An ^{1}H NMR-Chemometric model for the classification of Italian Extra Virgin Olive Oils

SISSG CONGRESS

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SISSG OLI e GRASSI ALIMENTARI INNOVAZIONE e SOSTENIBILITÀ NELLA PRODUZIONE E NEL CONTROLLO

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EVOOs: a precious Italian product

Main olive oil producers

Spain (1129233 tons)

Italy (336581 tons)

Greece (290476 tons)

Tunisia (239500 tons)

Turkey (217800 tons)

Morocco (204200 tons)

[FAOSTAT DATA, referred to 2019]

Consumes

2020

Italian consume + 7.4%

Global export + 15.6%

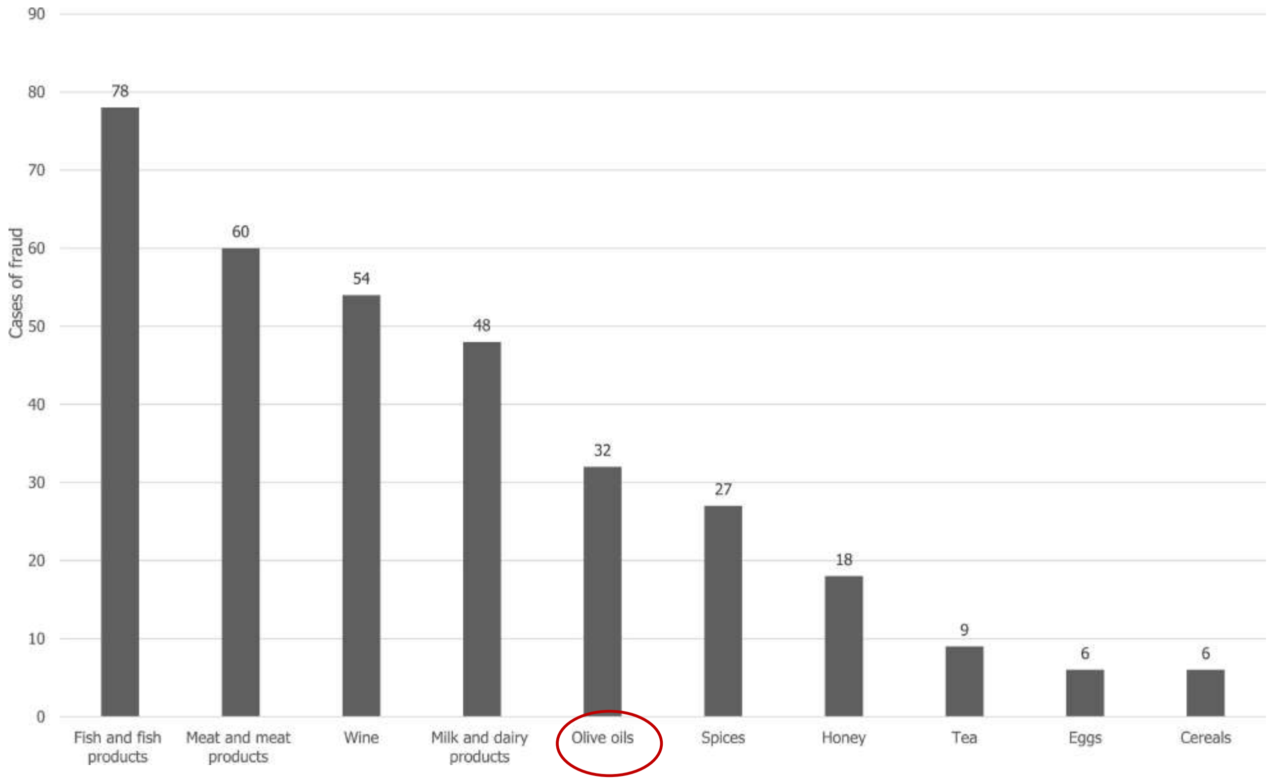
European export + 24.7%

1.815 million dollars market in 2026 (+ 24% respect to 2020)

[ABSOLUTE REPORTS, 2021]

HIGH RISK OF FRAUDS

EVOOs: high risk of frauds



[E. Casadei et al. Emerging trends in olive oil fraud and possible countermeasures, Food Control, 2021]

Olive oil related frauds

Mislabeling

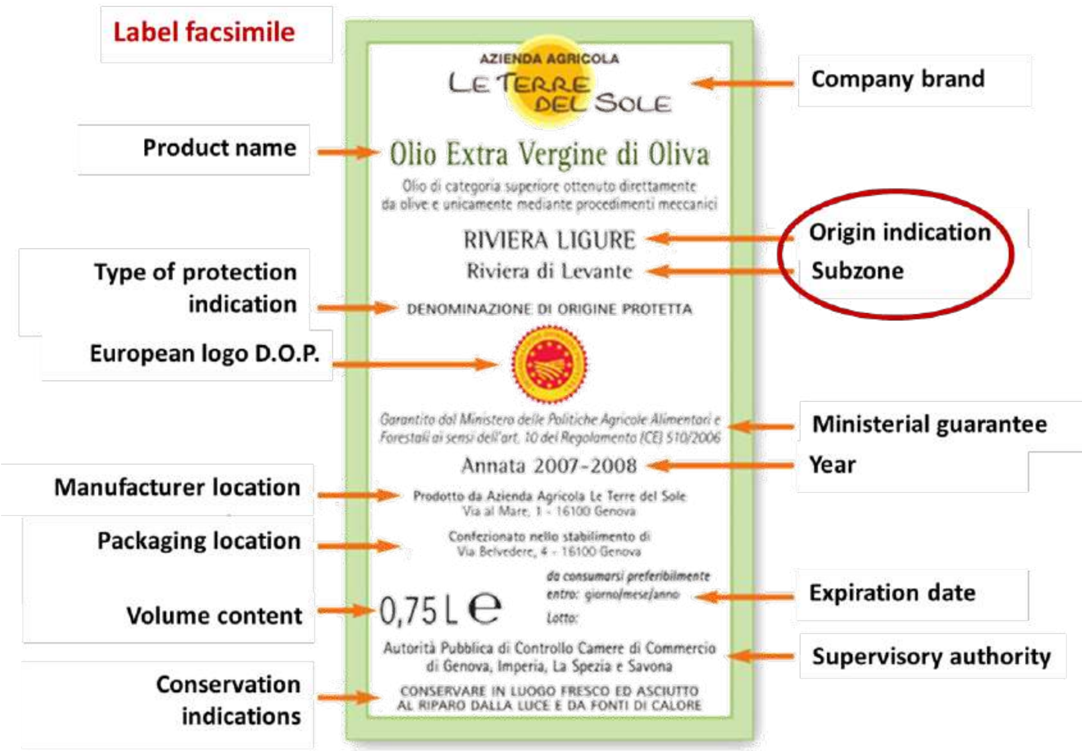
Dilutions

Unauthorized enhancements

False declarations

Geographical origin

EVOOs: label information



No official analyses are able to define the geographical origin of EVOOs

(Commission Implementing Regulation (EU) No 299/2013 of 26 March 2013)

Alternative and innovative analysis methods are requested

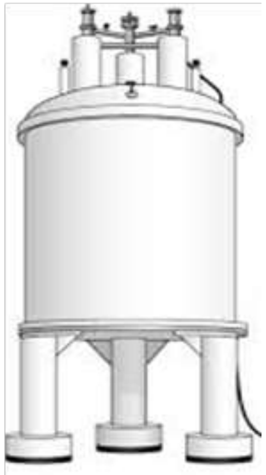
NMR ANALYSIS COMBINED WITH STATISTICAL ANALYSIS REPRESENTS AN IMPORTANT POTENTIAL TOOL

[COMMISSION REGULATION (EC) No 182/2009 amending Regulation (EC) No 1019/2002 on marketing standards for olive oil]

Aim of the work

Create a statistical model able to predict Italian EVOOs geographical origin by using data obtained from ^{1}H NMR analysis

NMR spectroscopy

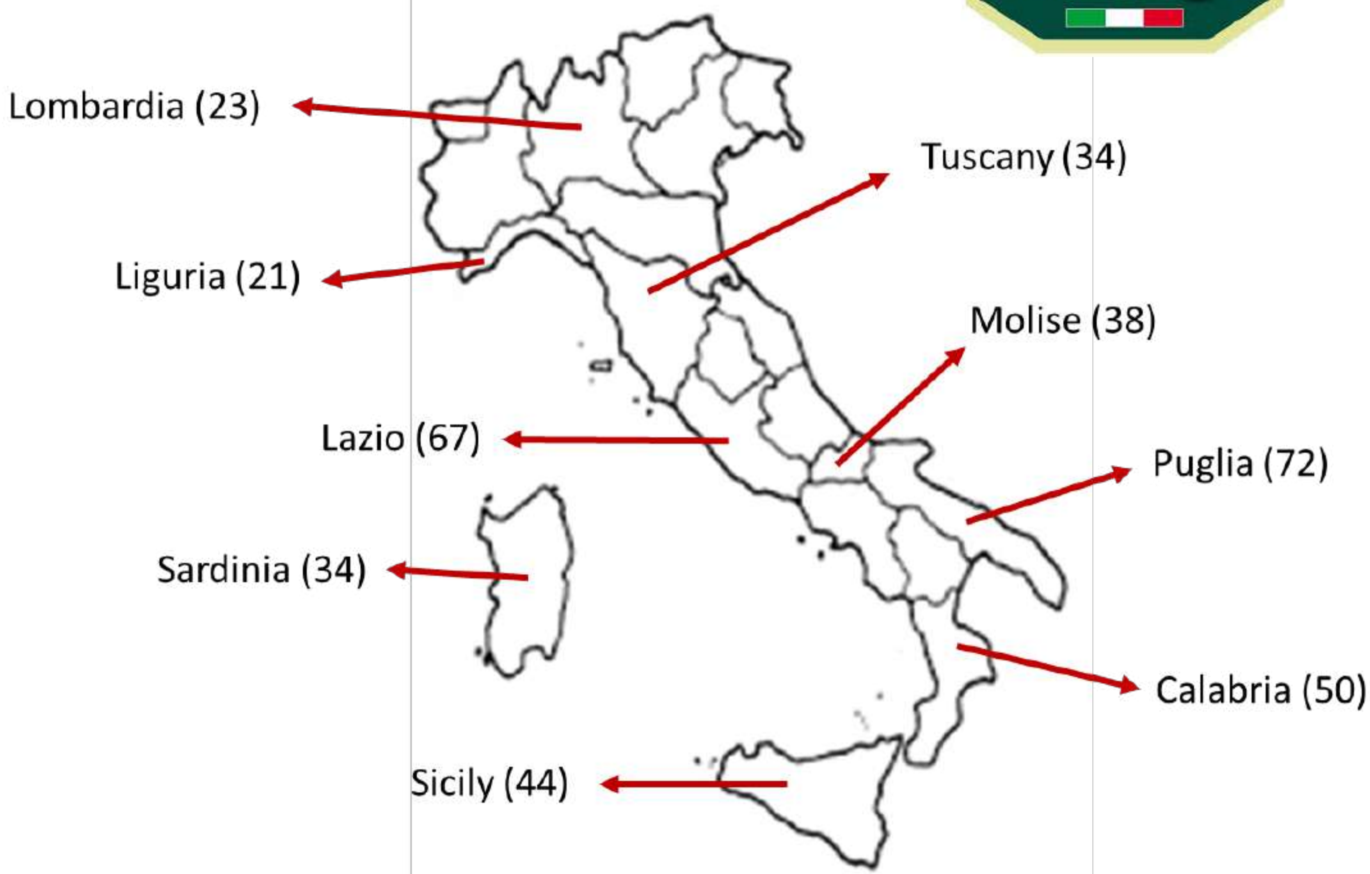


- Analysis methodology based on the magnetic properties of nuclei
- Non-destructive
- High reproducibility
- Short time for sample preparation
- Sample resolution with a single experiment (possibility to identify both polar and non-polar molecules)
- Quantification possibility
- Structural elucidation

Sample collection

383 EVOOs samples collected from nine Italian region in three sequential years

Samples were preserved at a temperature range of 10-15°C until analysis, far from light and heat sources



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^{1}H NMR analysis

Sample preparation

20 µL of olive oil
20 µL DMSO-d_6
700 µL $CDCl_3$

Bruker AVANCE 600

Spectra acquisition

The ^{1}H NMR spectra were acquired at 301K on a Bruker AVANCE 600 using the following experimental conditions:

- number of scan 1024;
- 90°pulse
- TD 64K data points
- relaxation delay 0.5 s
- acquisition time 2.96 s
- spectral width 18.5 ppm

Spectra processing

- The ^{1}H NMR spectra were phased manually
- 0.3 Hz as line-broadening factor
- Zero filling (size=64K)
- Calibration: $CDCl_3$ signal set at 7.26 ppm
- Baseline correction: Cubic Spline Baseline Correction

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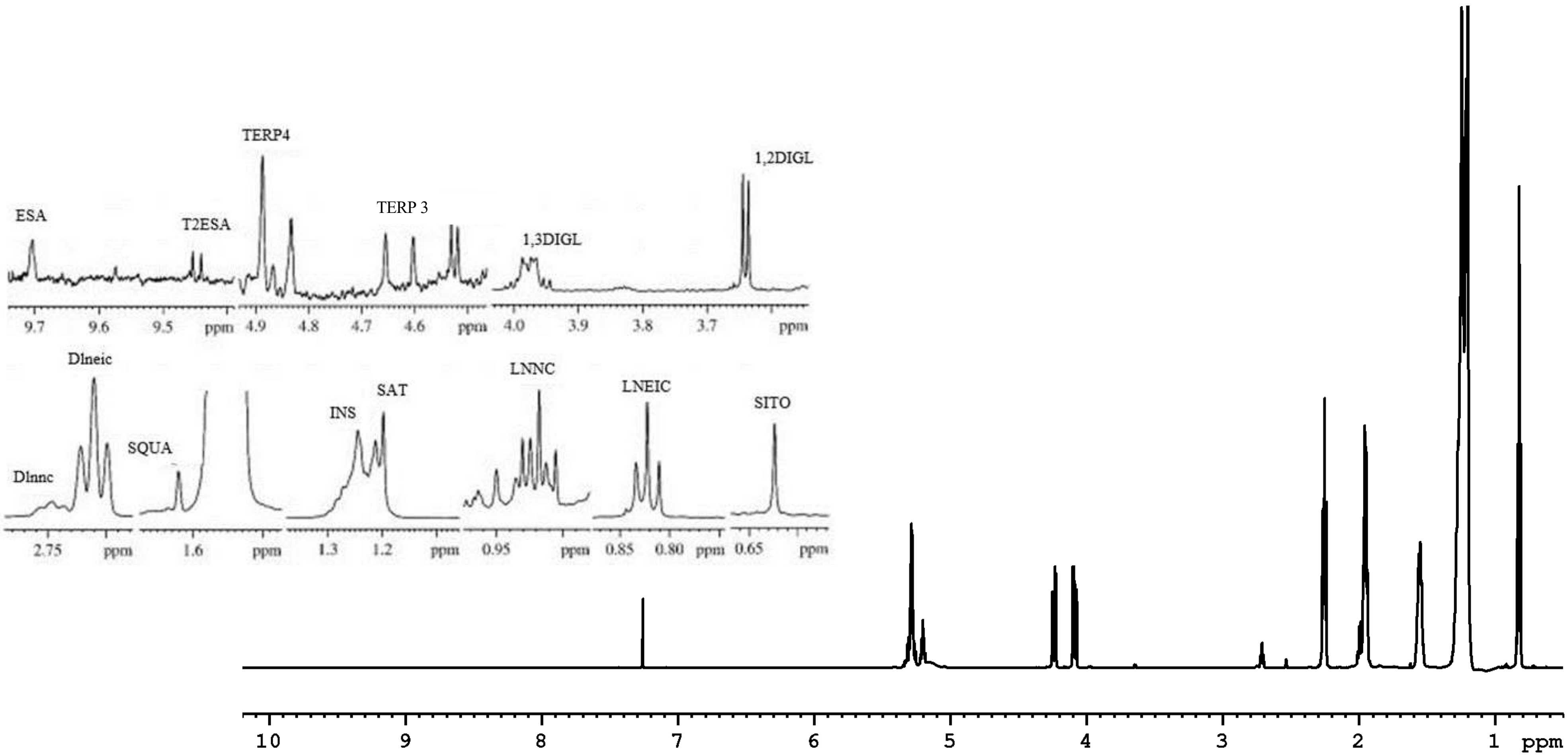
Metabolites identification



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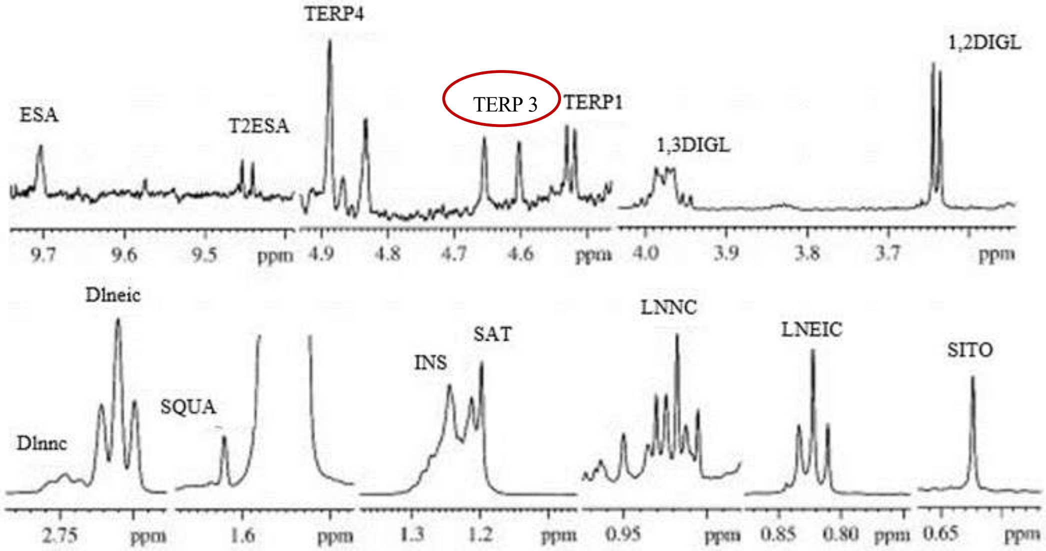

Assignment	^1H chemical shift (ppm)
Methyl 18 of β-sitosterol (SITO)	0.623
Linoleic acid methyl group (LNEIC)	0.843
Linolenic acid methyl group (LNNC)	0.910
Methylenic protons of the saturated fatty acids chains (SAT)	1.197
Methylenic protons of the unsaturated fatty acids chains (INS)	1.244
Squalene (SQUA)	1.620
Diallylic protons of the linoleic acid (Dlneic)	2.710
Diallylic protons of the linolenic acid (Dlnnc)	2.746
Sn-1,2-diglycerids α methylenic protons (1,2DIGL)	3.636
Sn-1,3-diglycerids α methylenic protons (1,3DIGL)	3.988
Terpene 1 (TERP1)	4.541
Terpene 3 (TERP 3)	4.609
Terpene 4 (TERP4)	4.885
Trans-2-Hexenal (T2ESA)	9.454
Hexanal (ESA)	9.704

24-methylenecycloartanol ???

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Metabolites identification

γ-oryzanol (40% 3-O-ferulyl-24-methylenecycloartanol and 40% 3-O-ferulylcycloartenol)

↓ EtOAc-EtOH 3:1 *v/v* X2

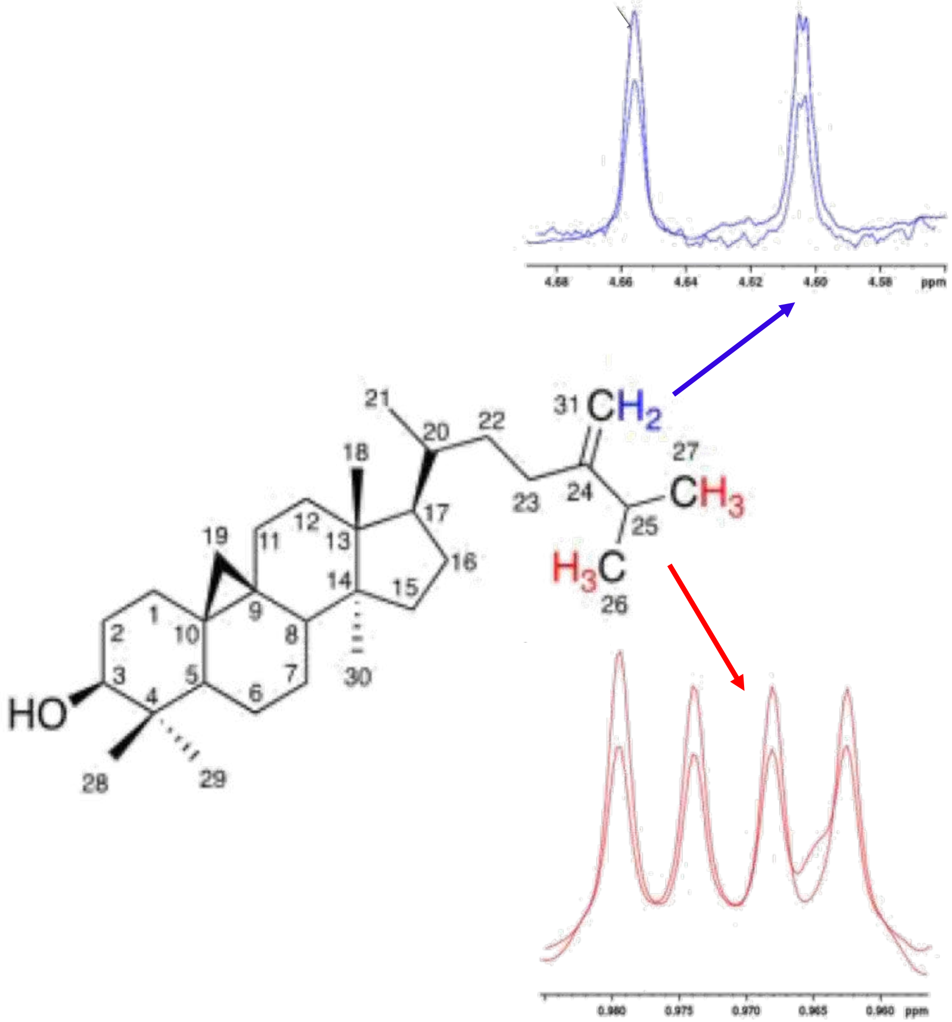
removal of 3-O-ferulylcycloartenol

↓ Acetone X3

3-O-ferulyl-24-methylenecycloartanol

↓ KOH aq. 10 N

24-methylenecycloartanol



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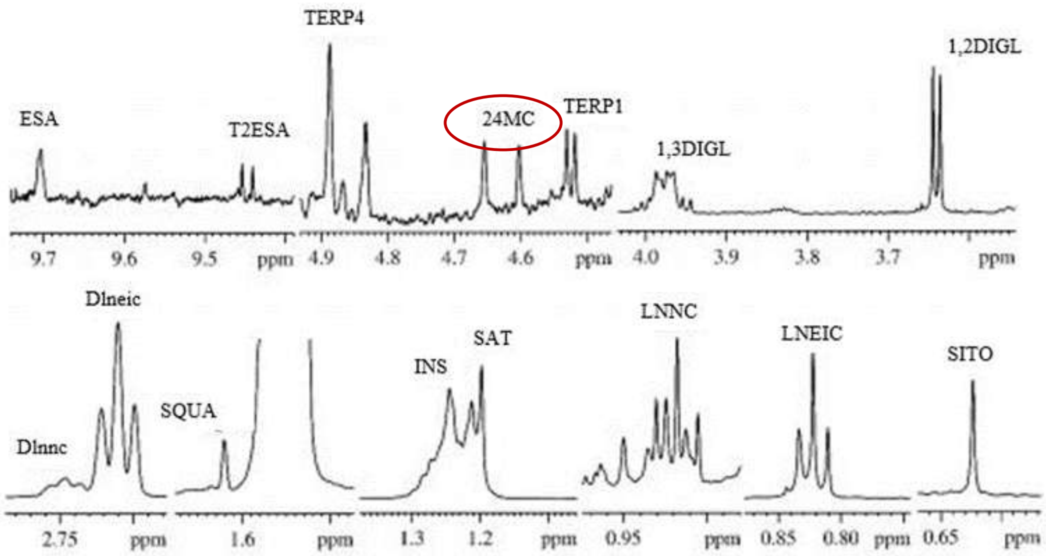

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Development of a statistical predictive model

Development of the linear discriminant analysis (LDA) model

The intensities of all the 15 identified NMR signals were selected for each of the 383 samples and used for the creation of a matrix (383 x 15) submitted to LDA analysis

Kennard-Stone algorithm was used to split the samples in training set (269 samples, used for model creation) and validation set (114 samples, used for model validation)

Region	Calibration fit %	Validation fit %
Calabria	36.11 (13/36)	40.00 (6/15)
Lazio	44.68 (21/47)	60.00 (12/20)
Liguria	80.00 (12/15)	66.67 (4/6)
Lombardy	75.00 (12/16)	100.00 (7/7)
Molise	81.48 (22/27)	63.64 (7/11)
Apulia	49.06 (26/53)	43.48 (10/23)
Sardinia	91.67 (22/24)	90.00 (9/10)
Sicily	74.19 (23/31)	61.54 (8/13)
Tuscany	60.00 (12/20)	66.67 (6/9)

LDA classification model to discriminate all the investigated region was not completely efficient

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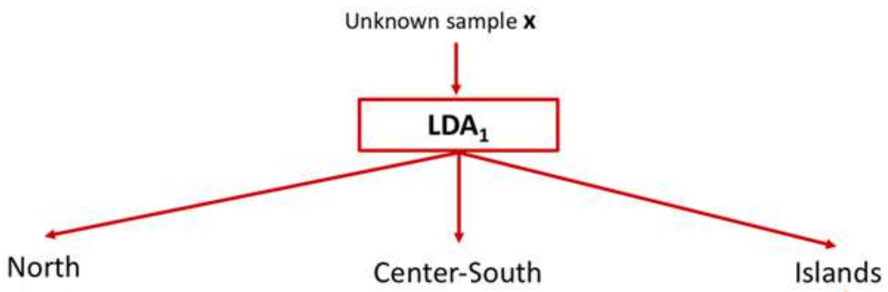
In a second stage, a hierarchical approach based on breaking the overall classification problem in a series of smaller sub-models to be sequentially applied was tested



Region	Calibration fit %	Validation fit %
macro-geographical area (LDA$_1$)		
North	80.65 (25/31)	84.62 (11/13)
Center-South	77.05 (141/183)	85.90 (67/78)
Islands	92.73 (51/55)	91.30 (21/23)

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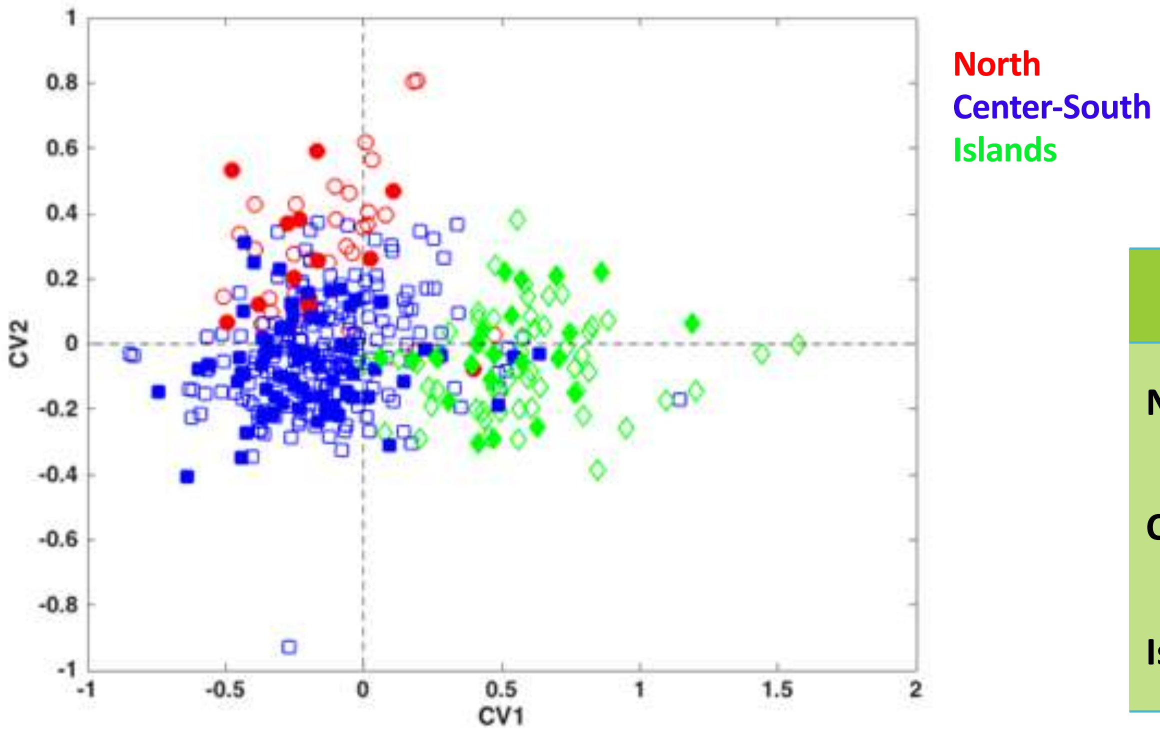
Development of a statistical predictive model

Development of the linear discriminant analysis (LDA) model

The LDA model built to discriminate among the three geographical macroareas provides very good classification ability not only on the training data, but also in validation ones



	High content	Low content
North	24MC, TERP1	SAT, LNEIC
Center-South	SAT, LNNC, SITO	TERP4, 24MC
Islands	TERP4, SQUA, LNEIC	TERP1, SITO

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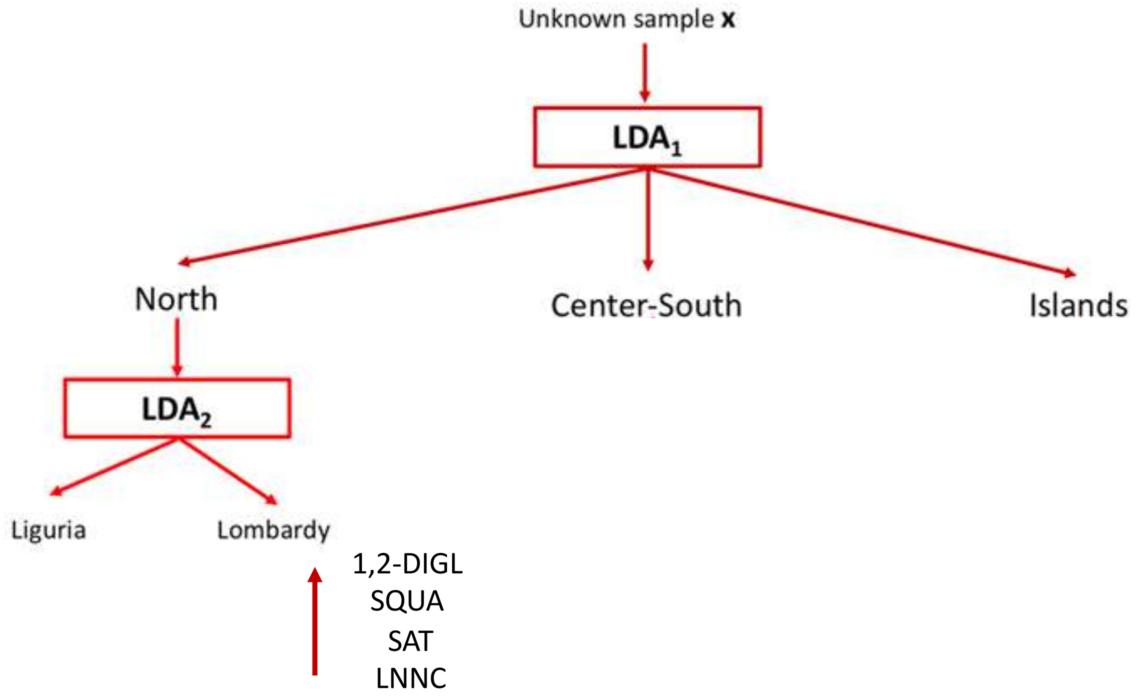
Development of a statistical predictive model

Development of the linear discriminant analysis (LDA) model

Additional models discriminating among the individual members of the three macro-geographical areas were built



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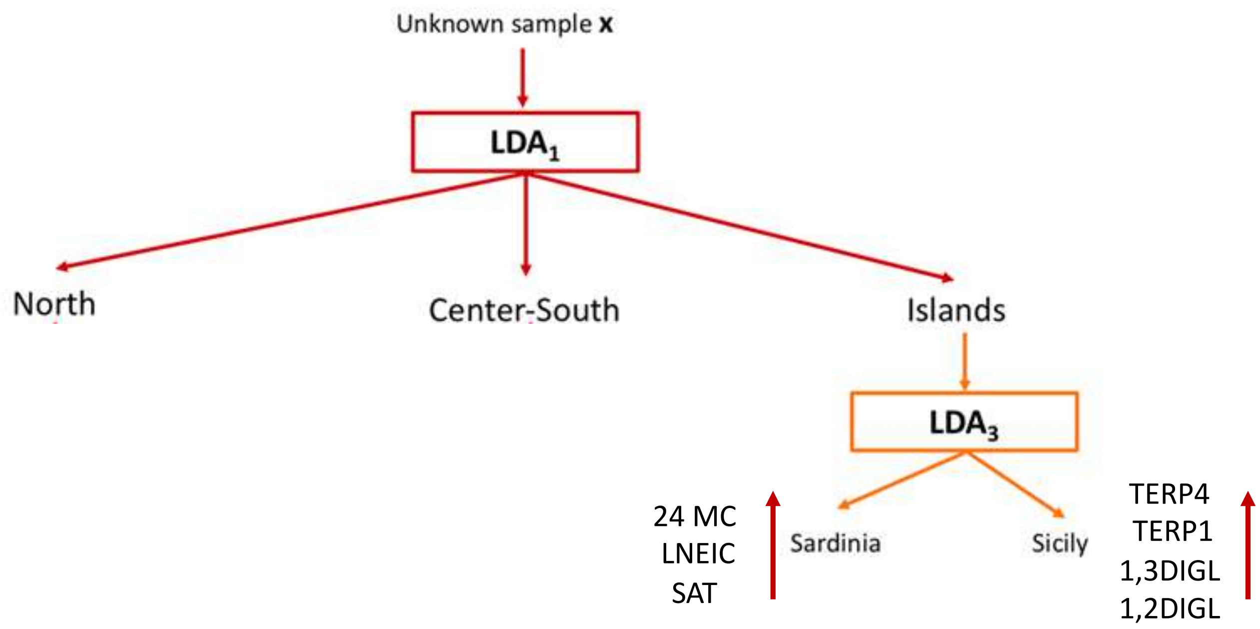

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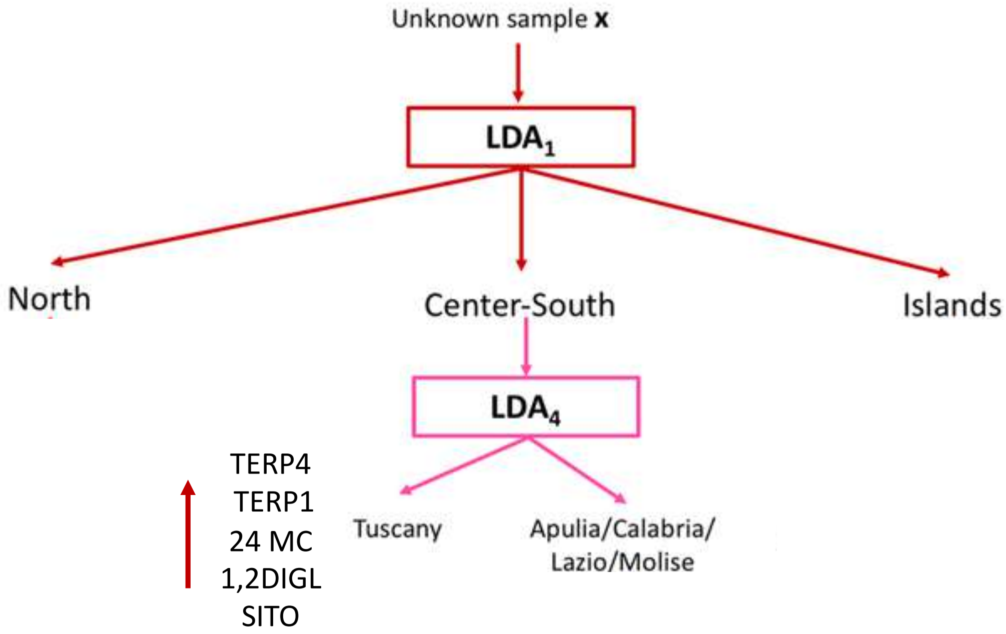

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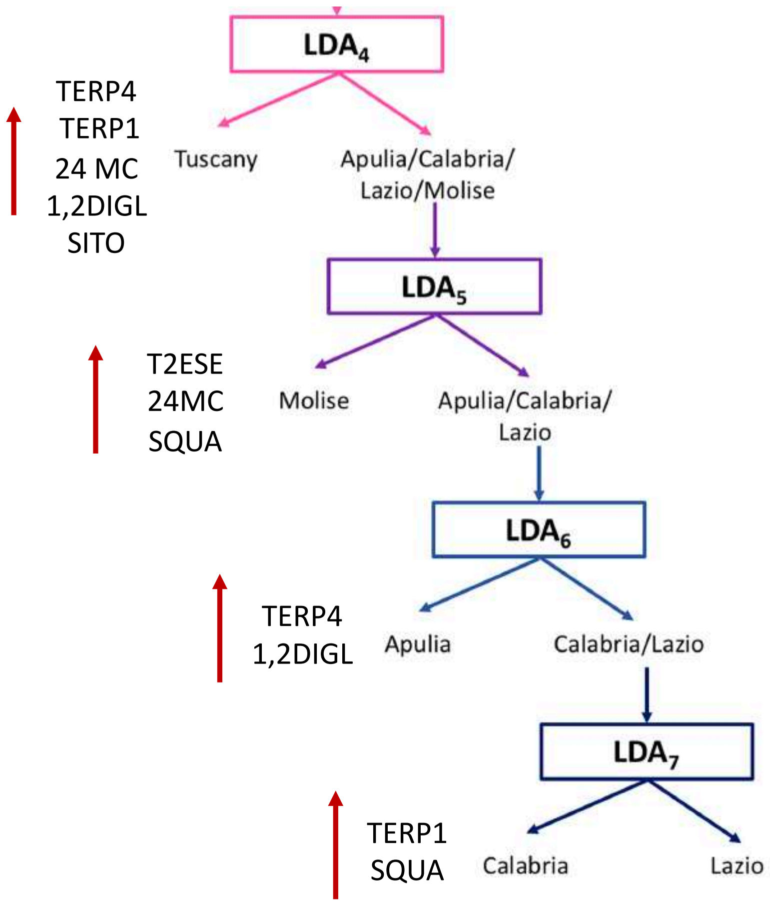

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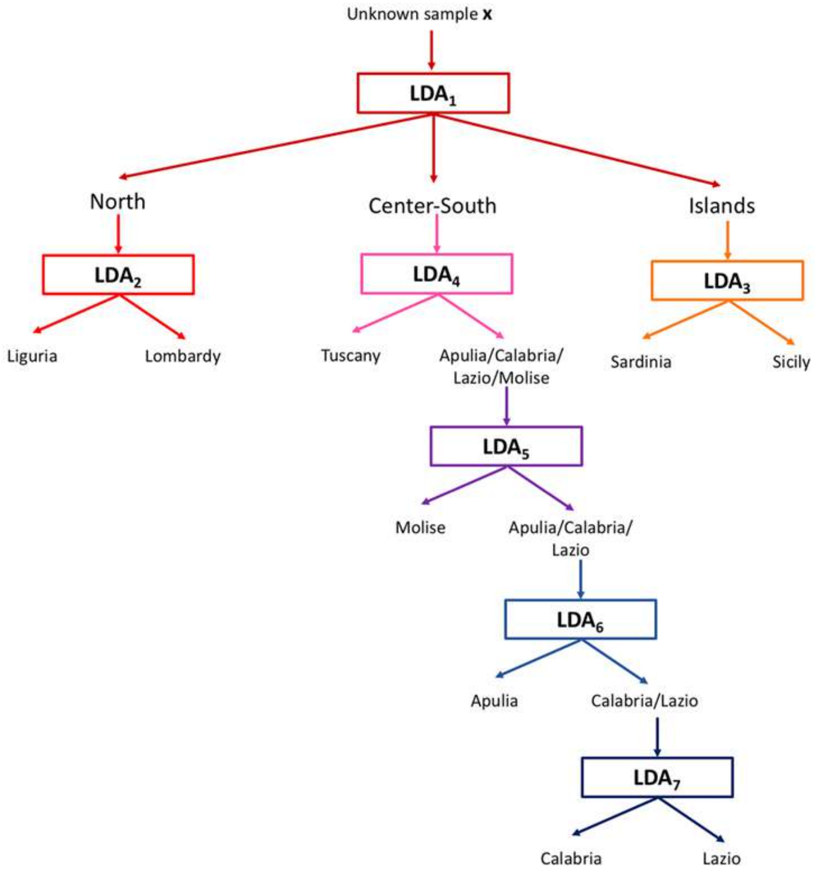

CONCLUSIONS

- NMR is a powerful tool for the characterization and the analysis of EVOOs
- When combined with statistical analysis, NMR data can be used for the creation of predictive models
- The model here presented showed to be effective in the prediction on regional EVOOs origin

FUTURE PERSPECTIVES

- Implementation of the created model by involving EVOOs from other Italian regions
- Use of NMR spectroscopy as an official analysis method for EVOOs quality determination

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GIDRM
Gruppo Italiano Discussione Risonanze Magnetiche

VII Workshop
Applicazioni della Risonanza Magnetica nella Scienza degli Alimenti

Dipartimento di Chimica e Tecnologie del Farmaco, Facoltà di Farmacia e Medicina, Sapienza Università di Roma, 23-24 giugno 2022

THANKS FOR YOUR KIND ATTENTION

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