



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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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

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 technique, 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

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**

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 \leq 0.8 GRAMS PER 100 GRAMS
MEDIAN OF DEFECTS = 0
MEDIAN OF FRUITY > 0
- **VIRGIN OLIVE OIL:** FREE ACIDITY \leq 0.8 GRAMS PER 100 GRAMS
MEDIAN OF DEFECTS < 3.5
MEDIAN OF FRUITY > 0
- **LAMPANTE VIRGIN OLIVE OIL:** FREE ACIDITY > 0.8 GRAMS PER 100 GRAMS OR
MEDIAN OF DEFECTS > 3.5
MEDIAN OF FRUITY \geq 0

Not fit for consumption

The panel test



**INTERNATIONAL
OLIVE
COUNCIL**

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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**

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

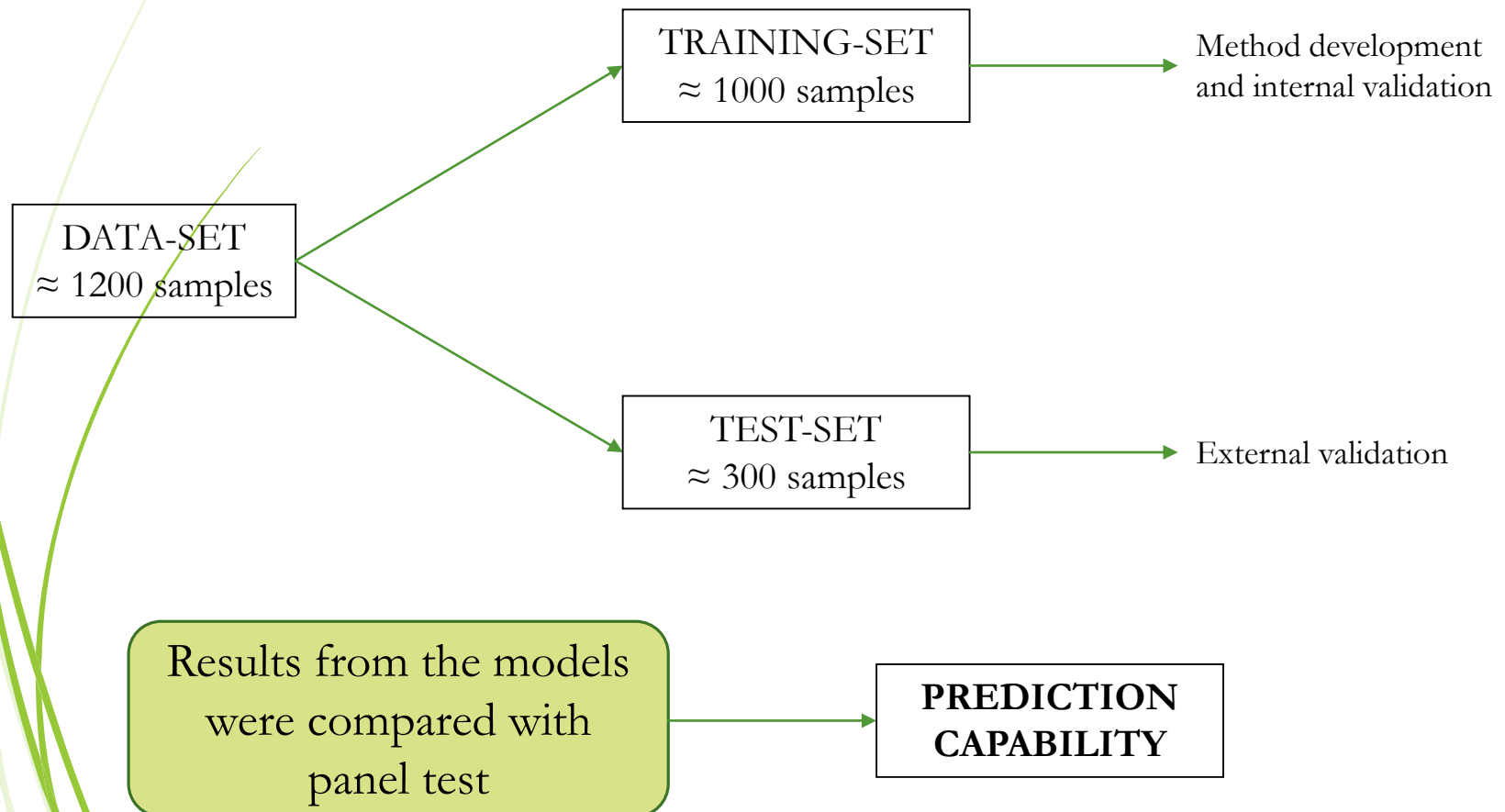
VOO = DE

Rancid defect = **OX**

Microbiological defect = **MI**

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

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

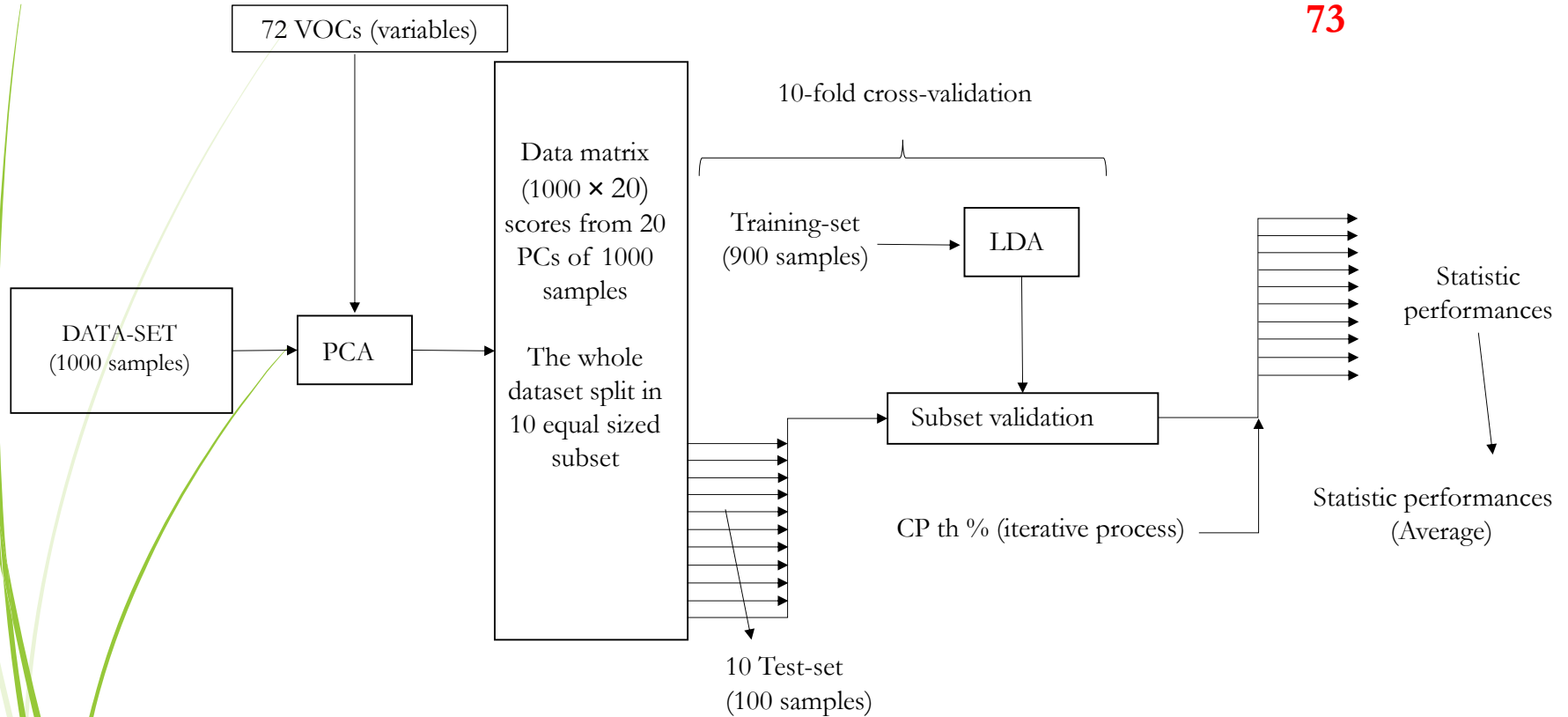


MODEL 1: PCA-LDA

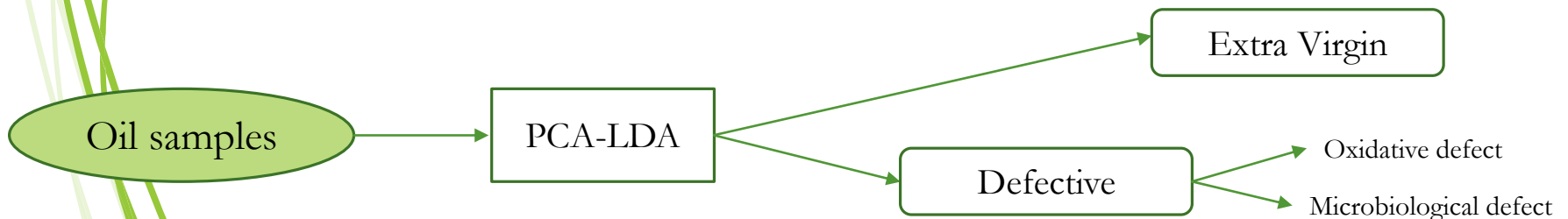
Porto – 2019-06-18

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 (%)	
			<i>Correct classification (wrong defect)</i>	<i>Misclassified</i>
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	CP th (%)	Not classified (%)	<i>Correct classification (wrong defect)</i>	<i>Misclassified</i>
1. PCA-LDA	5.3	5.3	83.5 (12.0)	16.5

Results obtained after external validation

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

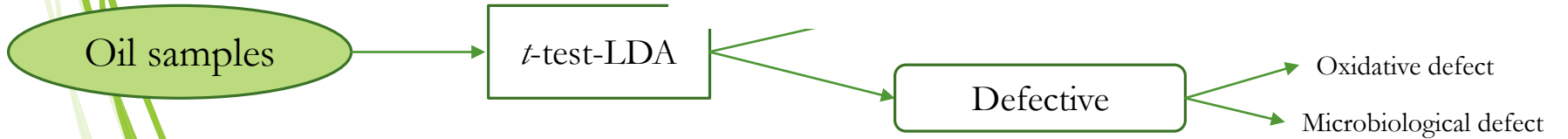
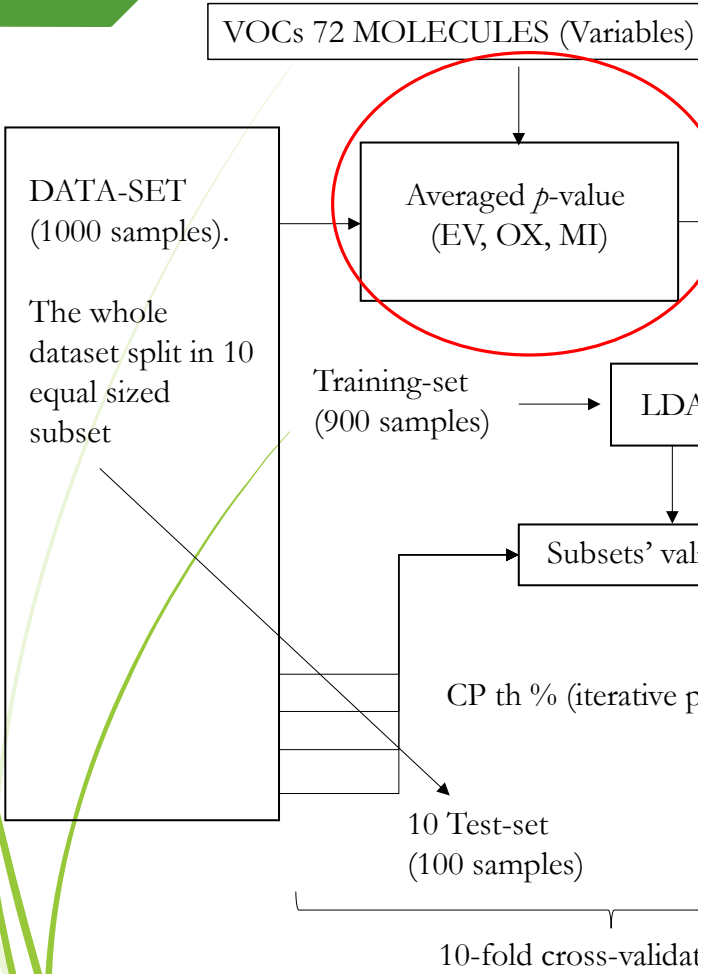
t-test-

VOC
Deca-2,4-dienal
<u>Propanol</u>
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
<u>Hexenal</u>
Guaiacol
<u>Octane</u>
Butanoic acid
E-2-octenal
E-2-pentenal
<u>Ethyl acetate</u>
<u>Ethanol</u>
<u>Methanol</u>
Isobutanol
<u>Ethyl propanoate</u>

- t*-test-run three times:
1. Discrimination **EV** and **OX**
 2. Discrimination **EV** and **MI**
 3. Discrimination **OX** and **MI**

Used Variables
73 → 23

Statistic performances
↓
Statistic performances (Average)



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

<i>t</i> -test-LDA Test-set n°	CP th (%)	Not classified (%)	Among the classified samples (%)	
			<i>Correct classification (wrong defect)</i>	<i>Misclassified</i>
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 validation

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

Results obtained after external validation

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

EV	MI-Fu	MI-Wi	MI-Mu	OX
Z-3-hexenal	Butanoic acid	Acetic acid	6-methylhept-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

EL 3: discriminant value

DATA-SET
(1295 samples)

TRAINING
(1000 samples)

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)

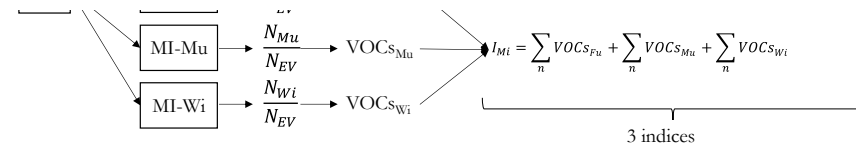
n° VOCs	DSV th		Non-classified (%)	Among the classified samples (%)	
	EV/DE	OX/MI		Correct classification	wrong defect
3	0.04	0.20	1.0	74.1 (10.8)	25.9
5	0.40	1.60	8.0	80.1 (13.8)	19.9
10	0.15	0.50	1.7	77.6 (11.5)	22.4

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

Decision criteria

3 indices

COMPARISON OF RESULTS FROM THE 4 APPROACHES

Model	Non-classified (%)	Among the classified samples (%)	
		<i>Correct classification (wrong defect)</i>	<i>Misclassified</i>
1. PCA-LDA	5.3	83.5 (12.0)	16.5
2. <i>t</i> -test-LDA	4.7	79.7 (10.1)	20.3
3. <i>t</i> -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 USING 10 VOLATILE MOLECULES

QUALITATIVE INFORMATION

MOLECULES USEFUL FOR DISCRIMINATING SPECIFIC CATEGORIES OR DEFECT DIFFERENT FROM THOSE USUALLY 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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