# Volatile fraction by HS-SPME-GC-MS and sensory evaluation of more than 1200 Virgin Olive Oil samples: methods to support Panel Test in Virgin Olive Oil classification

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# INTRODUCTION



Collaboration

The collaboration stems to satisfy the needs of a company leader in the olive oil field. These needs are linked to two main goals:

**NEEDS**

1. Quality evaluation
2. Legislative requirements

**QUALITY EVALUATION ➔**

- Raw materials selection
- Evolution of volatile compounds over time
- Blends & Products standardisation
- Detection of poor-quality virgin olive oils by only chemical analysis

**LEGISLATIVE REQUIREMENTS➔**

- Supporting panel test in virgin olive oil classification according to Reg. CE 2568/91
- Authentication of the geographical origin of virgin olive oils

**TOOLS**

1. Reliable chemical/analytical methods
2. Large data-sets (more than 1000 samples)
3. Suitable statistical tools

# The analytical method

It has been developed and validated with the purpose of a reliable quantification of VOCs in virgin olive oils

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## Multiple internal standard normalization for improving HS-SPME-GC-MS quantitation in virgin olive oil volatile organic compounds (VOO-VOCs) profile

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11 internal standards for area normalization allows overcoming some issues that usually limits quantification by HS-SPME-GC-MS techinque, as:

1. different absorption capacity of different fiber
2. fiber wearing
3. competition of molecules at different concentration in different samples
4. different affinity of different molecules for the coating material of the fiber

resulting in a more reliable quantitation of VOCs in wider ranges of calibration

# The developed researches 2017-2019

1. Focus on the **rancid** defect studying the evolution of VOCs in EVOOs differing for fatty acid composition for **definition of new volatile molecular markers** under typical household and market storage conditions.

2. **Development of 4 chemometric approaches for supporting panel test in virgin olive oil classification. 73 VOCs × 1223 samples. Oil samples were with median of defect < 1.5 (i.e. considered difficult to be classified by the panel test).**

3. Development of 3 chemometric approaches for authentication of geographic origin of virgin olive oil. 73 VOCs × 1223 samples (from all over the world)

4. **Application of one of the approaches developed at point 2 and analysis of the total content of tyrosol and hydroxytyrosol after acidic hydrolysis for assessment of extra virgin olive oil quality**

# Virgin olive oil classification

2. **Development of 4 chemometric approaches for supporting panel test in virgin olive oil classification**. 73 VOCs × 1223 samples. Oil samples were with median of defect < 1.5 (i.e. considered difficult to be classified by the panel test).

## CLASSIFICATION OF VIRGIN OLIVE OILS

| | |
|---|---|
| • **EXTRA VIRGIN OLIVE OIL**: | FREE ACIDITY ≤ 0.8 GRAMS PER 100 GRAMS<br>MEDIAN OF DEFECTS = 0<br>MEDIAN OF FRUITY > 0 |
| • **VIRGIN OLIVE OIL**: | FREE ACIDITY ≤ 0.8 GRAMS PER 100 GRAMS<br>MEDIAN OF DEFECTS < 3.5<br>MEDIAN OF FRUITY > 0 |
| •**LAMPANTE VIRGIN OLIVE OIL**: | FREE ACIDITY > 0.8 GRAMS PER 100 GRAMS OR<br>MEDIAN OF DEFECTS > 3.5<br>MEDIAN OF FRUITY ≥ 0 |

Not fit for consumption

# The panel test

INTERNATIONAL
OLIVE
COUNCIL

COI/T.20/Doc. No 15/Rev. 10
2018

ENGLISH
Original: FRENCH

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## SENSORY ANALYSIS OF OLIVE OIL

## METHOD FOR THE ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OIL

### 1. PURPOSE

The purpose of this international method is to determine the procedure for assessing the organoleptic characteristics of virgin olive oil and to establish the method for its classification on the basis of those characteristics.

# The panel test

**DRAWBACKS OF THE PANEL TEST**

- **subjectivity and emotionality**
- **slowness ➔ difficult to perform all the daily tests**
- **low reproducibility and legal uncertainty**
- **expensive**


NEED FOR A RELIABLE AND ROBUST ANALYTICAL METHOD **TO SUPPORT** THE PANEL TEST

# SAMPLES

TOTAL: **1223** commercial virgin olive oil samples

- 3 olive oil crops: 2016-17, 2017-18, 2018-19
- Provenance: Spain (34.5%), Italy (26.7), Greece (23.6%), Portuga (6.9%), Tunisia (6.7%), other (1.6%)
- Category after chemical and sensorial analysis:
  1. Lampante virgin olive oils (5 samples ➔ outliers)
  2. Extra Virgin olive oils, EVOO (562 samples)
  3. Virgin olive oils, VOO (656 samples)

N.B. almost all samples were considered difficult to be classified with accuracy by the panel test

**EVOO = EV**

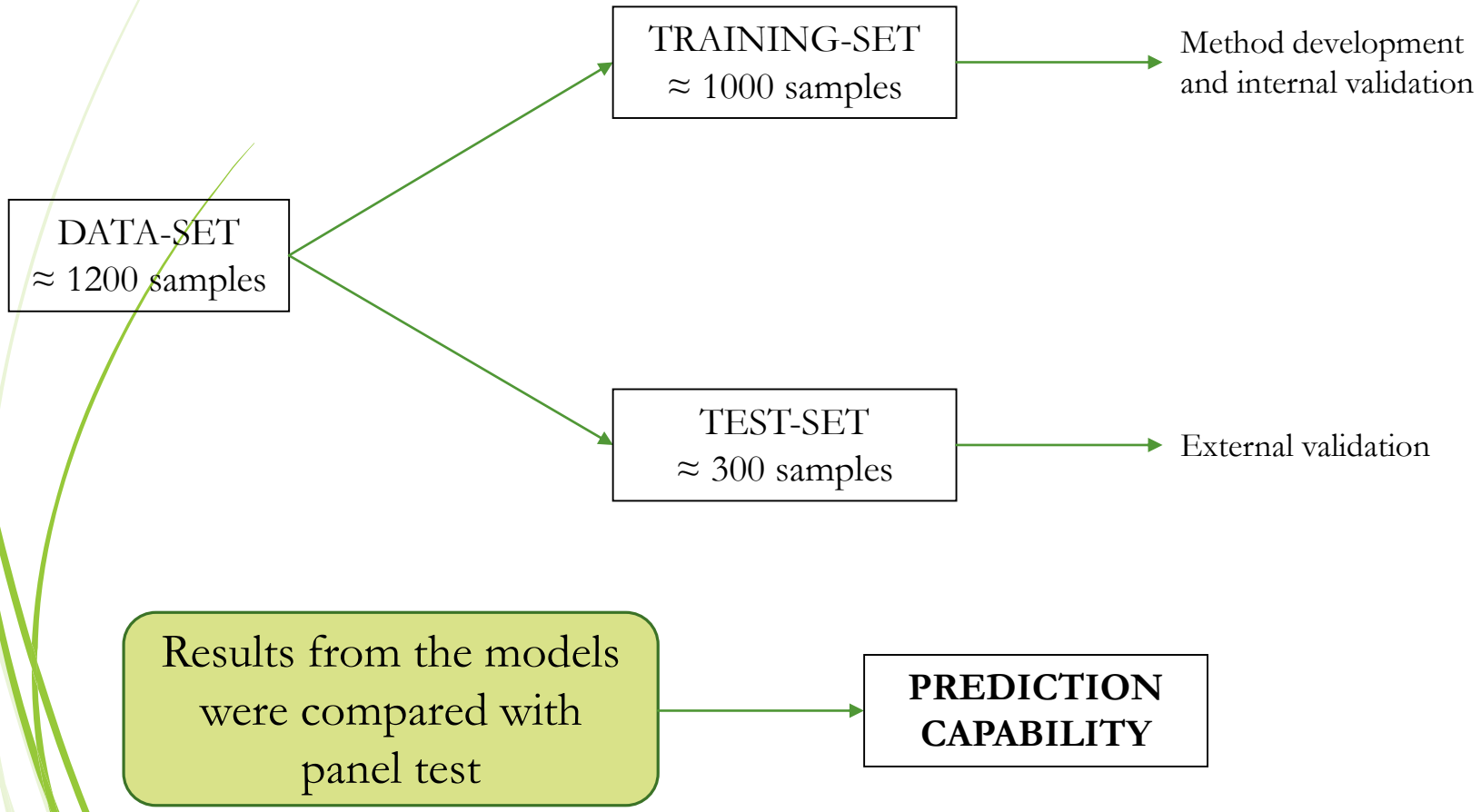
**VOO = DE** → Rancid defect **= OX**

→ Microbiological defect **= MI**

→ Rancid and microbiological defect **= OX/MI**

# RESULTS: THE MODELS

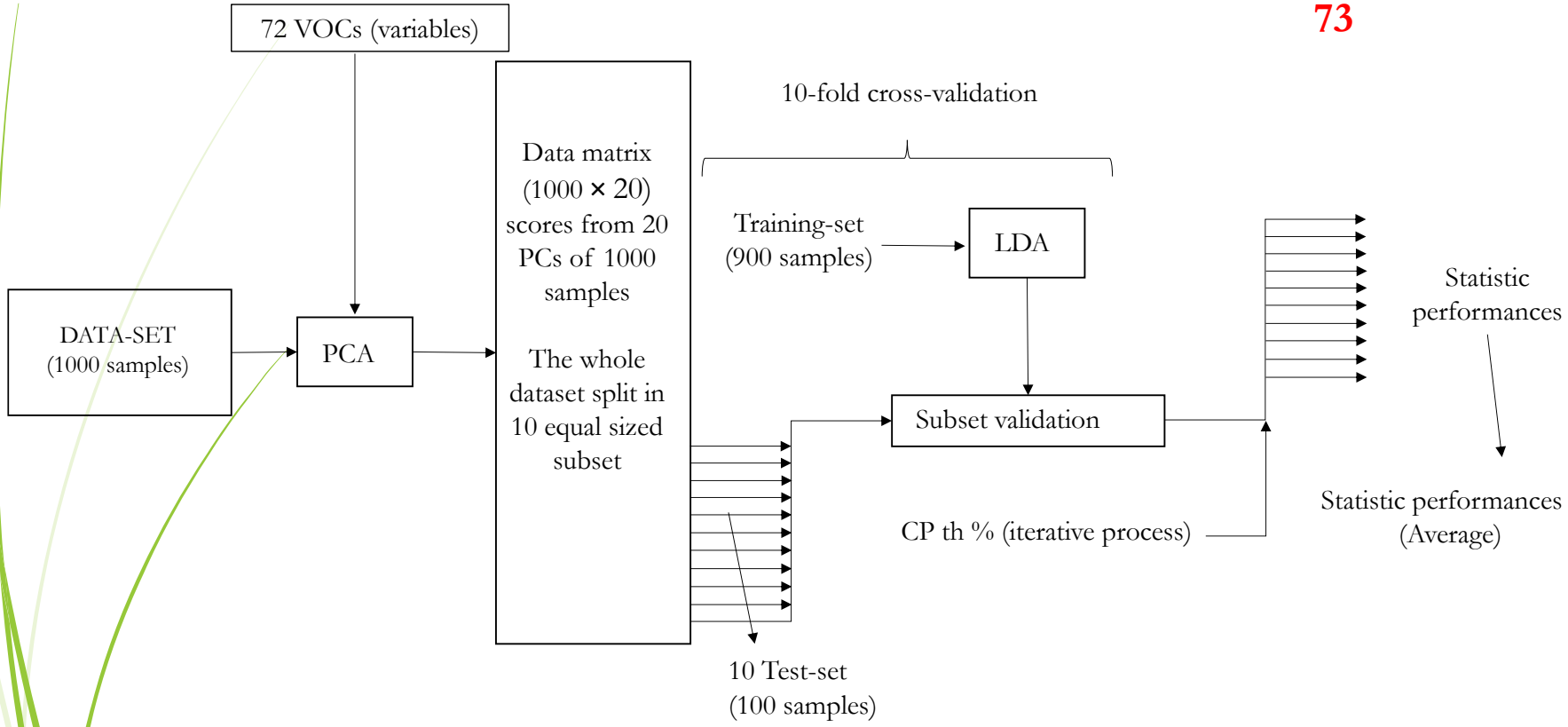
1. PCA-LDA
2. *t*-test-LDA
3. *t*-test-Discriminant Value
4. Chemical indices



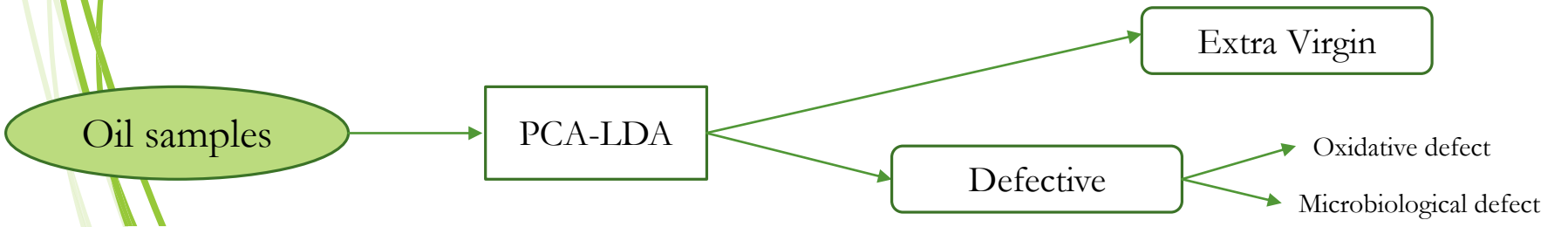
# MODEL 1: PCA-LDA

**PCA-LDA approach**

**Used Variables**
**73**




All variables initially retained ➔ no information excluded *a priori* ➔ The best results likely obtained



# MODEL 1: PCA-LDA - results

| **PCA-LDA** Test-set n° | CP th (%) | Not classified (%) | Among the classified samples (%) | |
|---|---|---|---|---|
| | | | *Correct classification (wrong defect)* | *Misclassified* |
| 1 | 39 | 10.0 | 82.9 (21.4) | 17.1 |
| 2 | 40 | 6.9 | 89.3 (11.6) | 10.7 |
| 3 | 42 | 7.7 | 85.0 (13.3) | 15.0 |
| 4 | 39 | 6.9 | 79.3 (9.1) | 20.7 |
| 5 | 41 | 6.2 | 82.8 (10.7) | 17.2 |
| 6 | 42 | 7.7 | 74.2 (11.7) | 25.8 |
| 7 | 43 | 8.5 | 79.0 (13.4) | 21.0 |
| 8 | 42 | 2.3 | 78.7 (13.4) | 21.3 |
| 9 | 42 | 3.1 | 88.1 (11.1) | 11.9 |
| 10 | 43 | 5.4 | 82.1 (8.9) | 17.9 |
| **Mean ± sd** | **41.3** | **6.5 ± 2.4** | **82.1 ± 4.6 (12.5 ± 3.5)** | **17.9 ± 4.6** |

High predictive capability
Robustness

| **Model** | (%) | *Correct classification (wrong defect)* | *Misclassified* |
|---|---|---|---|
| **1. PCA-LDA** | 5.3 | 83.5 (12.0) | 16.5 |

**Results obtained after external validatrion**

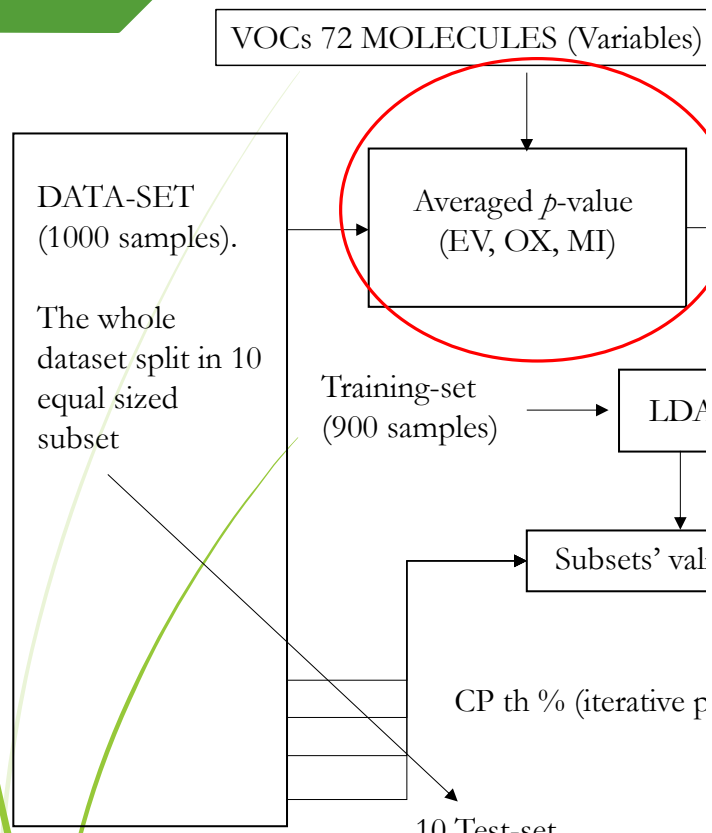
# OTHER MODELS

## AIMS

1. To propose simplified models, using a reduced number of volatile compounds and/or short statistical procedures

2. To gain qualitative information about the volatile molecules able to differentiate between samples

# MODEL 2: *t*-test-LDA




*t*-test-

| VOC |
|---|
| Deca-2,4-dienal |
| Propanol |
| Oct-1-en-3-ol |
| Heptanal |
| Pentan-2-ol |
| Nona-2,4-dienal |
| Isovaleraldehyde |
| 4-ethylphenol |
| *Z*-3-hexenal |
| Pent-1-en-3-ol |
| *E*-2-hexenal |
| Nonanal |
| Hexenal |
| Guaiacol |
| Octane |
| Butanoic acid |
| *E*-2-octenal |
| *E*-2-pentenal |
| Ethyl acetate |
| Ethanol |
| Methanol |
| Isobutanol |
| Ethyl propanoate |

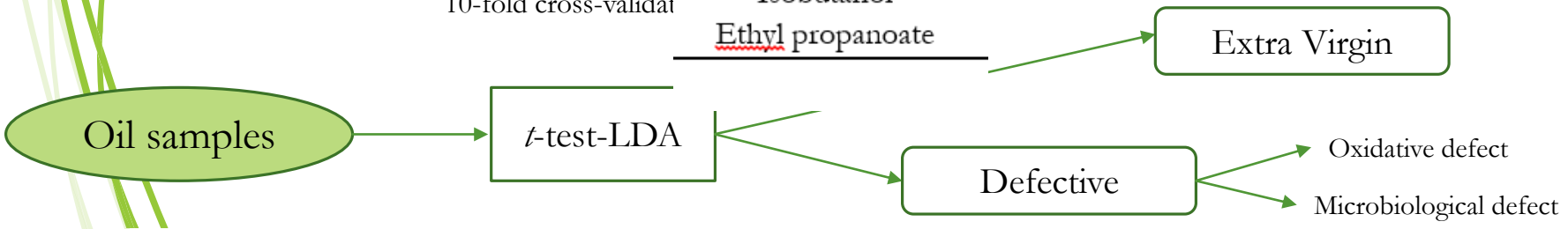
*t*-test-run three times:

1. Discrimination **EV** and **OX**
2. Discrimination **EV** and **MI**
3. Discrimination **OX** and **MI**

Statistic
performances

atistic performances
(Average)

**Used Variables**
73 ➔ **23**



# MODEL 2: *t*-test-LDA - results

| ***t*-test-LDA** Test-set n° | CP th (%) | Not classified (%) | Among the classified samples (%) | |
|---|---|---|---|---|
| | | | *Correct classification (wrong defect)* | *Misclassified* |
| 1 | 41 | 5.4 | 79.7 (18.7) | 20.3 |
| 2 | 42 | 10.0 | 82.1 (12.0) | 17.9 |
| 3 | 41 | 4.6 | 77.4 (8.9) | 22.6 |
| 4 | 40 | 10.8 | 82.8 (9.5) | 17.2 |
| 5 | 40 | 8.5 | 80.7 (8.4) | 19.3 |
| 6 | 40 | 5.4 | 70.7 (12.2) | 29.3 |
| 7 | 42 | 7.7 | 75.0 (10.8) | 25.0 |
| 8 | 40 | 3.1 | 76.2 (8.7) | 23.8 |
| 9 | 42 | 6.2 | 80.3 (9.0) | 19.7 |
| 10 | 42 | 2.3 | 81.1 (9.4) | 18.9 |
| **Mean ± sd** | **41** | **6.4 ± 2.8** | **78.6 ± 3.7 (10.8 ± 3.1)** | **21.4 ± 3.7** |

**Results obtained after full ten-fold cross validatrion**

| **Model** | Non-classified (%) | Among the classified samples (%) | |
|---|---|---|---|
| | | *Correct classification (wrong defect)* | *Misclassified* |
| **1. PCA-LDA** | 5.3 | 83.5 (12.0) | 16.5 |
| **2. *t*-test-LDA** | 4.7 | 79.7 (10.1) | 20.3 |

**Results obtained after external validatrion**

- Prediction capability only slightly lower than the PCA-LDA model
- Qualitative information on the VOCs more able in distriminating samples

| EV | MI-Fu | MI-Wi | MI-Mu | OX |
|---|---|---|---|---|
| Z-3-hexenal | Butanoic acid | Acetic acid | 6-methyhept-5-en-2-one | Octane |
| Hexa-2,4-dienal | Octane | Ethanol | 4-ethylphenol | 6-methylhept-5-en-2-one |
| *E*-2-hexenal | Ethyl butanoate | Ethyl acetate | Guaiacol | Heptanal |
| *E*-2-pentenol | Phenol | | Propanol | Deca-2,4-dienal |
| Isobutanol | 4-ethylguaiacol | | 2-methyl+3-methylbutanol | *E*-2-heptenal |
| Pent-1-en-3-ol | Isovaleraldehyde | | Pentan-2-ol | Nonanal |
| | Ethyl propanoate | | | *E*-2-octenal |
| | | | | *E*-2-decenal |
| | | | | Octanal |
| | | | | Valeraldehyde |
| | | | | Heptanol |
| | | | | Nonanol |
| | | | | Octanol |

DATA-SET
(1295 samples)

TRAINING
(1000 sam

# EL 3:

## scriminant value

| $I_{MI}$ | $I_{OX}$ | $I_{EV}$ | Classification (type of defect) |
|---|---|---|---|
| > 0.70 | > 1.00 | - | DE (OX + MI) |
| > 0.70 | < 1.00 | - | DE (MI) |
| < 0.70 | > 1.00 | - | DE (OX) |
| < 0.70 | < 1.00 | > 0.15 | EV |
| < 0.70 | < 1.00 | < 0.15 | Non-classified |

**CHEMICAL INDICES approach**

DATA-SET
(1218 samples)

VOCs 72 Molecules (variables)

| n° VOCs | DSV th *EV/DE* | DSV th *OX/MI* | Non-classified (%) | Among the classified samples (%) *Correct classification (wrong defect)* | *Misclassified* |
|---|---|---|---|---|---|
| 3 | 0.04 | 0.20 | 1.0 | 74.1 (10.8) | 25.9 |
| 5 | 0.40 | 1.60 | 8.0 | 80.1 (13.8) | 19.9 |
| 10 | 0.15 | 0.50 | 1.7 | 77.6 (11.5) | 22.4 |

M

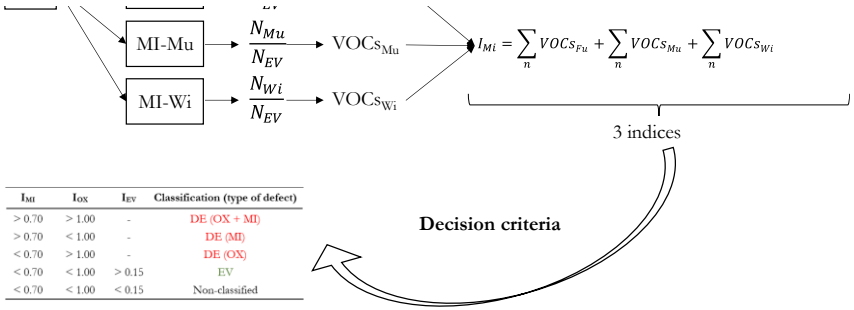
Chemical indices

TEST-SET
(295 samples)

**Used Variables**

73 ➔ 23 ➔ **10**

Predictive performance




| $I_{MI}$ | $I_{OX}$ | $I_{EV}$ | Classification (type of defect) |
|---|---|---|---|
| > 0.70 | > 1.00 | - | DE (OX + MI) |
| > 0.70 | < 1.00 | - | DE (MI) |
| < 0.70 | > 1.00 | - | DE (OX) |
| < 0.70 | < 1.00 | > 0.15 | EV |
| < 0.70 | < 1.00 | < 0.15 | Non-classified |

# COMPARISON OF RESULTS FROM THE 4 APPROACHES

| Model | Non-classified (%) | Among the classified samples (%) | |
|---|---|---|---|
| | | *Correct classification (wrong defect)* | *Misclassified* |
| **1. PCA-LDA** | 5.3 | 83.5 (12.0) | 16.5 |
| **2. *t*-test-LDA** | 4.7 | 79.7 (10.1) | 20.3 |
| **3. *t*-test-DSV** | 8.0 | 80.1 (13.8) | 19.9 |
| **4. chem-indices** | 8.7 | 77.0 (5.5) | 23.0 |

- The PCA-LDA model gave the best results
- All the model gave good results

Using a reliable analytical method and a large number of samples, allows building several robust models

THE THIRD MODEL SHOWED A VERY GOOD PREDICT CAPABILITY **ONLY <u>USING 10 VOLATILE MOLECULES</u>**

# QUALITATIVE INFORMATION

## MOLECULES USEFUL FOR DISCRIMINATING SPECIFIC CATEGORIES OR DEFECT DIFFERENT FROM THOSE USULLY REPORTED IN THE LITERATURE

**Extra virgin category**: iso-butanol and hexa-2,4-dienal

**Rancid defect**: alcohols as heptan-1-ol, octan-1-ol, nonan-1-ol

**Musty defect**: volatiles different from the C8 alcohols and ketones usually associated

## MOLECULES USEFUL FOR DISCRIMINATING BETWEEN EV AND DE IN ALL THE 3 PROPOSED APPROACHES

Octane

Pent-1-en-3-ol

Heptanal

Nonanal

4-ethylphenol

*Z*-3-hexenal

# Conclusions and future aims

The work provides useful chemometric tools only based on VOCs quantification, easily usable in testing laboratories for supporting panel test in virgin olive oil classification

- The PCA-LDA model is proposed as the best one for samples' classification
- The *t*-test-Discriminant Value approach could be a useful alternative simplifying the analytical work.
- The approach with chemical indices could be applied with the further goal of discriminating between the different types of defects in the defective samples.

# Future perspectives

In the next step, the proposed approaches have to be validated by interlaboratory tests and involving several panels, then the approaches will be suitable for routine use by the olive oil companies

# THANK YOU FOR YOUR ATTENTION

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