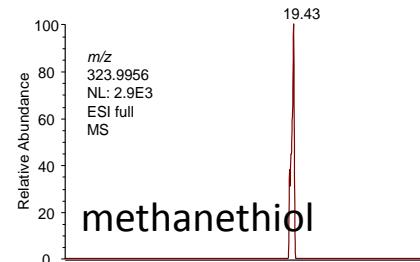
THIOLS

- Screening of thiols in VOO

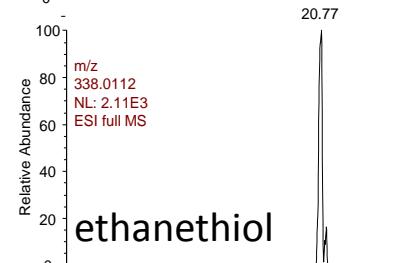
Sensory impact?



Generic descriptors:



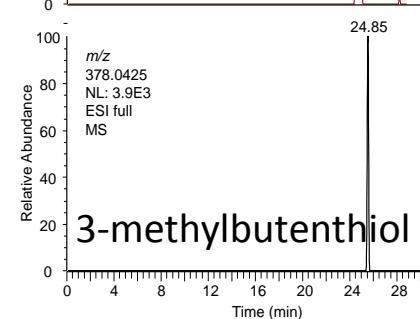
Cabbage-like



Onion-like



Meaty, broth-like



Skunk-like

ALKYLPHENOLS

- Development of the analytical method

- ✓ Low concentrations ($\mu\text{g}/\text{kg}$)
- ✓ Higher-boiling compounds than major VOO volatiles

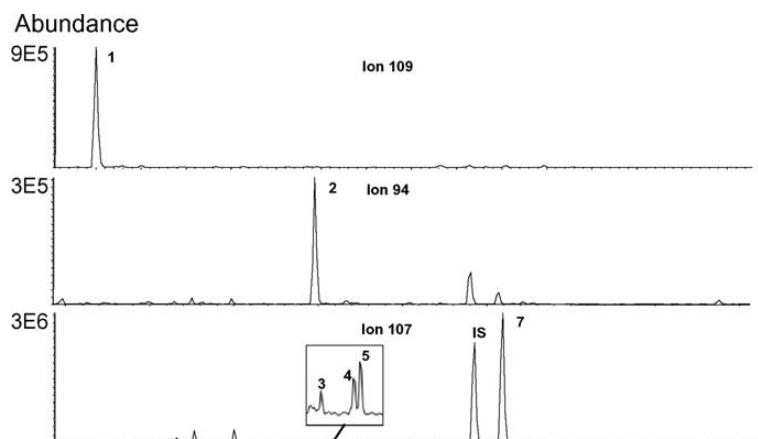
- ➔ Analysis: SPME-GC/MS
- ➔ Optimized extraction conditions:
 60°C , 30 min
MS → SIM mode



ALKYLPHENOLS

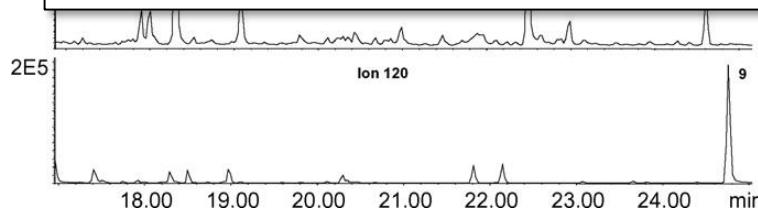
- Development of the analytical method

Nine phenols identified and quantified in VOO headspace



Alkylphenols odor threshold (OT) in oil:

0.01 – 0.4 mg/kg, > LOQ (0.0003-0.0026 mg/kg)



4-vinylguaiacol → Varnish

Generic descriptors:

guaiacol

Quantification:

phenol

IS: 2,3-dimethylphenol

o-, *m*-, *p*-
4-ethylp

RF by matrix-matched calibration
curves (sunflower oil)



ALKYLPHENOLS

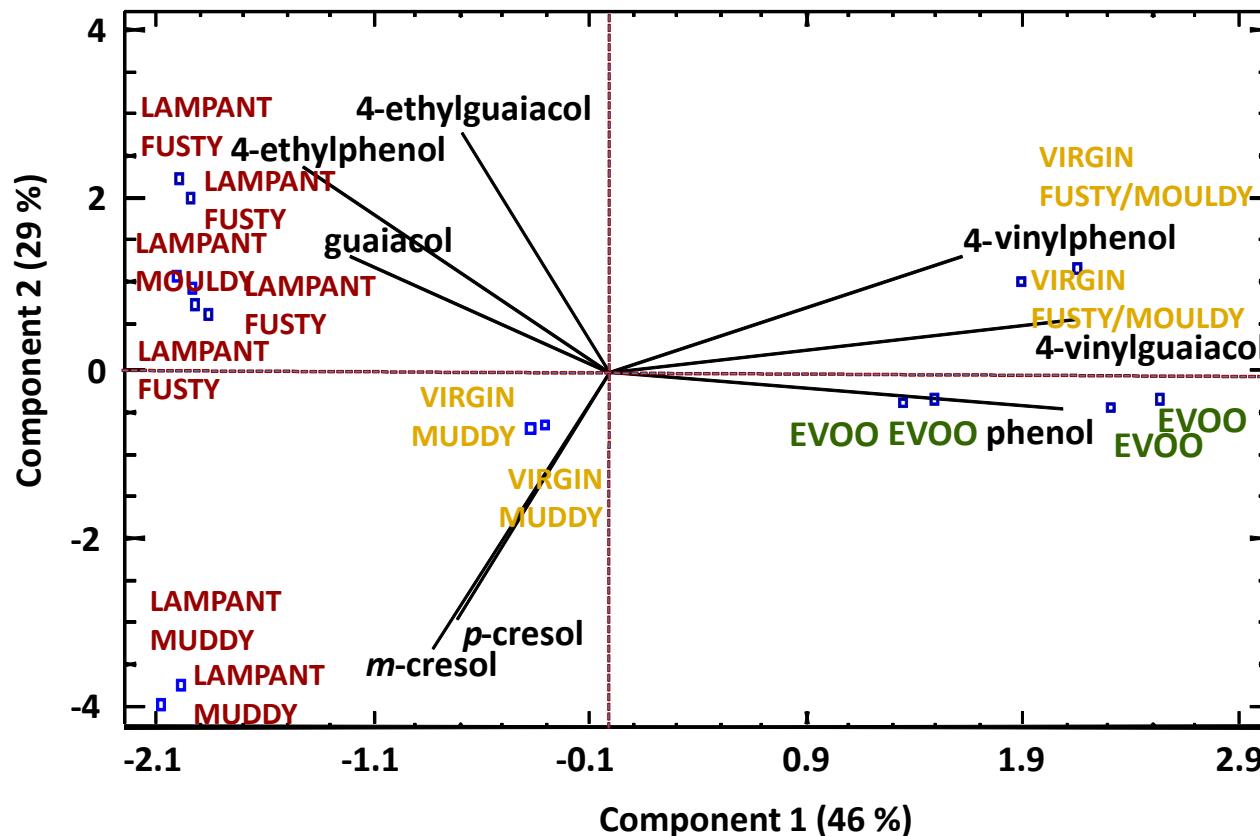
- Screening of alkylphenols in VOO and sensory impact

Odor Activity Values (OAVs)

ODT (mg/Kg)	EVOO	VOO	VOO	REF	REF	REF	
	control	fusty/ mouldy	muddy	fusty	mouldy	muddy	
Guaiacol	0.01		1	6	2-590	57	14
Phenol	0.1	1-2	1	2	2-41	5	2
<i>o</i> -Cresol	0.025				0-1		
<i>p</i> -Cresol	0.025		1	12	2-75	2	48
<i>m</i> -Cresol ^d	-			5	1-17	3	114
4-Ethylguaiacol	0.05		1	3	14-346	8	1
4-Ethylphenol	0.2		1	5	57-294	30	3
4-Vinylguaiacol	0.2			1	0-23	1	
4-Vinylphenol	0.4		2	6	5-201	8	2

ALKYLPHENOLS

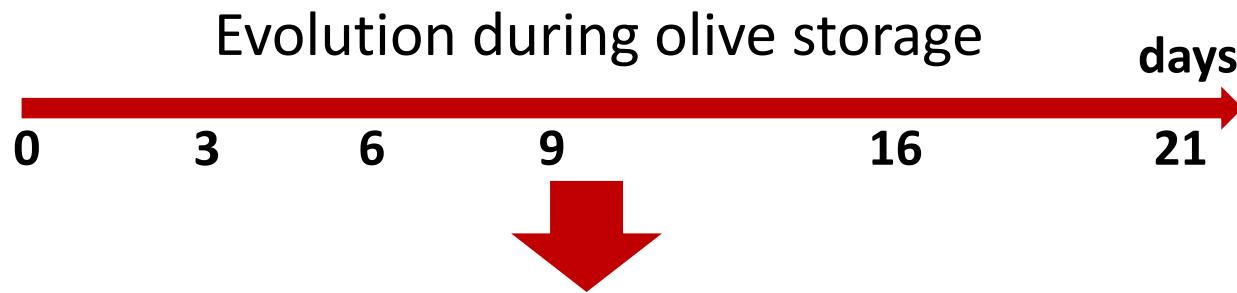
- Screening of alkylphenols in VOO and sensory impact





ALKYLPHENOLS

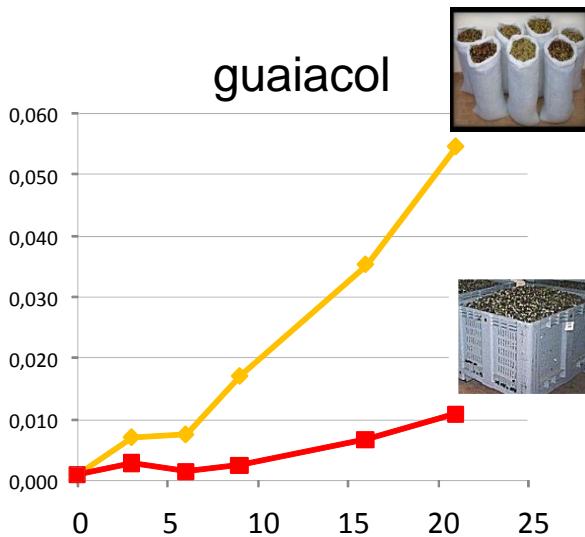
- Screening of alkylphenols in VOO and sensory impact



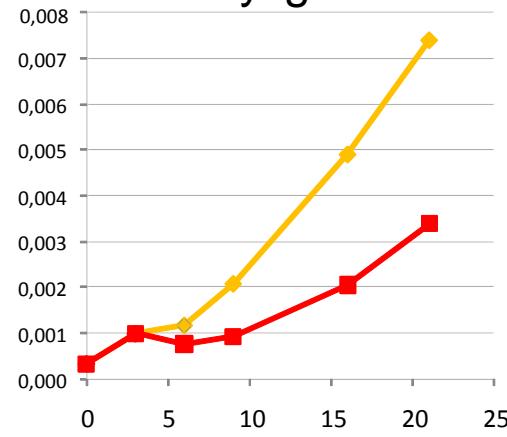
ALKYLPHENOLS

- Screening of alkylphenols in VOO and sensory impact

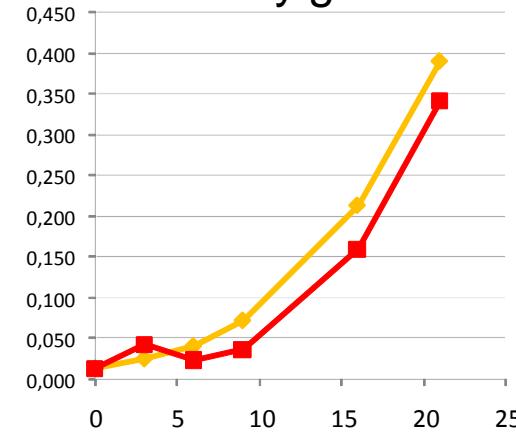
guaiacol



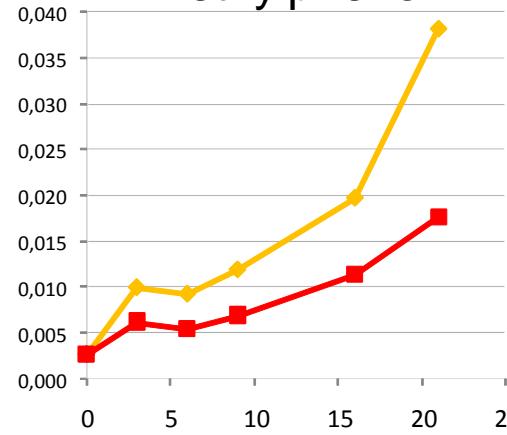
4-ethylguaiacol



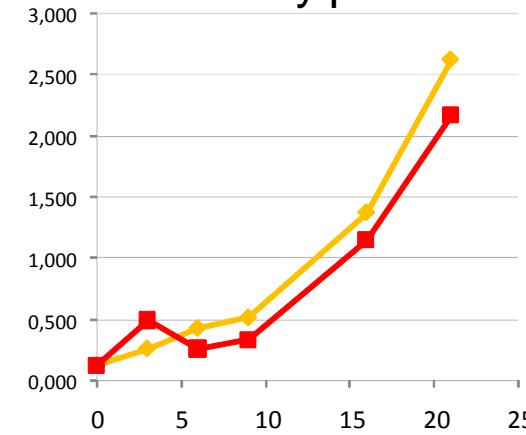
4-vinylguaiacol



4-ethylphenol

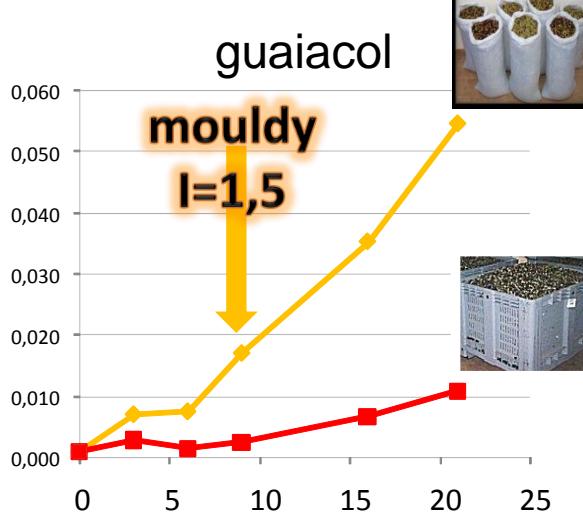


4-vinylphenol



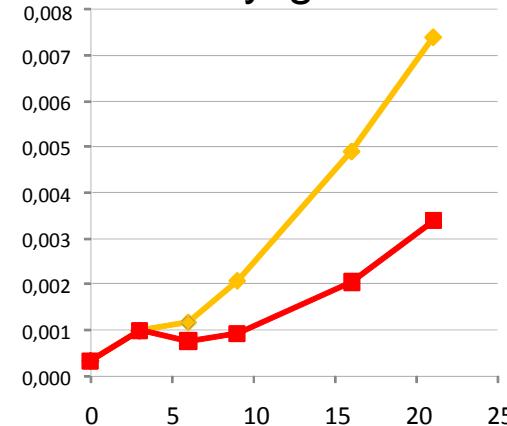
ALKYLPHENOLS

- Screening of alkylphenols in VOO and sensory impact



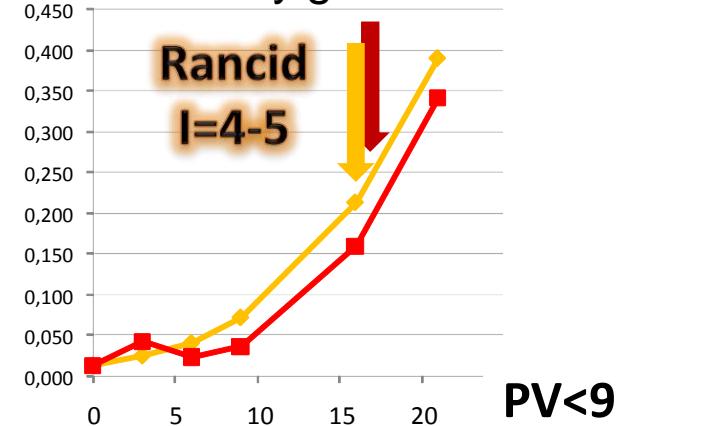
OAV>1

4-ethylguaiacol



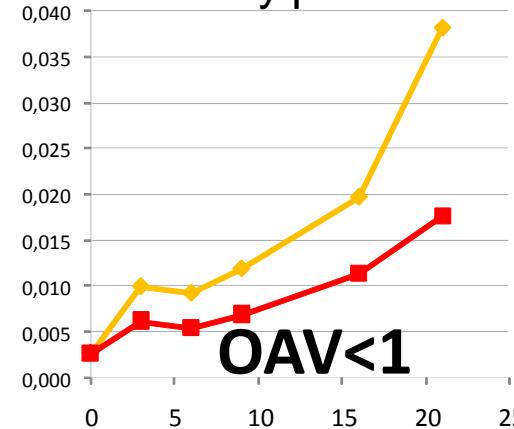
OAV<1

4-vinylguaiacol



PV<9
K₂₇₀<0,15
K₂₃₂<2

4-vinylphenol



OAV>1

OAV>1



- ## Summary

- ✓ Reliable, sensitive and selective methods, suitable for thiol and alkylphenol analysis
- ✓ THIOLS:
 - ✓ Thiols occurrence was not related to VOO quality and 4MMB presence was correlated to the perception of the “fig tree leaf note” → marker
 - ✓ 4 new thiols were identified in VOO, whose sensory impact on VOO is unknown.
- ✓ ALKYLPHENOLS:
 - ✓ Alkylphenols were above their odor threshold in defective oils, indicating their implication in sensory quality, and they were related to fermentative degradation of olives → marker
 - ✓ Guaiacol was associated to the mouldy defect
 - ✓ Vinylphenols were related to the perception of a “varnish” note, associated to the rancid defect by panellists.



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