Innovative Postgraduate Education in The Field of Environment Protection: Methods and Tools

Food authentication by fingerprinting approaches

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BIO Presenter

- Master degree in Physics
- PhD in Food Biotechnology
- Main research activity:

Food authentication,
Biochemistry,
Plant physiology,
Chemometrics.

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Modernized/New Developed Courses involved

- Environmental Statistics
- Food safety risk assessment

Prerequisites

- Chemistry
- Biology
- Experimetal errors theory and basic statistics

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Outcomes

- The students will acquire the capacity to analyze complex data matrixes with special reference to food quality and authentication

- The students will learn how to use and apply the multivariate statistical analysis to resolve food related problems

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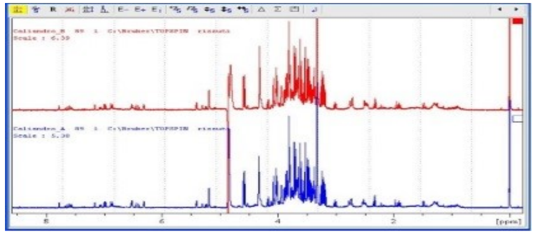
Involved analitical techniques

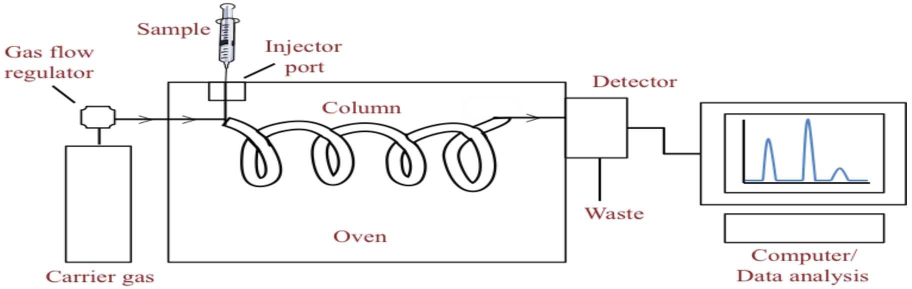
Spectroscopic

Identify functional groups



Separation

Convert a mixture of compounds into pure constituents



Mass Spectrometry

Identify heteromolecules, deduce molecular weight and formula

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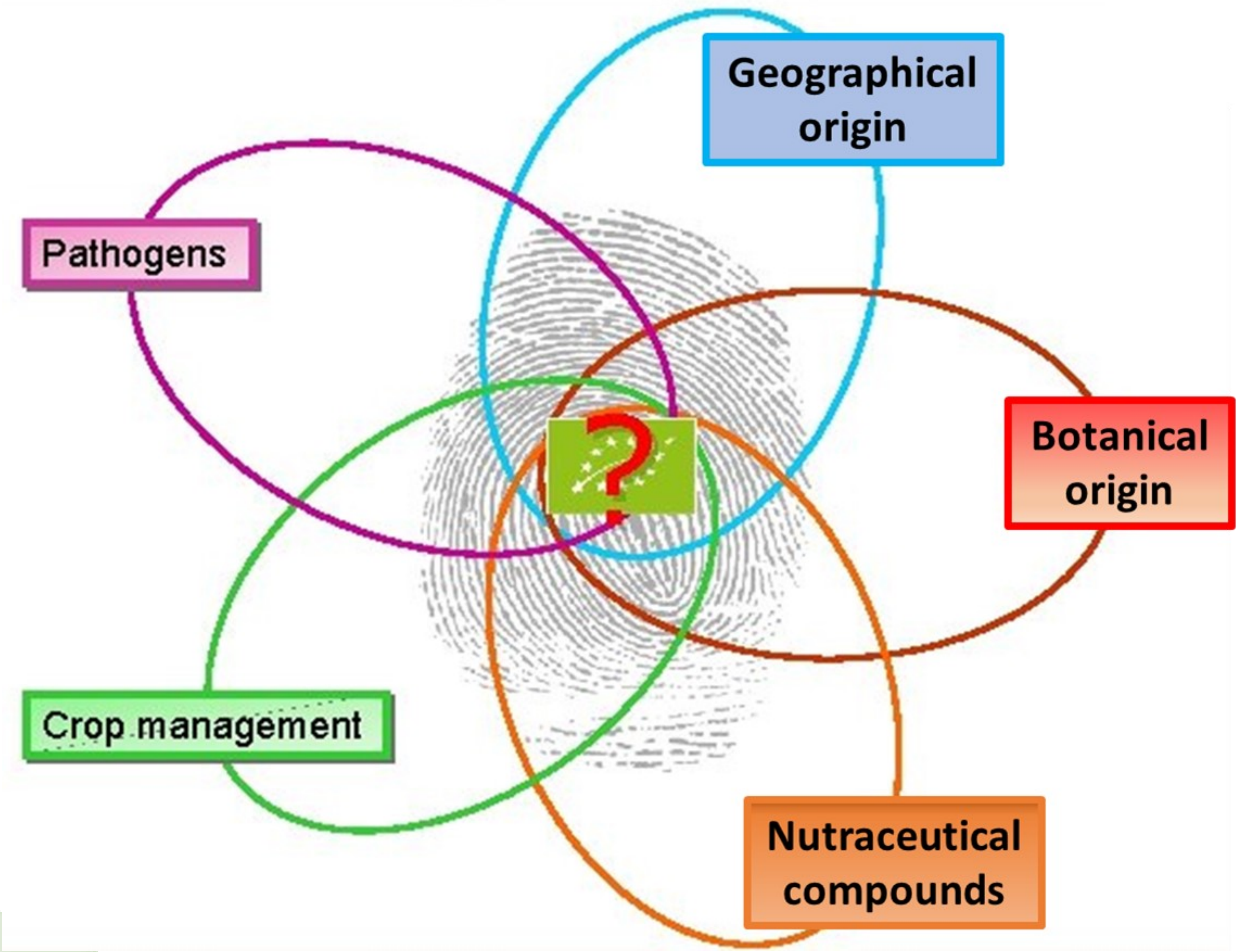
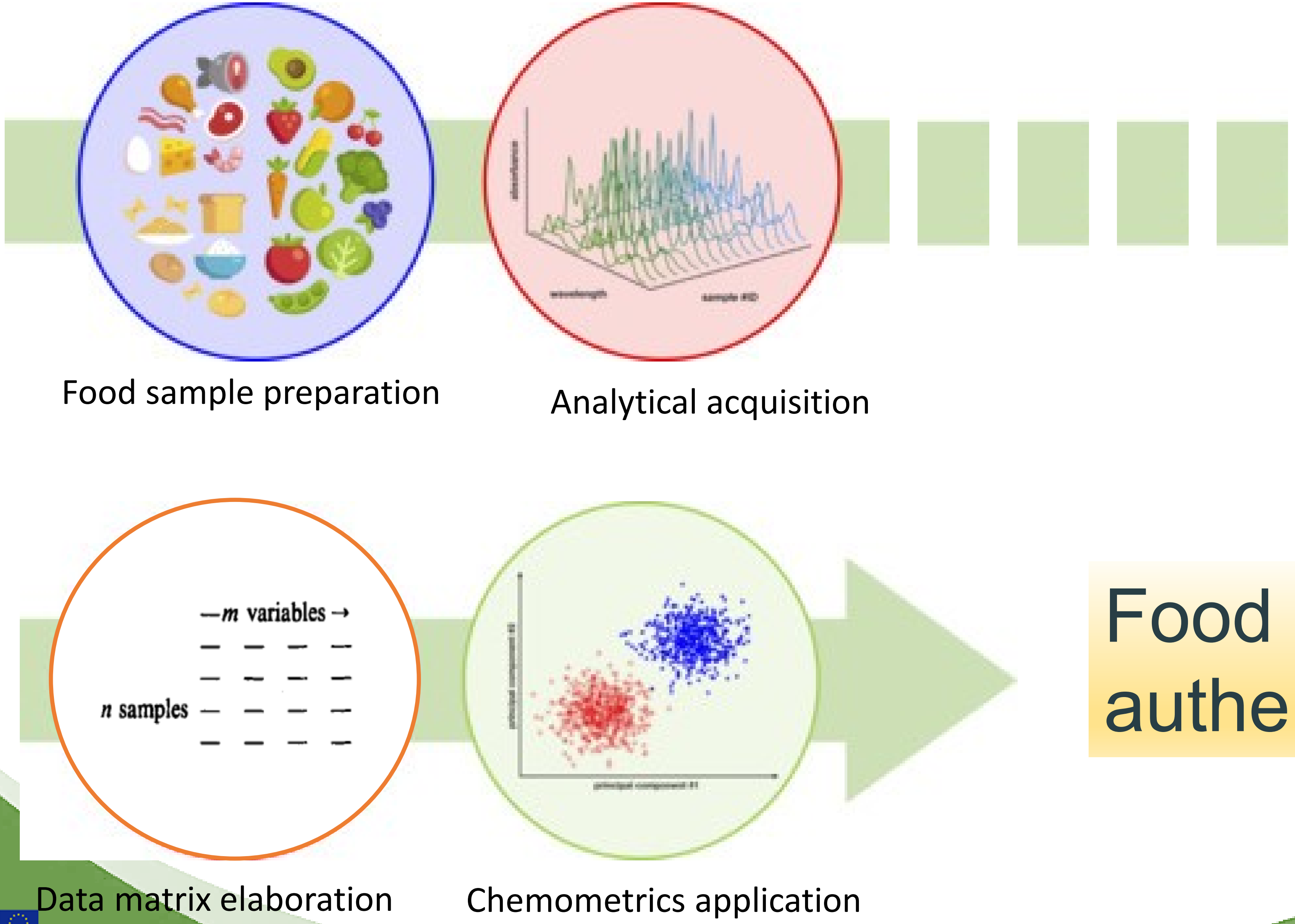
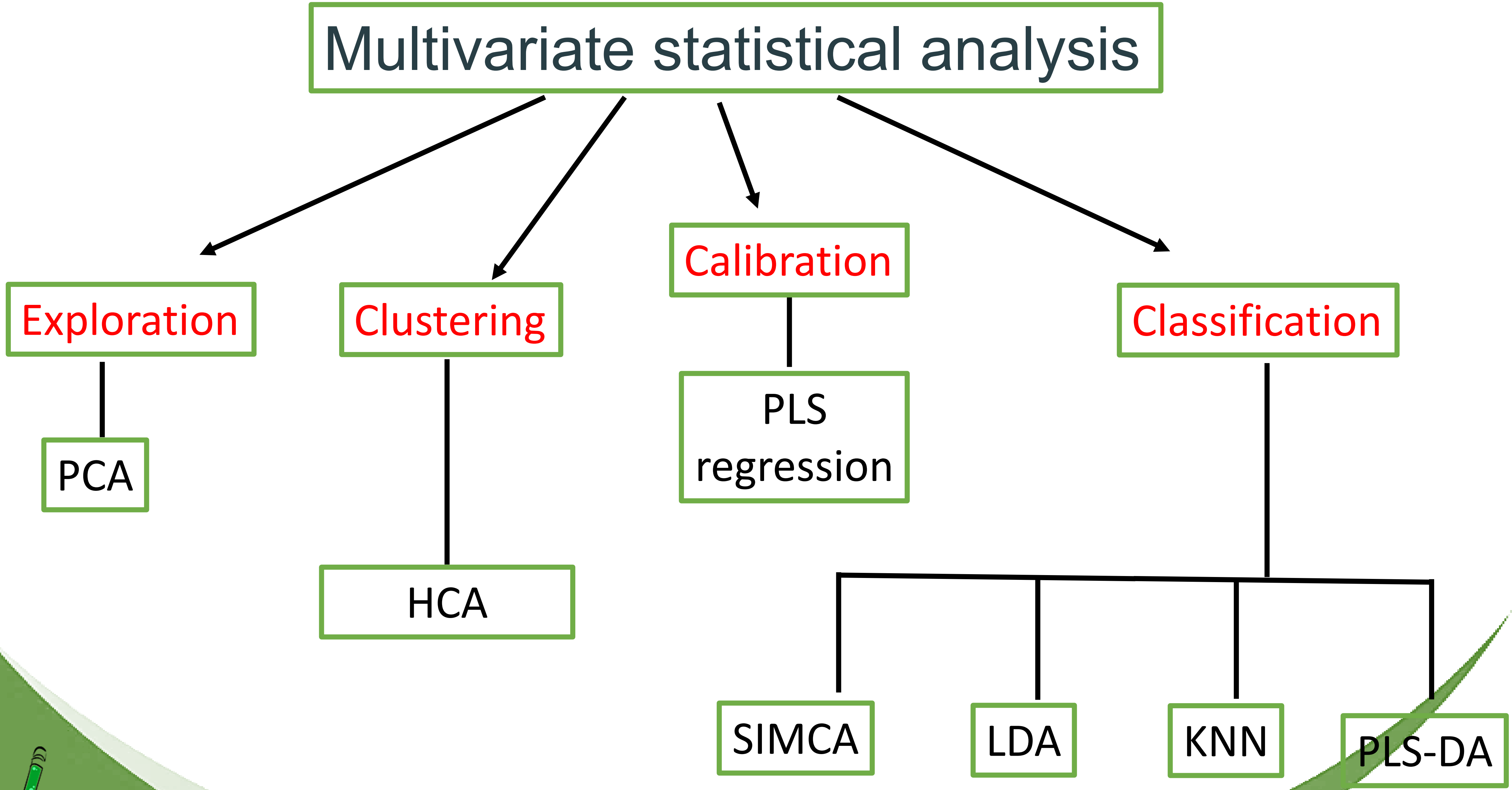
What can we extract by fingerptinting?



Analytical operations



Food authentication


Multivariate statistical analysis
Exploration
Clustering
Calibration
Classification
PCA
HCA
PLS regression
SIMCA
LDA
KNN
PLS-DA

Application 1: Quality evaluation of acerola phenotypes

Sampling:
- *103 phenotypes*
- *309 samples*
- *12 variables*

Fruit size characteristics and color parameters of 103 acerola phenotypes

Phenotypes	Fruit weight (g)	Fruit volume (cm³)	Pulp yield (%)	Longitudinal diameter (mm)	Transverse diameter (mm)	Brightness L	Color a	Color b
1	3.79 ± 0.04	3.9 ± 0.32	72.0 ± 0.7	16.3 ± 0.2	19.3 ± 0.2	41.7 ± 1.8	41.6 ± 1.1	31.4 ± 1.8
2	5.52 ± 0.27	5.6 ± 0.30	67.3 ± 2.9	18.9 ± 0.2	22.1 ± 0.2	44.2 ± 1.2	44.5 ± 0.9	31.6 ± 1.6
3	4.24 ± 0.28	4.7 ± 0.31	69.0 ± 2.6	17.9 ± 0.2	20.5 ± 0.3	47.2 ± 1.4	41.7 ± 1.5	37.8 ± 1.2
4	6.05 ± 0.16	5.5 ± 0.21	70.1 ± 1.0	18.3 ± 0.3	22.6 ± 0.4	34.7 ± 2.1	37.3 ± 2.4	21.0 ± 2.9
5	4.74 ± 0.14	4.8 ± 0.08	66.4 ± 5.0	18.0 ± 0.2	20.5 ± 0.2	29.1 ± 1.5	33.0 ± 2.8	15.5 ± 2.5
6	5.52 ± 0.14	5.6 ± 0.46	59.1 ± 9.0	19.4 ± 0.3	22.1 ± 0.3	34.6 ± 1.4	34.7 ± 2.5	17.5 ± 1.9
7	6.20 ± 0.21	6.5 ± 0.24	63.9 ± 0.5	18.8 ± 0.3	23.1 ± 0.2	28.0 ± 0.7	22.3 ± 1.2	9.3 ± 1.1
8	4.62 ± 0.14	4.8 ± 0.22	75.3 ± 1.5	18.7 ± 0.2	20.3 ± 0.2	34.5 ± 1.8	41.1 ± 1.8	21.8 ± 3.0
9	7.26 ± 0.51	6.8 ± 0.60	75.3 ± 0.8	20.4 ± 0.4	24.1 ± 0.5	36.0 ± 5.0	29.8 ± 2.3	16.1 ± 2.0
10	5.08 ± 0.18	5.0 ± 0.25	69.6 ± 1.3	18.6 ± 0.9	20.9 ± 0.4	29.5 ± 1.2	24.8 ± 1.9	10.6 ± 1.4
11	8.21 ± 0.39	8.5 ± 0.50	66.3 ± 1.6	21.3 ± 0.2	26.0 ± 0.3	30.4 ± 2.1	29.2 ± 2.5	15.0 ± 2.7
12	3.95 ± 0.11	4.2 ± 0.19	77.8 ± 0.7	16.7 ± 0.2	19.4 ± 0.3	37.0 ± 1.9	33.3 ± 2.6	21.3 ± 2.6
13	8.85 ± 0.33	8.4 ± 0.13	74.7 ± 3.5	21.6 ± 0.2	26.6 ± 0.3	36.1 ± 2.7	37.1 ± 3.1	25.1 ± 3.8
14	4.79 ± 0.32	4.5 ± 0.10	77.7 ± 0.4	18.8 ± 0.2	20.3 ± 0.3	39.7 ± 1.5	46.1 ± 3.0	32.5 ± 3.4
90	5.48 ± 0.22	6.0 ± 0.23	80.5 ± 2.2	18.0 ± 0.2	22.0 ± 0.3	27.1 ± 0.9	18.6 ± 2.4	8.0 ± 0.7
91	5.63 ± 0.12	6.0 ± 0.23	74.5 ± 1.2	18.0 ± 0.2	22.1 ± 0.4	27.8 ± 1.2	26.8 ± 1.5	9.0 ± 0.9
92	4.14 ± 0.19	3.9 ± 0.19	76.4 ± 1.3	16.8 ± 0.4	19.5 ± 0.3	29.1 ± 2.9	18.3 ± 2.0	11.9 ± 2.2
93	4.49 ± 0.18	4.8 ± 0.43	90.1 ± 1.0	16.6 ± 0.2	20.5 ± 0.2	21.5 ± 0.7	19.1 ± 1.6	5.0 ± 0.6
94	4.55 ± 0.08	5.2 ± 0.16	89.1 ± 0.5	15.8 ± 0.2	19.6 ± 0.3	26.3 ± 1.6	27.2 ± 1.0	9.9 ± 1.4
95	4.48 ± 0.15	5.2 ± 0.37	83.7 ± 0.4	16.3 ± 0.3	20.6 ± 0.4	35.0 ± 2.2	33.0 ± 1.8	16.0 ± 1.6
96	4.54 ± 0.21	4.4 ± 0.16	85.7 ± 0.5	17.3 ± 0.2	20.8 ± 0.3	26.5 ± 1.2	27.4 ± 1.7	8.6 ± 0.7
97	4.72 ± 0.19	4.8 ± 0.33	81.8 ± 0.9	16.8 ± 0.2	19.6 ± 0.3	27.9 ± 2.0	24.3 ± 2.2	8.2 ± 1.7
98	5.75 ± 0.39	4.7 ± 0.30	81.8 ± 0.4	17.7 ± 0.4	20.9 ± 0.5	33.0 ± 1.6	32.2 ± 1.7	14.0 ± 1.3
99	4.11 ± 0.23	4.2 ± 0.20	82.3 ± 2.3	16.6 ± 0.3	18.2 ± 0.4	31.3 ± 3.0	18.0 ± 1.7	10.3 ± 1.9
100	5.96 ± 0.20	6.2 ± 0.12	68.8 ± 4.8	21.5 ± 0.2	21.3 ± 0.3	34.2 ± 1.4	20.4 ± 1.7	11.6 ± 0.9
101	4.57 ± 0.15	4.5 ± 0.35	85.0 ± 1.4	17.4 ± 0.3	20.4 ± 0.4	33.4 ± 2.6	19.5 ± 1.6	12.3 ± 2.5
102	6.28 ± 0.14	6.6 ± 0.35	77.8 ± 1.6	17.4 ± 0.2	22.6 ± 0.4	40.9 ± 2.3	17.9 ± 1.9	20.5 ± 1.9
103	4.65 ± 0.07	4.3 ± 0.19	82.4 ± 0.7	15.2 ± 0.2	19.2 ± 0.3	31.7 ± 1.9	17.6 ± 2.2	11.9 ± 1.5

Analytical acquisition and data matrix elaboration

- **Morphological + chemical traits**
- **data matrix: $M_{103 \times 12}$**
- **Multivariate classification analysis**
- **HCA (hierarchical cluster analysis)**

Biochemical characteristics of the fruits of 103 acerola phenotypes

Phenotypes	Vitamin C Content (mg/100 g pulp)	Soluble Solids Content (° Brix) (SSC)	Titratable Acidity (%) (TA)	pH	SSC/TA ratio	Total Polyphenols (mg/kg gallic acid)
1	1600 ± 100	7.10 ± 0.2	0.40 ± 0.10	3.47 ± 0.01	18.62 ± 1.97	1367 ± 14
2	550 ± 150	7.43 ± 0.2	1.30 ± 0.10	2.95 ± 0.05	5.76 ± 0.35	1236 ± 14
3	1000 ± 10	7.14 ± 0.2	1.90 ± 0.10	3.48 ± 0.01	3.77 ± 0.18	917 ± 3
4	2100 ± 100	11.86 ± 0.3	3.35 ± 0.15	3.34 ± 0.01	3.54 ± 0.08	1134 ± 36
5	1375 ± 25	9.22 ± 0.2	3.40 ± 0.10	3.28 ± 0.00	2.71 ± 0.08	1150 ± 34
6	1300 ± 100	7.10 ± 0.2	1.90 ± 0.10	3.23 ± 0.01	3.75 ± 0.16	1456 ± 24
7	650 ± 150	9.48 ± 0.4	3.20 ± 0.10	3.15 ± 0.00	2.97 ± 0.15	1088 ± 18
8	575 ± 25	7.94 ± 0.5	2.60 ± 0.10	2.82 ± 0.02	3.05 ± 0.17	1192 ± 28
9	790 ± 10	7.04 ± 0.1	2.90 ± 0.10	2.92 ± 0.02	2.43 ± 0.06	1582 ± 13
10	1100 ± 100	9.20 ± 0.3	1.95 ± 0.15	2.99 ± 0.01	4.73 ± 0.09	84 ± 2
11	1210 ± 10	12.12 ± 0.5	2.20 ± 0.10	3.16 ± 0.02	5.53 ± 0.30	1160 ± 17
12	950 ± 50	9.38 ± 0.2	2.30 ± 0.10	3.23 ± 0.03	4.08 ± 0.08	1438 ± 71
13	1400 ± 100	9.50 ± 0.2	2.90 ± 0.10	2.87 ± 0.01	3.28 ± 0.12	1202 ± 20
14	2025 ± 75	7.62 ± 0.4	3.40 ± 0.10	2.72 ± 0.02	2.19 ± 0.07	1853 ± 95
91	1095 ± 95	8.02 ± 0.3	3.60 ± 0.10	3.86 ± 0.02	2.23 ± 0.07	891 ± 34
92	1445 ± 255	10.08 ± 0.2	3.55 ± 0.05	3.66 ± 0.01	2.84 ± 0.06	1476 ± 125
93	1180 ± 20	8.35 ± 0.2	3.48 ± 0.08	3.54 ± 0.01	2.40 ± 0.07	1382 ± 15
94	1150 ± 150	9.13 ± 0.2	3.63 ± 0.02	3.48 ± 0.03	2.52 ± 0.05	2260 ± 48
95	1145 ± 5	10.14 ± 0.5	3.06 ± 0.06	3.85 ± 0.02	3.31 ± 0.17	1223 ± 31
96	1075 ± 25	7.12 ± 0.2	3.82 ± 0.02	3.37 ± 0.01	1.86 ± 0.06	1811 ± 50
97	800 ± 100	8.61 ± 0.3	3.11 ± 0.01	3.57 ± 0.02	2.77 ± 0.10	1558 ± 83
98	1350 ± 50	11.27 ± 0.5	3.63 ± 0.03	3.46 ± 0.02	3.10 ± 0.13	1403 ± 62
99	2625 ± 75	7.75 ± 0.2	3.10 ± 0.10	3.31 ± 0.01	2.50 ± 0.07	1197 ± 47
100	1295 ± 5	7.46 ± 0.1	3.63 ± 0.02	3.66 ± 0.01	2.06 ± 0.04	635 ± 0
101	1000 ± 50	6.50 ± 0.2	3.45 ± 0.05	3.37 ± 0.03	1.88 ± 0.05	1173 ± 20
102	675 ± 25	9.93 ± 0.2	3.12 ± 0.11	3.49 ± 0.01	3.19 ± 0.06	967 ± 41
103	870 ± 20	11.17 ± 0.3	3.81 ± 0.01	3.36 ± 0.00	2.93 ± 0.07	1681 ± 104

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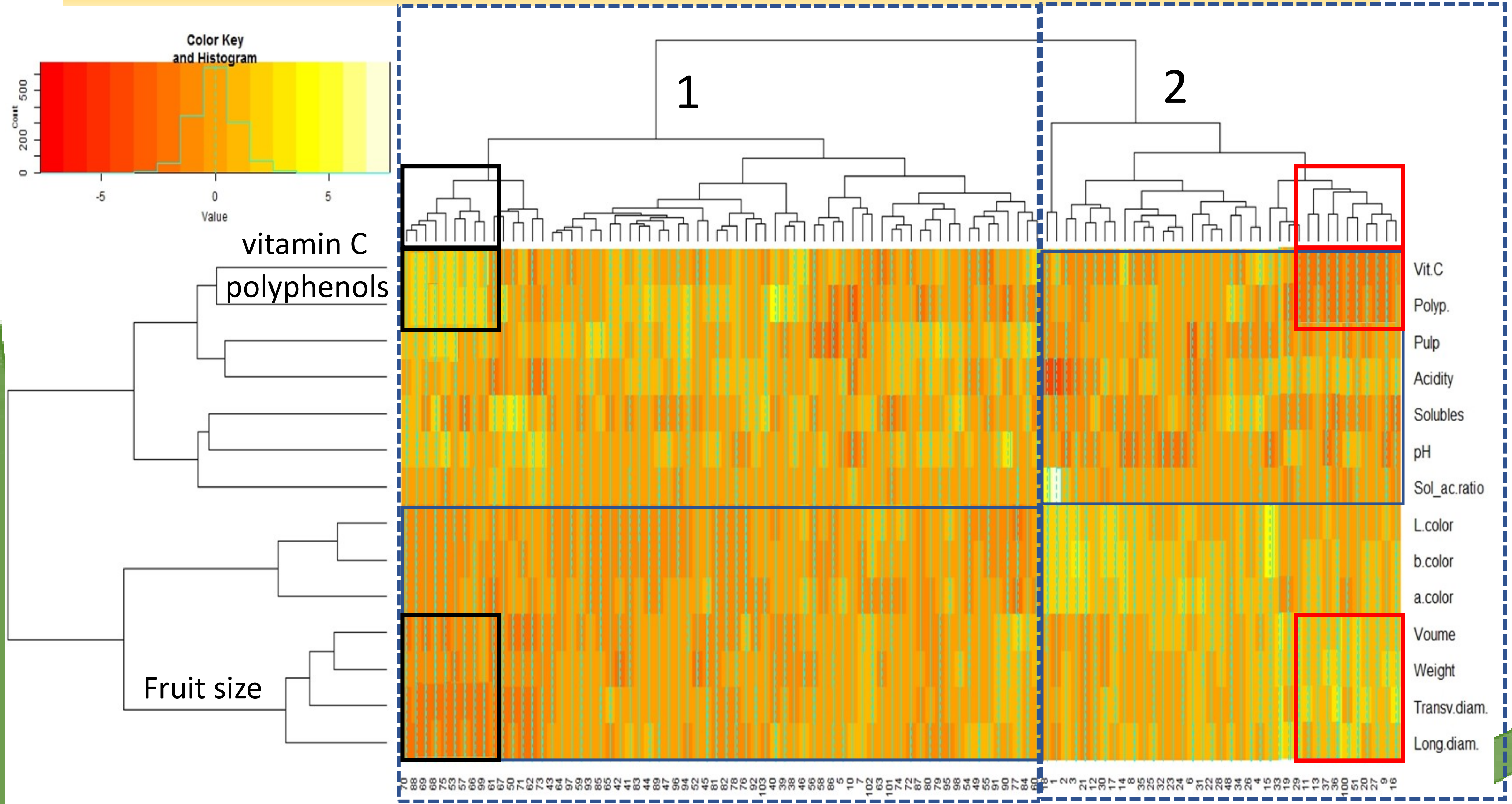
Unsupervised clustering: HCA

Heat map row and column dendrogram, based on hierarchical cluster analysis



103 acerola samples

Standardization:

$$z = \frac{x - \mu}{\sigma}$$

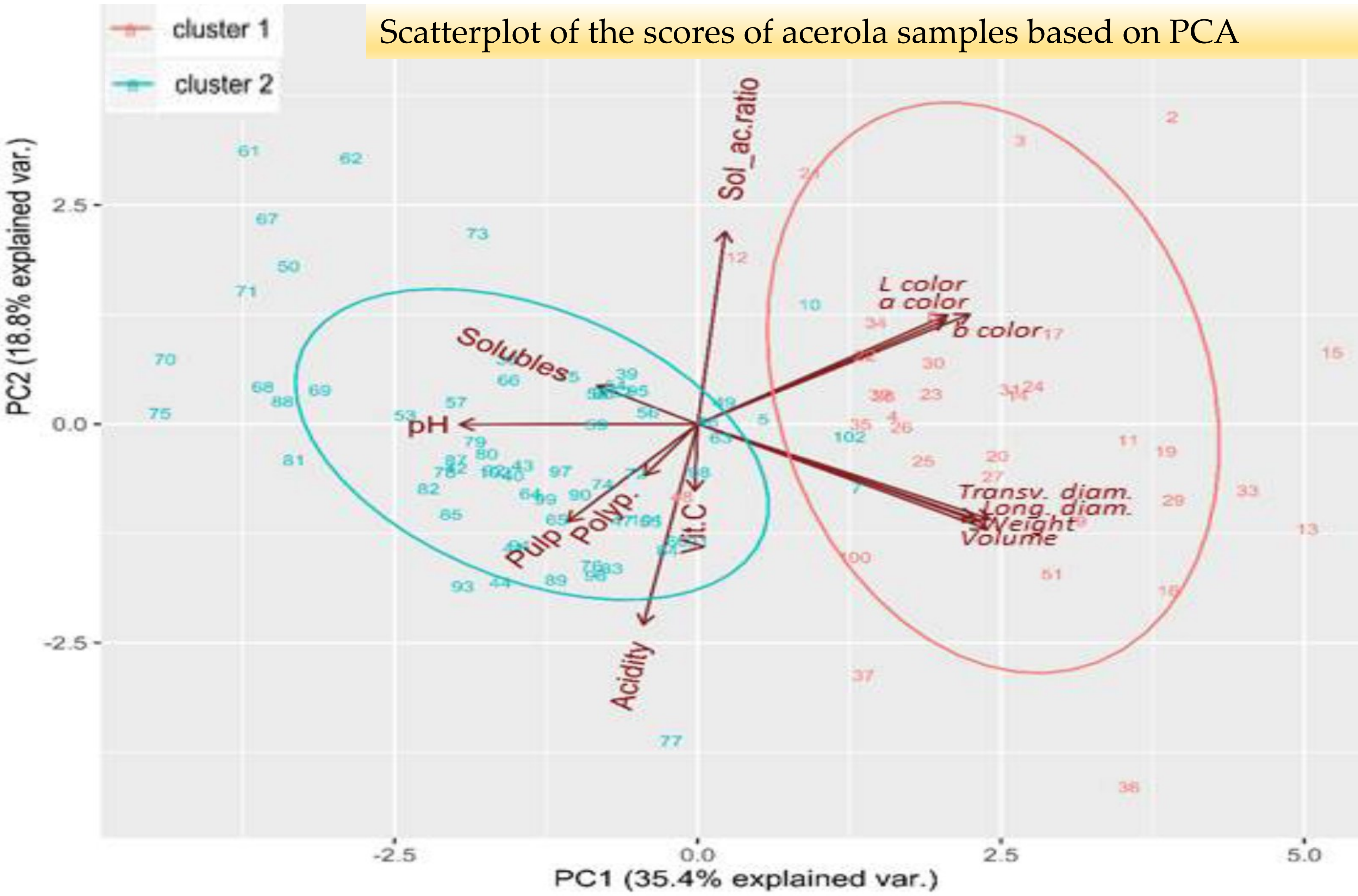
with mean:

$$\mu = \frac{1}{N} \sum_{i=1}^{N} (x_i)$$

and standard deviation

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - \mu)^2}$$

Explorative analysis: PCA



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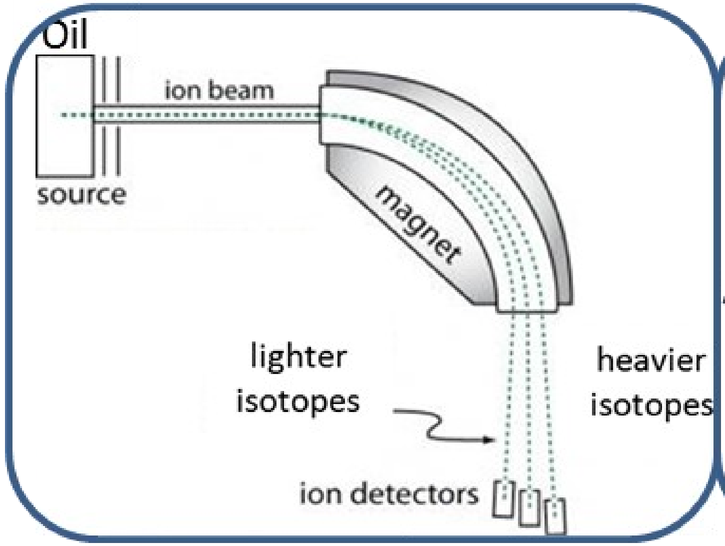
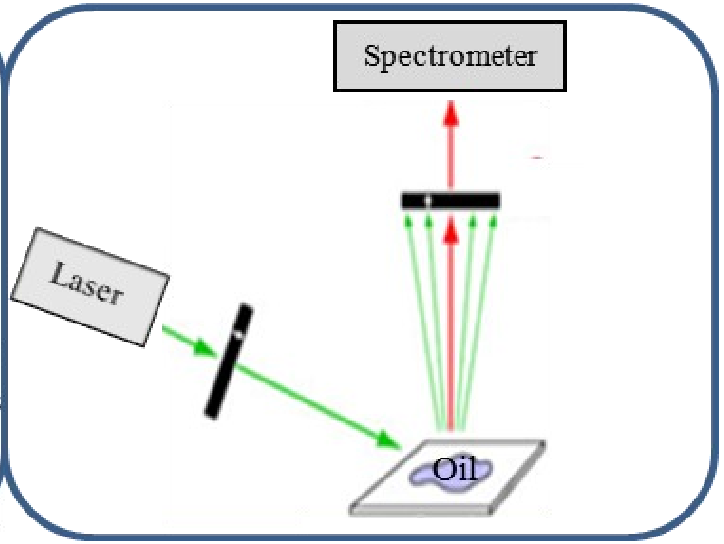
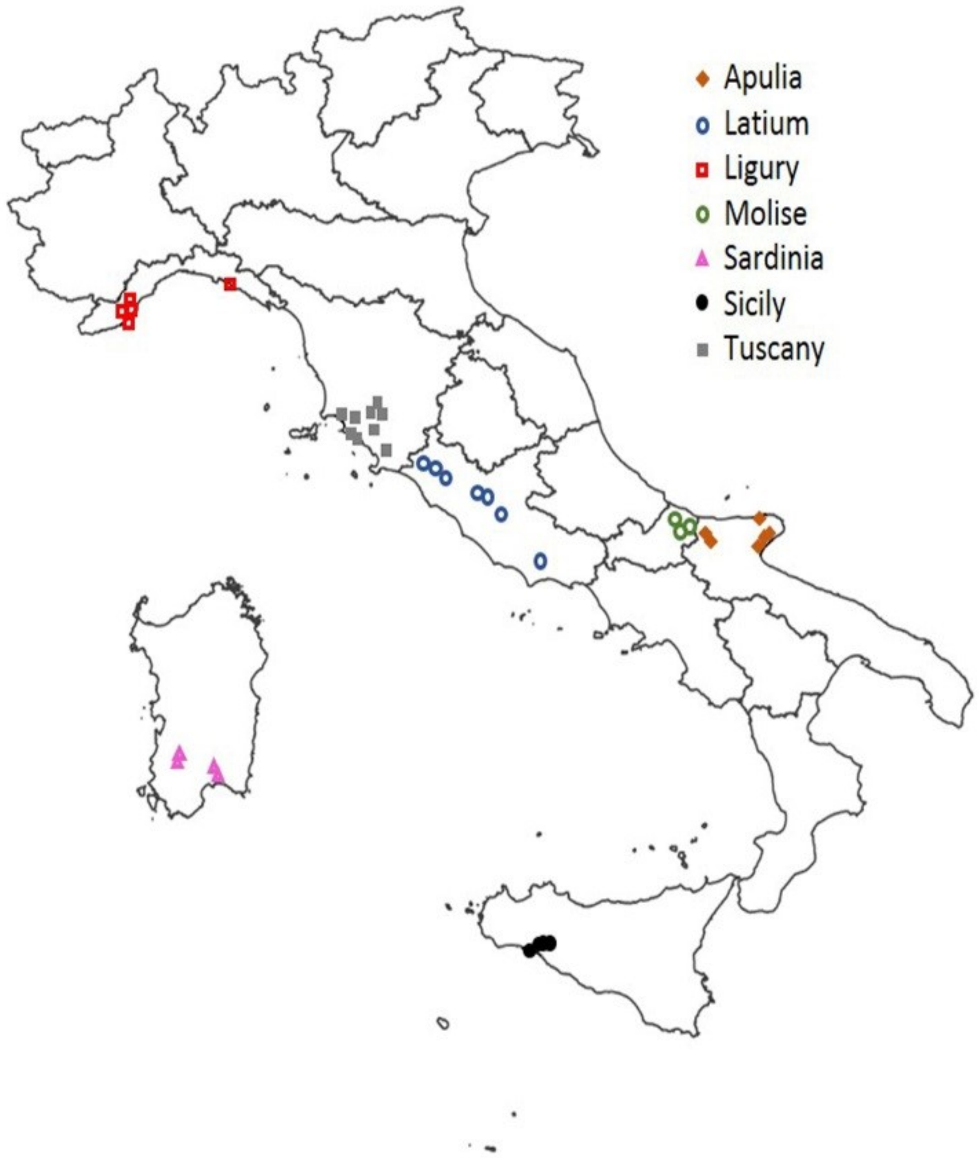
Application 2: geographical traceability of olive oil



Isotope Ratio Mass Spectrometry



Resonant Raman Spectroscopy



Sampling:

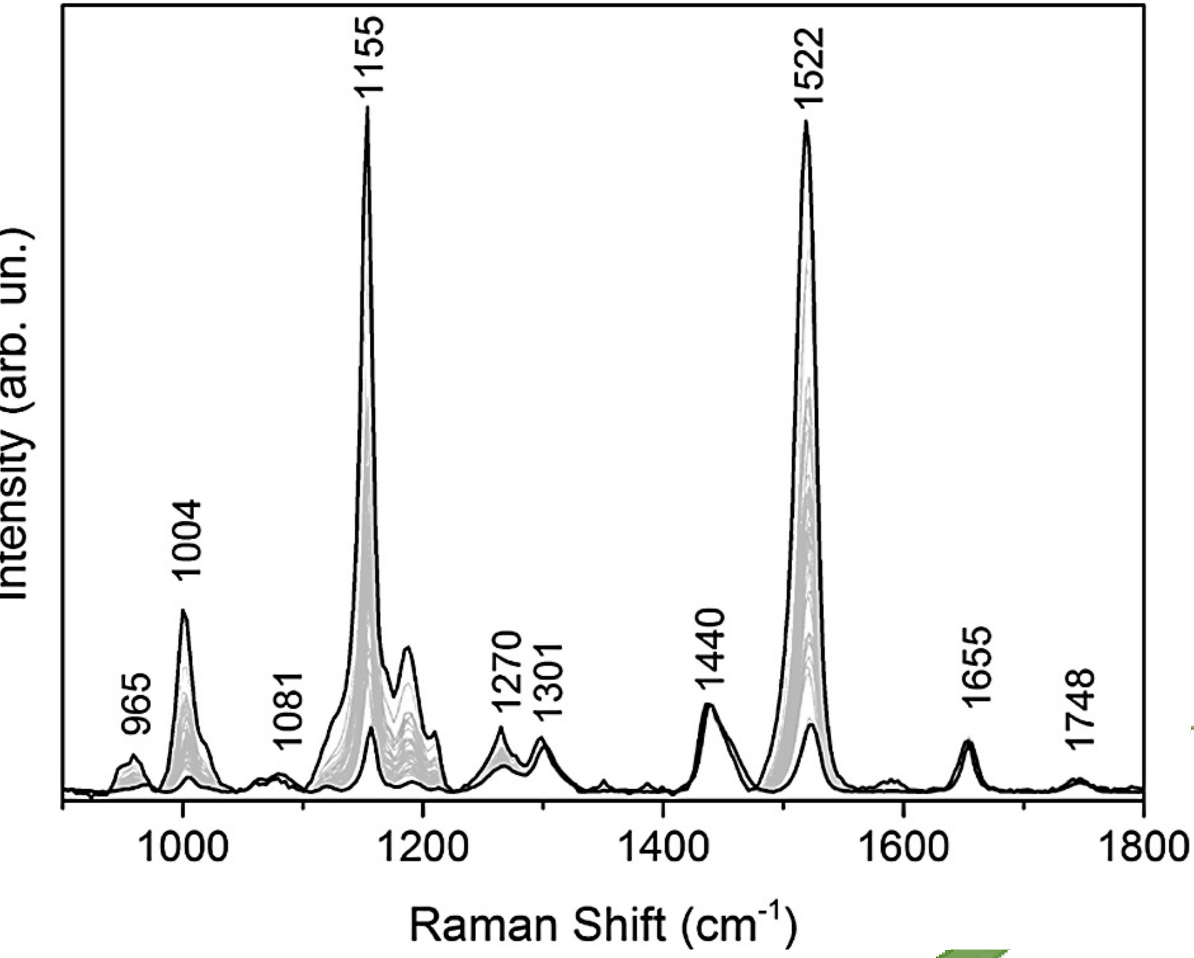
- *40 samples*
- *7 Italian regions*
- *More than 500 variables*

Analytical acquisition and data matrix elaboration

Stable isotope composition of oils

$\delta^{13}C$					$\delta^{18}O$				
Mean	SE	Min	Max	CI	Mean	SE	Min	Max	CI
−30.1	0.3	−30.4	−29.8	–	21.5	1.4	20.1	22.8	–
−29.2	0.2	−30.0	−28.4	0.3	22.4	0.2	21.4	23.4	0.5
−29.8	0.1	−31.0	−28.5	0.2	22.9	0.1	21.7	24.2	0.2
−29.9	0.2	−31.1	−28.0	0.5	22.2	0.1	21.0	22.9	0.3
−30.3	0.2	−31.3	−29.0	0.4	21.9	0.2	20.7	23.9	0.5
−30.0	0.2	−31.3	−28.9	0.3	23.3	0.2	20.9	24.9	0.4
−29.1	0.2	−29.9	−28.4	0.6	24.1	0.2	23.5	24.5	0.4
−29.3	0.1	−30.2	−28.5	0.2	24.5	0.2	21.1	26.3	0.5
−31.6	0.1	−32.2	−31.3	0.3	19.1	0.1	18.7	19.5	0.3
−31.3	0.1	−31.6	−30.2	0.3	20.9	0.1	20.3	21.4	0.3
−29.9	0.2	−30.9	−29.1	0.5	22.3	0.1	21.7	22.8	0.3
−29.8	0.3	−31.4	−28.4	0.6	22.2	0.3	20.5	23.2	0.6
−30.4	0.2	−30.9	−29.0	0.4	21.6	0.3	20.9	23.8	0.6
−29.9	0.1	−31.1	−28.8	0.2	22.0	0.1	20.8	23.2	0.2
−30.2	0.2	−31.2	−28.9	0.3	22.9	0.2	20.9	25.5	0.4
−29.2	0.1	−29.9	−28.3	0.2	23.5	0.1	22.7	24.2	0.3
−29.2	0.1	−30.0	−28.8	0.3	23.9	0.3	22.9	24.9	0.6
−30.7	0.2	−31.6	−29.8	−0.3	20.7	0.1	20.0	21.1	−0.2
−29.5	0.2	−30.3	−28.5	−0.4	22.8	0.2	22.1	24.4	−0.5
−28.5	0.1	−29.4	−27.7	0.3	23.6	0.1	22.9	24.7	0.2
−28.7	0.1	−29.9	−27.8	−0.2	24.3	0.1	23.3	25.3	−0.2
−29.3	0.1	−29.9	−28.2	−0.4	22.7	0.2	21.3	23.4	−0.5
−29.0	0.2	−30.5	−27.8	0.5	23.3	0.2	21.7	24.5	0.4
−−28.7	0.2	−30.2	−27.2	−0.5	24.4	0.2	23.3	25.2	−0.4
−28.2	0.1	−28.8	−27.5	−0.2	25.1	0.3	24.2	27.9	−0.7
−30.7	0.2	−31.6	−29.8	−0.3	20.7	0.1	20.0	21.1	−0.2
−29.5	0.2	−30.3	−28.5	−0.4	22.8	0.2	22.1	24.4	−0.5
−28.5	0.1	−29.4	−27.7	0.3	23.6	0.1	22.9	24.7	0.2
−28.7	0.1	−29.9	−27.8	−0.2	24.3	0.1	23.3	25.3	−0.2
−29.3	0.1	−29.9	−28.2	−0.4	22.7	0.2	21.3	23.4	−0.5
−29.0	0.2	−30.5	−27.8	0.5	23.3	0.2	21.7	24.5	0.4
−−28.7	0.2	−30.2	−27.2	−0.5	24.4	0.2	23.3	25.2	−0.4
−28.2	0.1	−28.8	−27.5	−0.2	25.1	0.3	24.2	27.9	−0.7

Raman spectra of oils

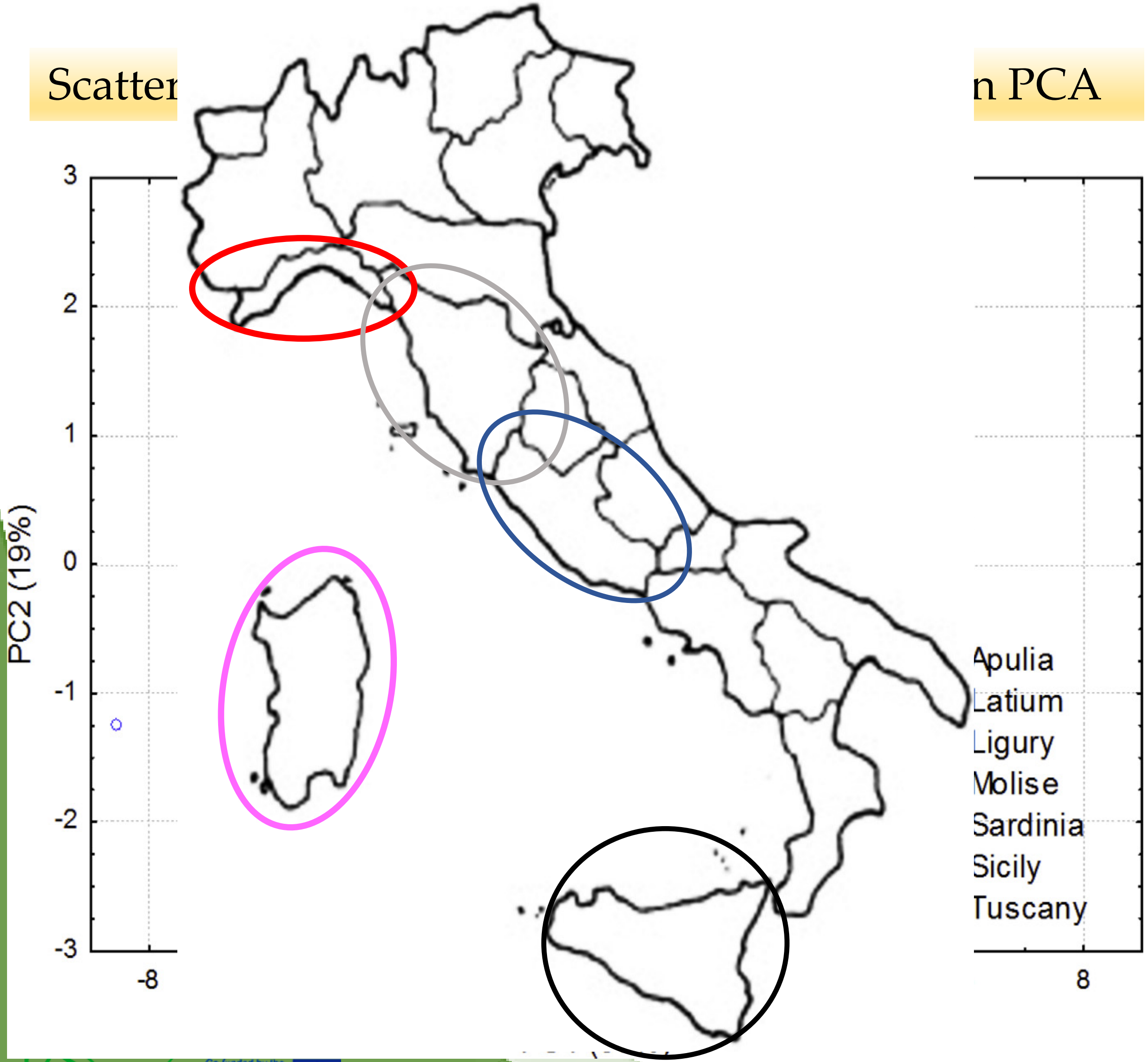


- Complex chemical matrix: $M_{40 \times 500}$
- Multivariate classification analysis

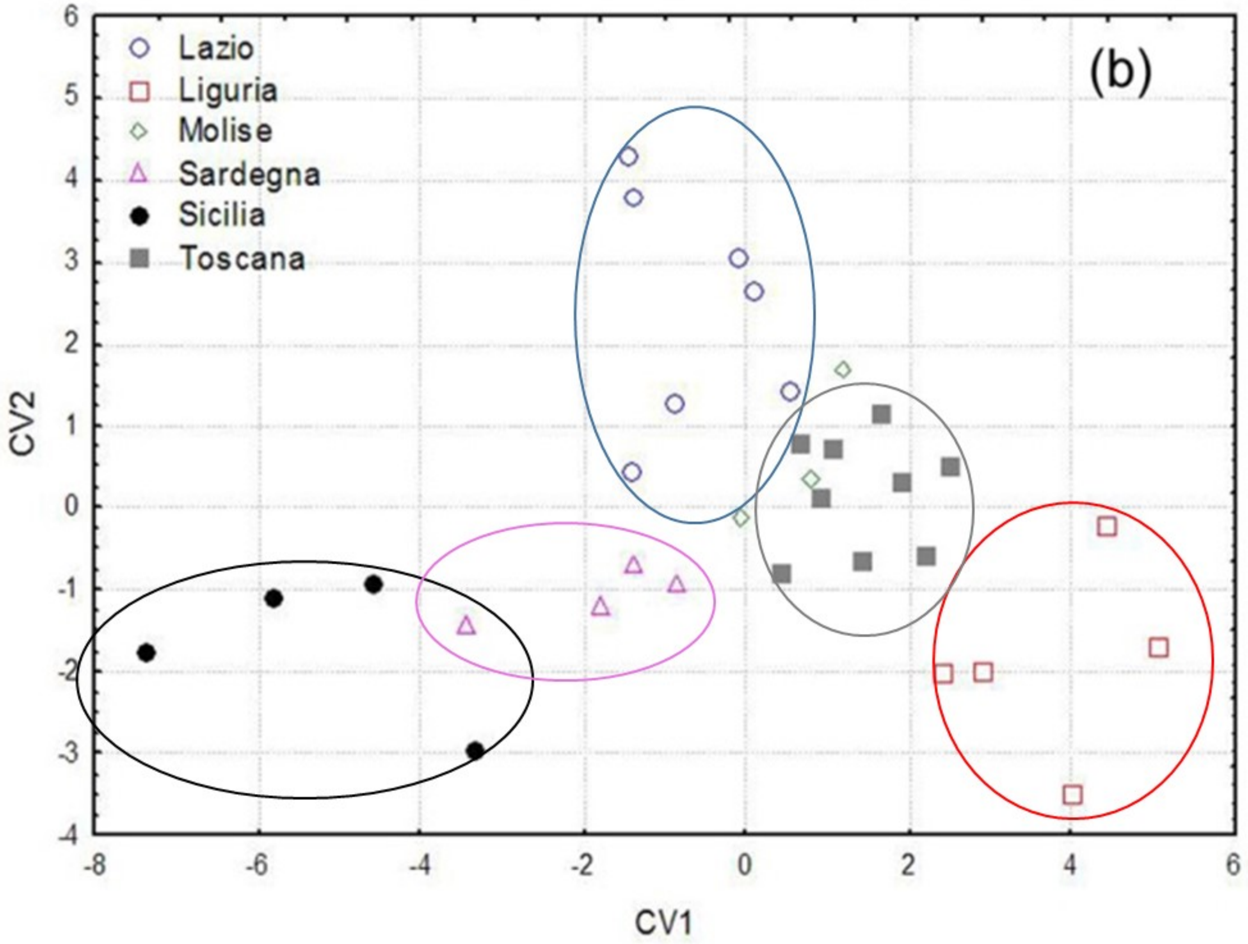
From unsupervised exploration…

…to supervised classification

Scatter… n PCA



Scatterplot of the scores of oil samples based on LDA



Conclusions

- Food authentication by fingerprinting approach enable the characterization of foodstuff in terms of nutraceutical compounds, geographical and botanical origin, crop management, etc.
- The great amount of data provided by the different analytical approaches needs the use of multivariate statistical methods
- The application of a particular statistical tool varied in relation to the specific case study in order to understand and model the information contained in the complex matrixes
- It is of the great importance to develop other novel analytical approaches to define efficient markers and indicators for food authenticity and quality

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THANK YOU !

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