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?

minor compound

low odor threshold
Volatile thiols

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

  - Very low odor thresholds

Alkylphenols

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

  - Relatively low odor thresholds

sensory impact in VOO even at low concentrations
Volatile thiols

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

Alkylphenols

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

4MMB (Guth & Grosch, 1993)
• **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:

\[ \text{Derivatization:} \]

\[ \text{C}_{11} \text{H}_{20} \text{O}_{3} \text{NSSe?} \]

\[ \text{C}_{19} \text{H}_{24} \text{O}_{2} \text{NSSe?} \]

\[ \ldots \]

ESI-HRMS analysis:

- LRMS: 410
  - Not resolved peaks
  - Low accuracy
  - No possible formulae

- HRMS: 410.0687
  - Separated Peaks
  - Better accuracy and precision
  - Formulae determined

Only one possible candidate \( \text{C}_{19} \text{H}_{24} \text{O}_{2} \text{NSSe} \)

- Development of the analytical method

\[ \text{THIOLS} \]
THIOLS

• Development of the analytical method

Sample (2g)  Ebselen solution

Derivatized thiols

One-step LLE/derivatization

RP-HPLC

ESI-HRMS

Orbitrap Exactive-HCD
R: 50,000 (m/z 200 FWHM)
$\Delta \leq 2$ ppm
ESI+
**THIOLS**

- Development of the analytical method

**4MMB**

Full scan

\[ m/z = 410.0687 \]

(1.9 ng/kg)

Quantification:

- **IS:** methoxy-\( \alpha \)-toluenethiol
- **RF by matrix-matched calibration curves (VOO)**

**Method performances (4MMB):**

4MMB odor threshold in oil:

\[ 0.5 \text{ ng/kg, (p<0.05) > LOQ (0.05ng/kg) } \]
THIOLS

- Screening of thiols in VOO and sensory impact

**Thiols analysis**

- Derivatization-ESI-LC-HRMS

**Sensory analysis**

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

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”
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

\[ y = 8.1568x + 11.202 \]

\[ R^2 = 0.8361 \]
**THIOLS**

- Screening of thiols in VOO

**Sensory impact?**

**Generic descriptors:**

- Cabbage-like
- Onion-like
- Meaty, broth-like
- Skunky
ALKYLPHENOLS

• Development of the analytical method

✓ Low concentrations (μg/kg)

✓ Higher-boiling compounds than major VOO volatiles

Analysis: SPME-GC/MS

Optimized extraction conditions:
60ºC, 30 min
MS → SIM mode
**ALKYLPHENOLS**

- Development of the analytical method

Nine phenols identified and quantified in VOO headspace

**Generic descriptors:**

- Woody, smoky, earthy
- Faecal
- Horse-like, stable
- Clove-like
- Varnish

**Quantification:**

- IS: 2,3-dimethylphenol
- RF by matrix-matched calibration curves (sunflower oil)

**Method performances:**

- Precision: RSD(%) 4.2 – 9.9%
- Sensitivity: LOQ 0.3 – 2.6 mg/kg

**Alkylphenols odor threshold (OT) in oil:**

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

- Alkylphenols
  - guaiacol
  - phenol
  - o-, m-, p-cresol
  - 4-ethylphenol
  - 4-ethylguaiacol
  - 4-vinylguaiacol

- Varnish
**ALKYLPHENOLS**

- Screening of alkylphenols in VOO and sensory impact

<table>
<thead>
<tr>
<th>Odor Activity Values (OAVs)</th>
<th>EVOO</th>
<th>VOO</th>
<th>VOO</th>
<th>REF</th>
<th>REF</th>
<th>REF</th>
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<tbody>
<tr>
<td>ODT (mg/Kg)</td>
<td>control</td>
<td>fusty/mouldy</td>
<td>muddy</td>
<td>fusty</td>
<td>mouldy</td>
<td>muddy</td>
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<tr>
<td>Guaiacol</td>
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<td>6</td>
<td>2-590</td>
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<td>Phenol</td>
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<td>2</td>
<td>2-41</td>
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<td>o-Cresol</td>
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<td>5</td>
<td>1-17</td>
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<td>114</td>
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<tr>
<td>m-Cresol</td>
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<td>-</td>
<td>5</td>
<td>1-17</td>
<td>3</td>
<td>114</td>
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<tr>
<td>4-Ethylguaiacol</td>
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<td>3</td>
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<tr>
<td>4-Ethylphenol</td>
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<td>5</td>
<td>57-294</td>
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<td>4-Vinylguaiacol</td>
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<td>1</td>
<td>0-23</td>
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<tr>
<td>4-Vinylphenol</td>
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<td>2</td>
<td>6</td>
<td>5-201</td>
<td>8</td>
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ALKYLPHENOLS

• Screening of alkylphenols in VOO and sensory impact
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- Screening of alkylphenols in VOO and sensory impact
ALKYLPHENOLS
• Screening of alkylphenols in VOO and sensory impact

![Graphs showing the concentration of various alkylphenols over time with sensory impact indicators.]

- guaiacol
- 4-ethylguaiacol
- 4-vinylguaiacol
- 4-ethylphenol
- 4-vinylphenol

OAV > 1
OAV < 1
PV < 9
K_{270} < 0.15
K_{232} < 2
Rancid I = 4-5
• 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.
In collaboration with:

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The Official Tasting Panel of Virgin Olive Oils of Catalonia

Generalitat de Catalunya
gen.cat.cat