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High-Resolution Mass Spec

SMA®t-EVOO-Tools: Providing insights into Greek Extra Virgin Olive Oil Authenticity and Geographical Origin

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o   The **European Union is the largest producer, consumer and exporter of olive oil**.

o   The EU produces about **67%** of the world's olive oil. **About 4 million hectares,** mainly in the Mediterranean countries of the EU, **are destined for the cultivation of olive trees**, combining olive groves cultivated traditionally, intensively and over-intensively.

o   **Italy and Spain are the largest consumers of olive oil in the EU**, with an annual consumption of around 500 000 tonnes each, **while Greece has the highest per capita consumption in the EU, with around 12 kg per person per year.**

o   **Overall, the EU accounts for around 53% of world consumption.**

There are eight different categories of olive oils :

o   Virgin olive oil

o   Extravirgin olive oil

o   Lampante olive oil

o   Refined olive oil

o   Unrefined pomace oil

o   Refined pomace oil

o   Olive pomace oil

The European Community classifies olive oil according to three elements:

✓ the type of extraction used to obtain the oil,

✓ its composition, the percentage of free acidity (a parameter measured through chemical analysis),

✓ organoleptic analysis

Virgin olive oils

There are three different categories of virgin olive oils

✓ **Extra virgin olive oil** is the category with the highest quality. From an organoleptic point of view, it has no defects and is fruity. **The acidity level must not exceed 0,8 %.**

✓ **Virgin olive oil** may have some organoleptic defects, but at a very low level. **Its acidity must not exceed 2 %.**

✓ **Less-favoured olive oil** is virgin olive oil of inferior quality **with an acidity of more than 2 %,** without fruity characteristics and with substantial organoleptic defects. Less-favoured olive oil is not intended to be placed on the market at retail level. It is refined or used for industrial purposes.

EVOO is considered as one of the most important ingredients of a well-balanced nutrition

Extra Virgin Olive Oil BENEFITS

- Rich in healthy fats
- Reduces the risk of stroke and protects against heart disease
- Can eliminate harmful bacteria in the stomach
- Excellent source of antioxidants
- Reduces the risk of type II diabetes

Attracts particular attention due to its high nutritional value

Bioactive compounds are included in the minor fraction of 2% w/w

Extra · Virgin · Olive · Oil

EVOO has profitable health effects since is aassociated with the prevention of cardiovascular disease, diabetes, cancer, age-related cognitive decline, and lower incidence of metabolic syndrome

European Union (EU) has proceeded to establishment of relative regulation frameworks and health claims

Compliance checks

❑ According to the legal framework, **EU countries must carry out a minimum number of checks annually,** depending on the volume of olive oil placed on their country's market, to ensure that marketing standards for olive oils and olive-pomace oils are met.

❑ The purpose of the checks is **to verify compliance of the labelling and packaging with legal requirements and of the category of oil with the declared category**.

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In 2021, Member States performed 5 195 labelling checks and the results are as follows

	Number of label checks	Number of non-conform labels	Share of non-conform labels (%)
Producing Member States	4 787	633	13.22%
Non-producing Member States	408	103	25.25%
Total for 2021	5 195	736	14.17%

Source: Member States communications in ISAMM

Results of labelling checks for different types of olive oil
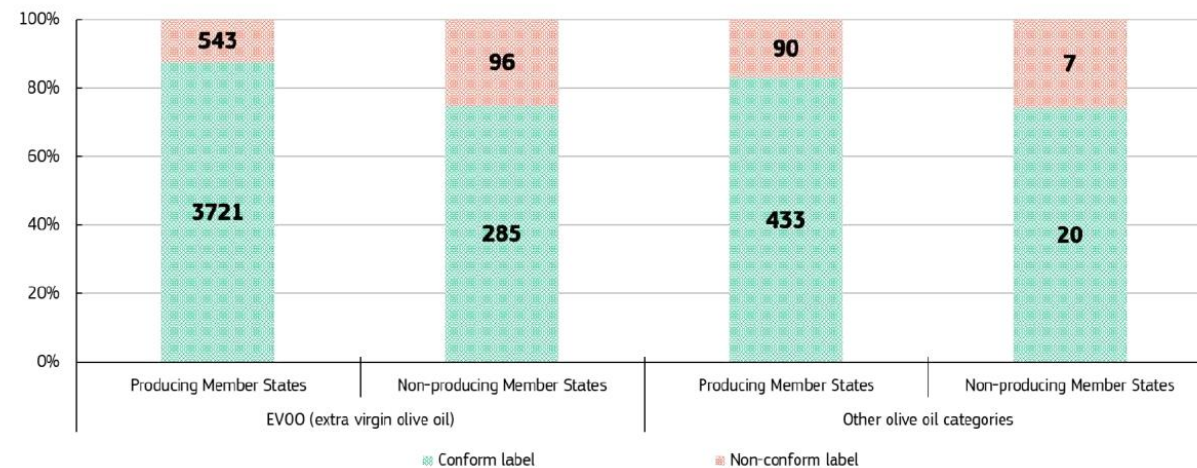
Source: Member States communications in ISAMM

In 2021, Member States performed 2 833 category checks and the results are as follow:

	Number of category checks	Number of checks detecting a non-conform category	Share of checks detecting a non-conform category (%)
Producing Member States	2 514	803	31.94%
Non-producing Member States	319	135	42.32%
Total for 2021	2 833	938	33.11%

Source: Member States communications in ISAMM

Results of category checks for different types of

Source: Member States communications in ISAMM

Food adulteration / authenticity

o   Food adulteration and authenticity **are major issues for the agri-food sector and public health.**

o   Among the various agri-food products, **Extra Virgin Olive Oil (EVOO),** due to its high economic value compared to other foods**, is both in Greece and Europe the food with the highest adulteration rates,** mainly with seed oils and olive-pomace oil or refined olive oil, according to a recent relevant study on the implementation of compliance controls in the olive oil sector across the EU.

o   Therefore, **the continuous intensive control of the authenticity and the certification of the geographical origin of olive oil are important and necessary practices** for the current conditions of economic competition.

o To date, **the authenticity and quality of extra virgin olive oil is evaluated using conventional chemical and organoleptic tests performed in chemical analytical laboratories and specialized tasting panels.**

o However, **all of these conventional chemical tests require the use of qualified personnel**, make use of a large volume of toxic organic solvents, are expensive and extremely time-consuming.

o In particular, **organoleptic tests require a team of 8-12 tasters (olive oil tasters)** selected who have great specialization and long-term training, **thus becoming very time-consuming and expensive.**

NEED FOR NEW RELIABLE METHODS

o   **The need therefore for new, more environmentally friendly, reliable and possibly rapid methods** capable of ensuring the quality and authenticity and verifying the geographical origin of virgin olive oil is an urgent requirement.

o   In this direction, **modern instrumental analytical techniques,** such as high resolution liquid chromatography (LC-HRMS) assisted by sophisticated chemometric tools can offer alternative and very attractive solutions.
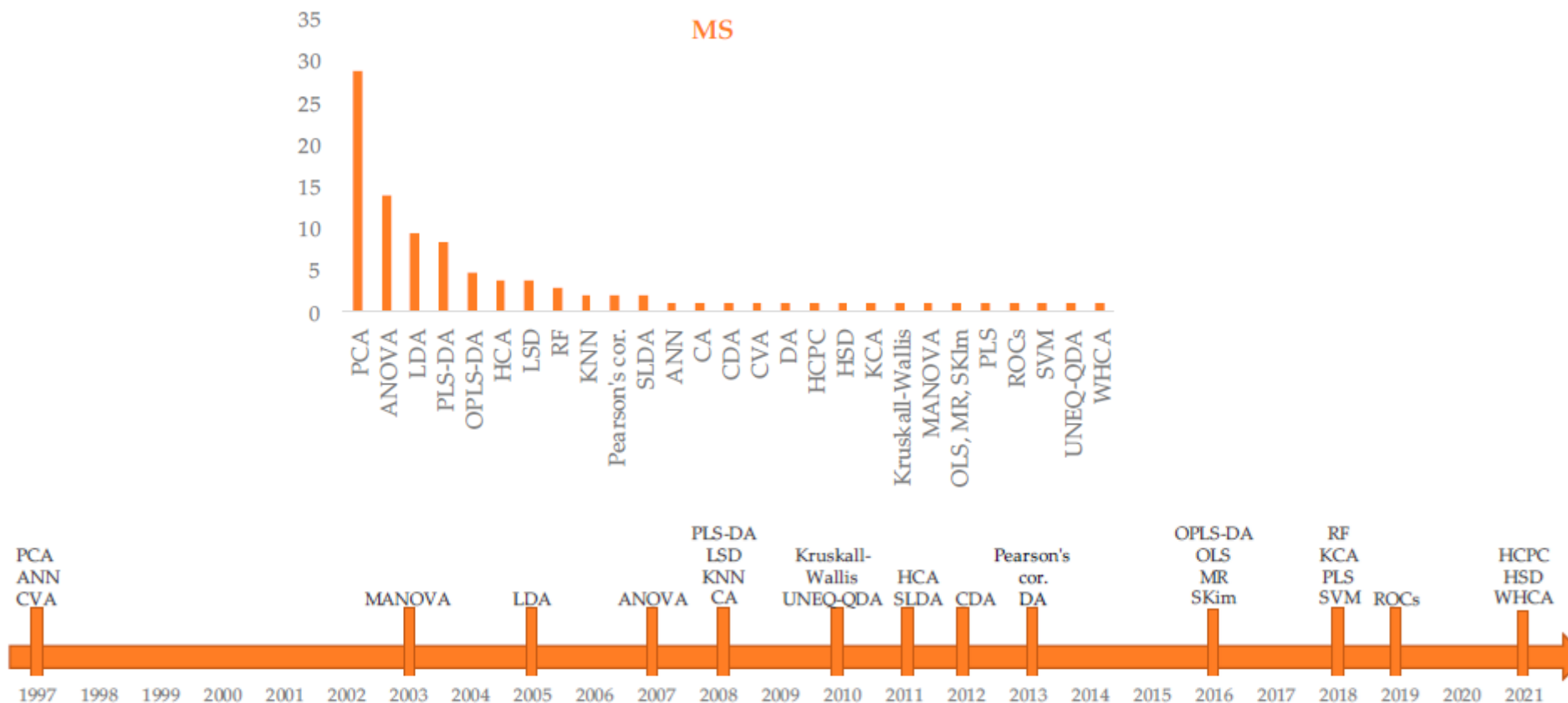
Calò, F.; Girelli, C.R.; Wang, S.C.; Fanizzi, F.P. Geographical Origin Assessment of Extra Virgin Olive Oil via NMR and MS Combined with Chemometrics as Analytical Approaches. Foods **2022**, 11, 113. https://doi.org/10.3390/foods11010113

CHEMOMETRIC ANALYSIS – HRMS-ARCGIS

o Therefore, **the coupling use of modern analytical techniques and chemometric modeling is a promising tool** for the development of models of quality and authenticity of extra virgin olive oil.

o In this direction, **apart from chemical techniques and chemometric modeling, the mapping of olive oil's authenticity characteristics in areas of interest (local products), including local varieties, and the creation of a reliable database is a powerful complementary tool.**

STRATEGIC GOAL

SMA®t EVOO- Tools

Design and development of three (3) "smart tools"

- **SMA®t-EVOO-Tool-1,** Mapping Tool
- **SMA®t-EVOO-Tool-2,** Quality/Authenticity Tool
- **SMA®t-EVOO-Tool-3,** Geographical Origin Tool

SMA®t EVOO- Tools

Partners

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Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης Τμήμα Χημείας

➤ Εργαστήριο Ελέγχου Ρύπανσης Περιβάλλοντος
Τμήμα Χημείας, Πανεπιστημιούπολη
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Σύγχρονη Αναλυτική

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Θέρμη, Θεσσαλονίκη
Τηλ.: 2310-465600,
2310-465700

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Center for Interdisciplinary Research and Innovation, AUTh

Department of Chemistry, AUTh
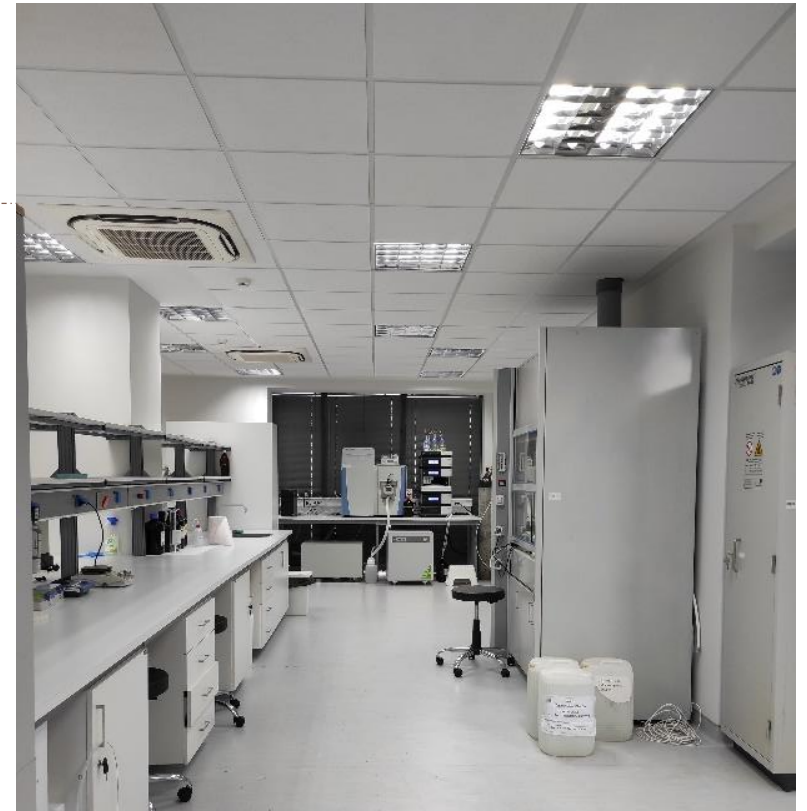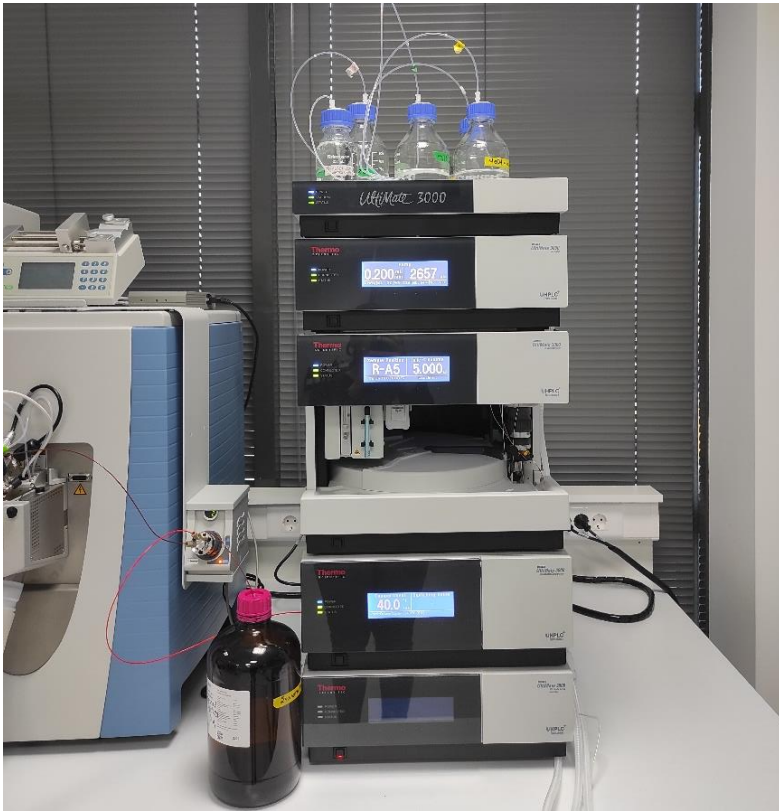
High-Resolution Mass Spectrometry Unit,
Center for Interdisciplinary Research and Innovation, AUTh
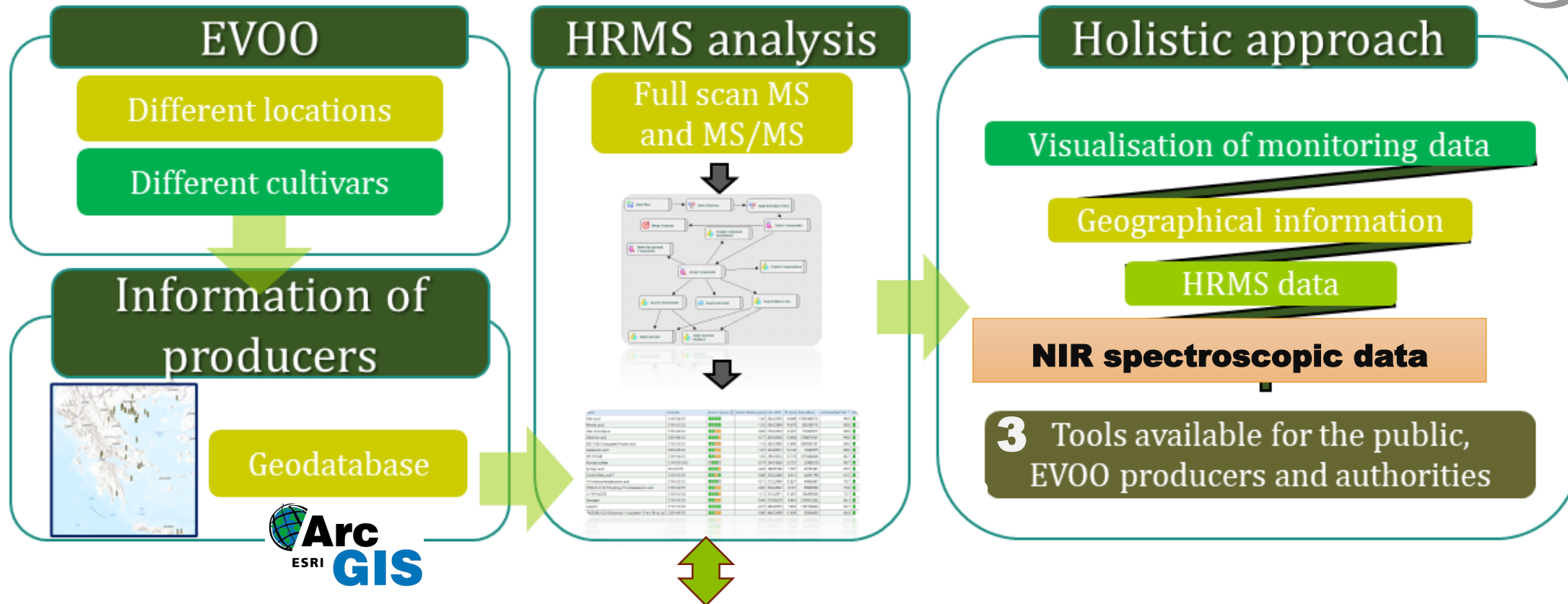
AUTh Infrastructure
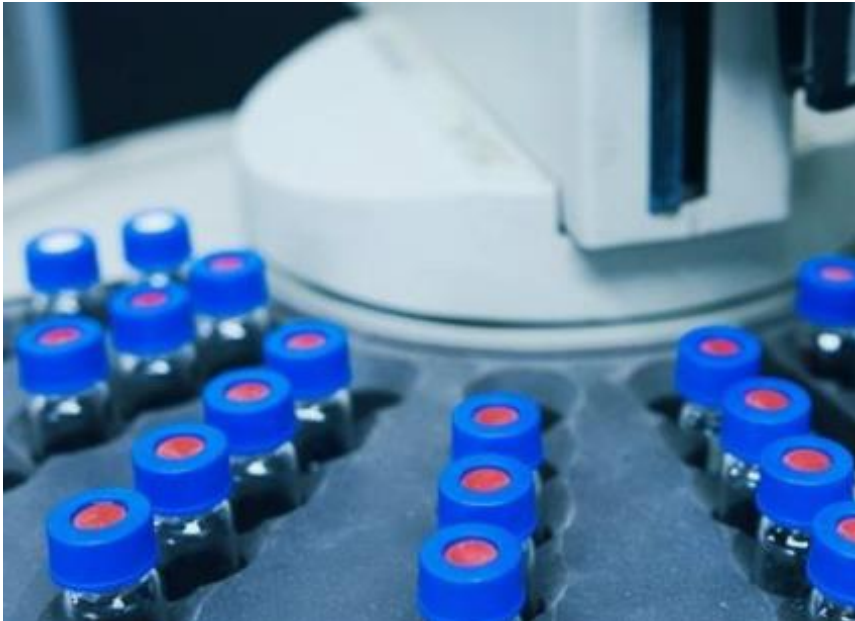
ΣΥΓΧΡΟΝΗ ΑΝΑΛΥΤΙΚΗ
MODERN ANALYTICS

ΤΜΗΜΑ ΧΗΜΕΙΑΣ
ΑΡΙΣΤΟΤΕΛΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ
ΚΕΔΕΚ

ΣΥΓΧΡΟΝΗ
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ΜΑΚΕΔΟΝΙΑΣ

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Ταμείο
Περιφερειακής Ανάπτυξης

ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ
Ε.Π. Περιφέρειας Κεντρικής Μακεδονίας

ΕΣΠΑ
2014-2020

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

SMA®t EVOO- Tools Project at a glance

NIR spectroscopic data

Communication network - partners

Method application Workflow

Sample collection

Different geographical origin

Extraction
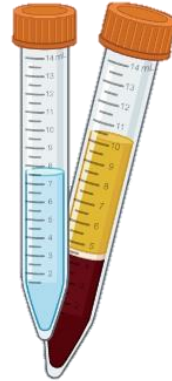
Liquid liquid extraction

Analysis
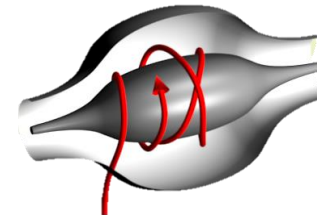
UHPLC LC-Q Exactive Focus Orbitrap MS/MS

Data curation

Xcalibur
TraceFinder
Compound Discoverer
Open data source softwares

Liquid liquid extraction

2 g sample

5 mL MeOH:H$_2$O 80:20 — Vortex 1 min

Ultrasonic extraction — 15 min

Centrifugation 5 min, 400 rpm — Collect the upper layer

Injection to LC-Orbitrap MS/MS — Regenerated cellulose filters

Why HRMS?

Discoverer software – Open source software

Databases / In silico prediction

EVOO samples

- Extraction of samples

Analysis

- HRMS
- LC-MS/MS
- Appropriate Ionization
- Optimization of LC-MS parameters

Data processing

- Peak detection and alignment
- Blanks subtraction
- Grouping
- Replicating
- Profile Building
- Series

Prioritization

- Isotopic pattern
- Mass defect
- Time and spatial trends

Identification

- Database
- Suspect search
- Formula assignment
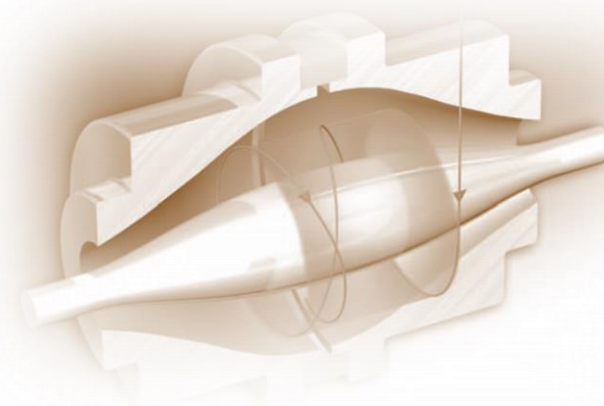- Fragments
- Analytical & Meta information

01 02 03 04 05
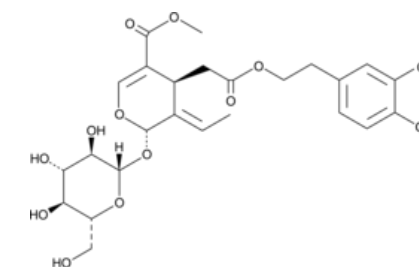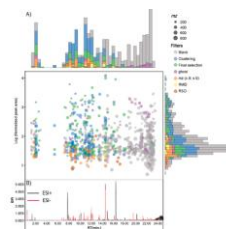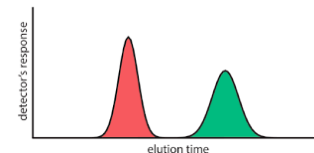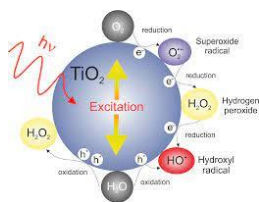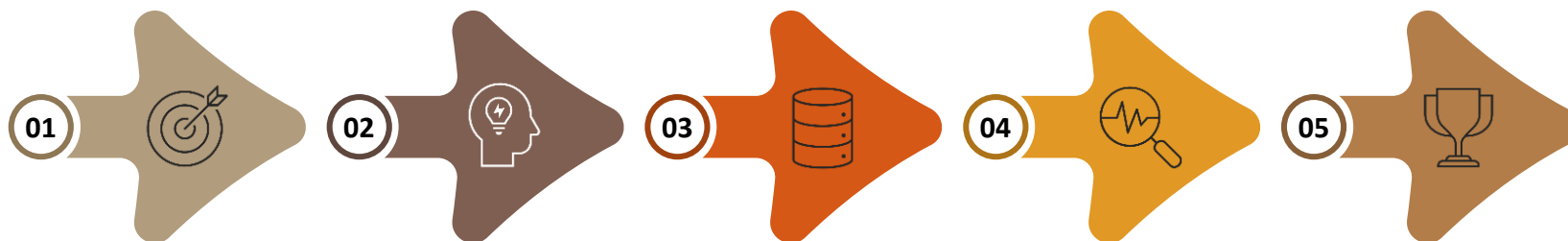
Why HRMS?

Scan speed up to 12 Hz

Resolution up to 700 (FWHM), at m/z 20

Mass accuracy: sub ppm for routine analysis

Mass range: m/z 50-200

Simultaneous acquisitions (eg. FS, FS-ddMS, SIM, PRM, vDIA)

Dynamic linear range

HCD fragmentation

switching polarity within a single run

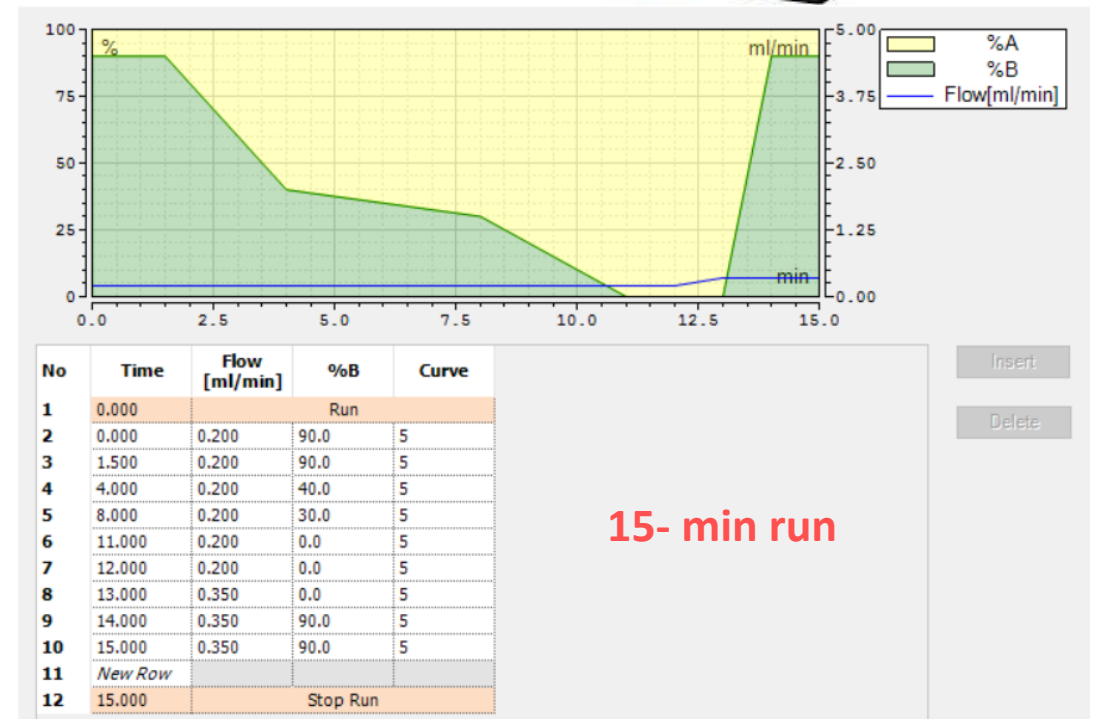## Orbitrap Q Exactive Focus

UHPLC – Orbitrap MS/MS analysis

- o LC System; UHPLC Ultimate 3000 (Thermo-Dionex)

- o Column: Thermo Hypersil Gold aQ C18 (50 x 2.1, 1.9 μm)

- o Elution solvents (A) MeOH+0,1% FA || (B) H_2O+0,1% FA

- o Flow rate: 200μL min^{-1}

- o Injection volume: 5μL

- o Ion Max heated electrospray ionization (HESI-II) probe

- o MS/MS System: Thermo Q Exactive Focus Orbitrap

- o Software: Xcalibur 4.1 and Trace Finder 4.1 EFS

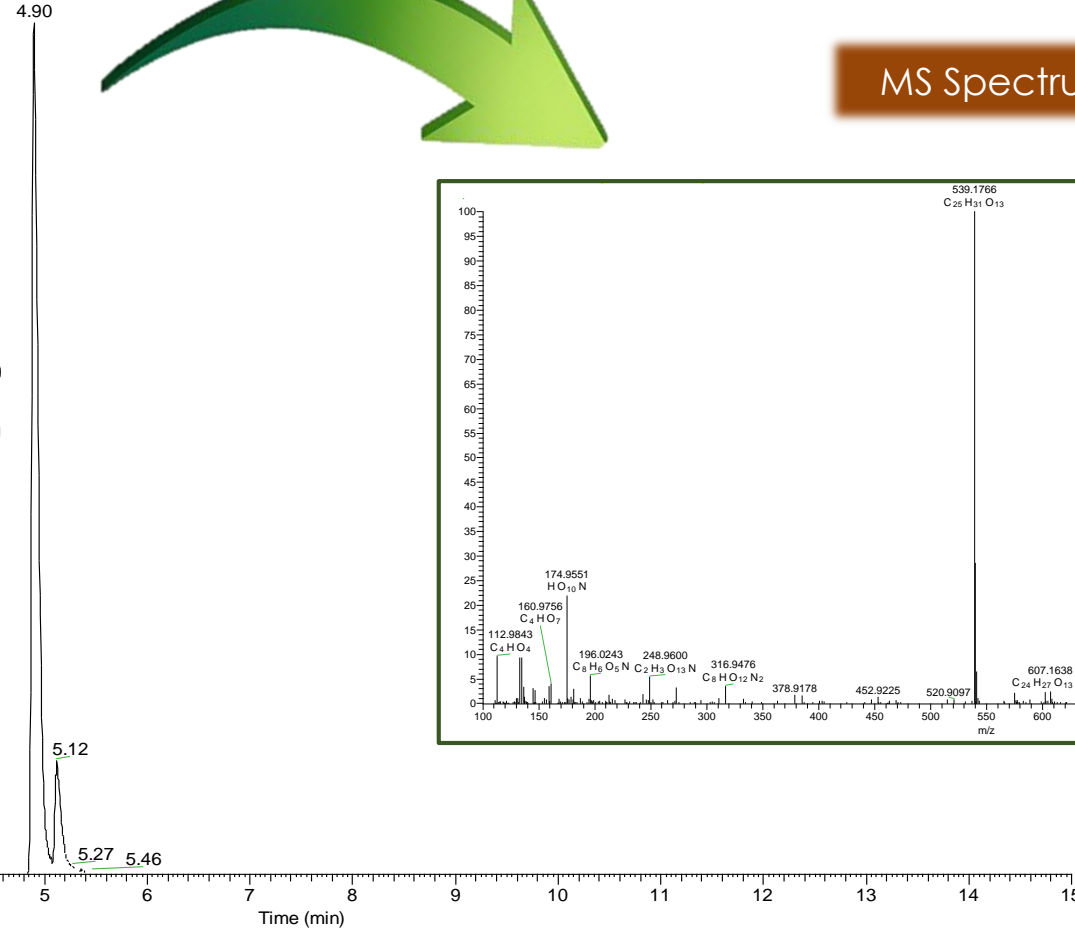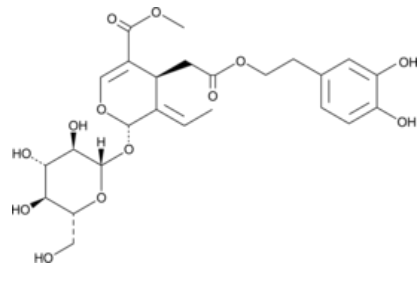- o **Compound Discoverer 3.2**

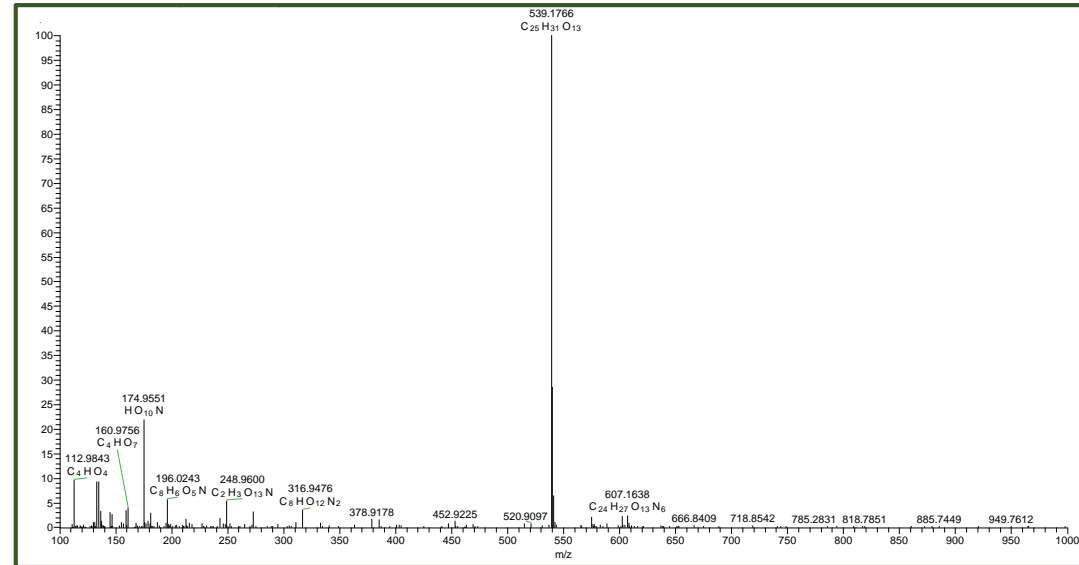- o **Method: ddMS, Discovery mode**

15- min run

No	Time	Flow [ml/min]	%B	Curve
1	0.000		Run	
2	0.000	0.200	90.0	5
3	1.500	0.200	90.0	5
4	4.000	0.200	40.0	5
5	8.000	0.200	30.0	5
6	11.000	0.200	0.0	5
7	12.000	0.200	0.0	5
8	13.000	0.350	0.0	5
9	14.000	0.350	90.0	5
10	15.000	0.350	90.0	5
11	New Row			
12	15.000		Stop Run	

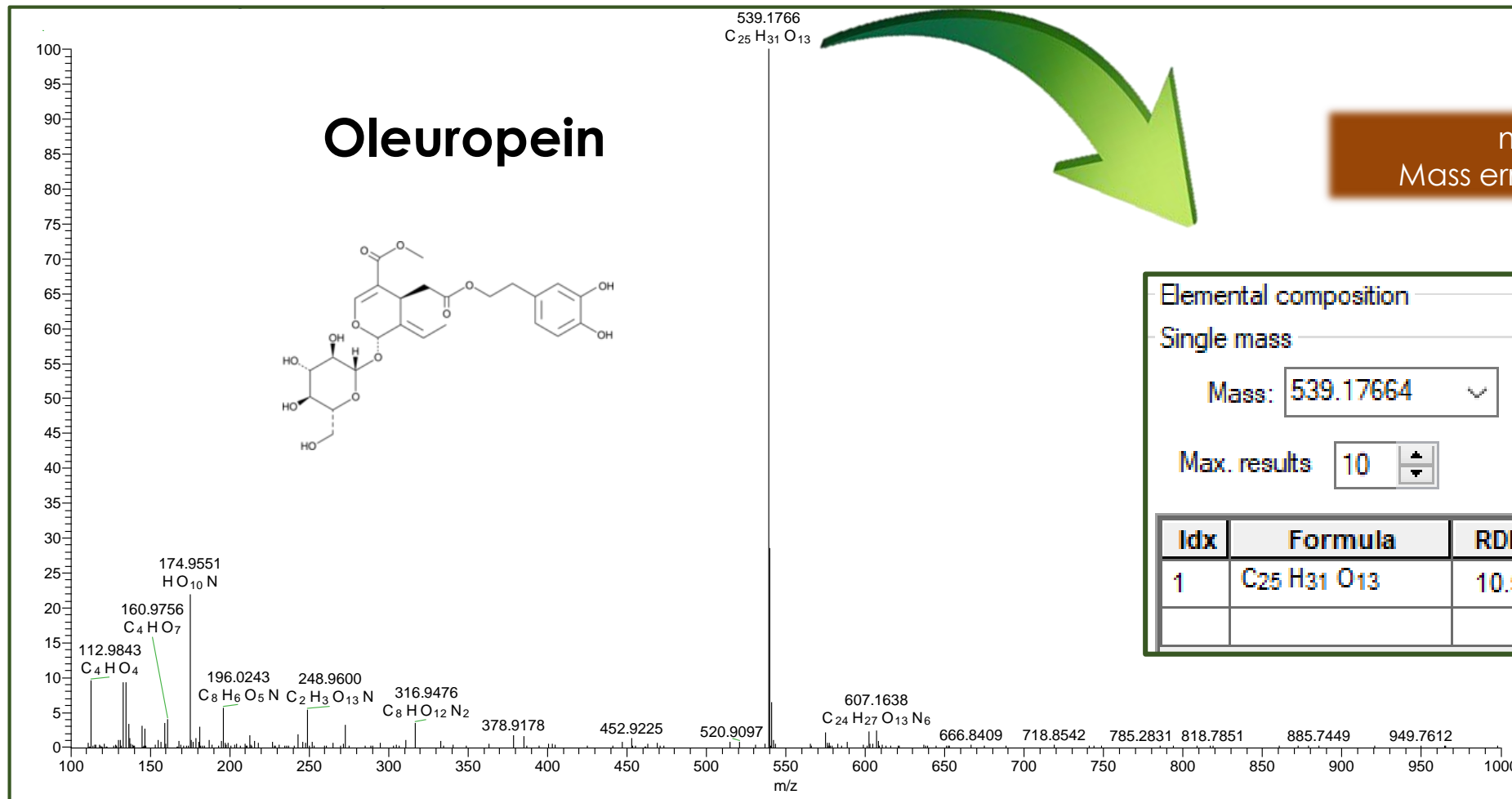# Orbitrap: Compound Identification

RT: 0.00 - 15.00

Oleuropein

MS Spectrum

Orbitrap: Compound Identification

Orbitrap: Compound Identification

F: FTMS - c ESI sid=5.00 d Full ms2 539.1765@hcd38.33 [50.0000-570.0000]

MS/MS Spectrum of selected ion

Oleuropein

95.0489 C_6H_7O -2.6539 ppm

111.0074 $C_5H_3O_3$ -2.1572 ppm

139.0388 $C_7H_7O_3$ -0.9853 ppm

149.0233 $C_8H_5O_3$ -0.3415 ppm

165.0548 $C_9H_9O_3$ 0.9596 ppm

179.0554 $C_6H_{11}O_6$ 2.3493 ppm

191.0343 $C_{10}H_7O_4$ 1.9409 ppm

223.0605 $C_{11}H_{11}O_5$ 1.9353 ppm

241.0718 $C_{12}H_9O_2N_4$ -0.8689 ppm

275.0922 $C_{16}H_{11}ON_4$ -1.9773 ppm

307.0821 $C_{16}H_{11}O_3N_4$ -1.4456 ppm

327.0863 $C_{18}H_{15}O_6$ -0.0334 ppm

345.0970 $C_{18}H_{17}O_7$ 0.2174 ppm

377.1239 $C_{20}H_{17}O_4N_4$ -1.3248 ppm

403.1245 $C_{18}H_{19}O_7N_4$ -0.7781 ppm

469.1325 $C_{19}H_{23}O_{11}N_3$ -0.4968 ppm

507.1513 $C_{25}H_{23}O_8N_4$ 0.4644 ppm

539.1766 $C_{25}H_{31}O_{13}$ 1.3324 ppm

m/z

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F: FTMS - c ESI sid=5.00 Full ms2 539.1765@hcd38.33 [50.0000-570.0000]

Oleuropein

Literature available characteristic fragments

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m/z

Orbitrap: Design of experiments

Improving response on the complex matrix of EVOO by implementing a design of experiments (DoE)
Two major bioactive compounds, namely **oleuropein** and **hydroxytyrosol** were selected as model compounds, as well as **apigenin**, **luteolin**, and **oleocanthal**
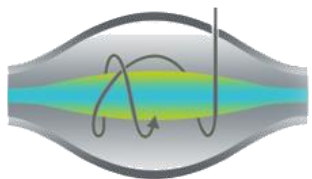
❑ Auxiliary heater temperature, AGC target and in-source CID: **FS-MS experiment (1st DoE)**
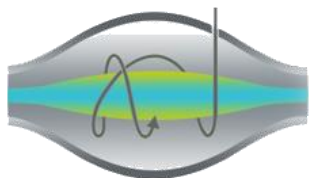❑ AGC target, in-source CID target and isolation window: **MS/MS experiments (2nd DoE)**

✓ **Response surface modelling**, and especially two different central composite designs (CCD) were selected as the best DoE for a stepwise optimization of MS and MS/MS parameters
✓ Area and number of fragments as response of analytes to employ the **desirability function (DF) approach**

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- ✓ Area and number of frag~~ments were used in a~~ **desirability function (DF) a~~pproach~~**

	Parameters	Levels
MS	Auxiliary heater temperature	200, 300, 400 °C
	AGC target	2e5, 5e5, 1e6
	in-source CID	5, 10, 15 eV
MS/MS	AGC target	5e4, 1e5, 2e5
	in-source CID	5, 10, 15 eV
	isolation window	1, 1.5, 2 m/z

ΤΜΗΜΑ ΧΗΜΕΙΑΣ
ΑΡΙΣΤΟΤΕΛΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ
ΚΕΔΕΚ
ΣΥΓΧΡΟΝΗ ΑΝΑΛΥΤΙΚΗ
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❑ Auxiliary heater temperature, AGC target and in-source CID: **FS-MS experiment (1ˢᵗ DoE)**
❑ AGC target, in-source CID target a_____
   MS/MS experiments (2ⁿᵈ DoE)

✓ **Response surface modell**_____te
   designs (CCD) were selec_____f
   MS and MS/MS paramete_____
✓ Area and number of frag_____
   desirability function (DF) _____

	Parameters	Levels
MS	Auxiliary heater temperature	200, 300, 400 °C
	AGC target	2e5, 5e5, 1e6
	in-source CID	5, 10, 15 eV
MS/MS	AGC target	5e4, 1e5, 2e5
	in-source CID	5, 10, 15 eV
	isolation window	1, 1.5, 2 m/z

MS PARAMETERS
Oleuropein

Monitored response: Peak area
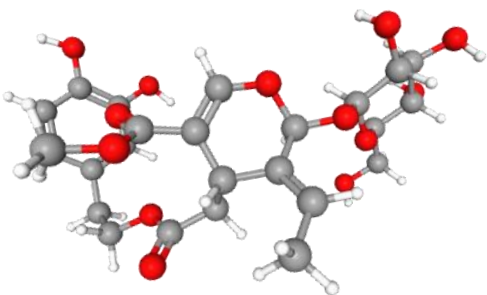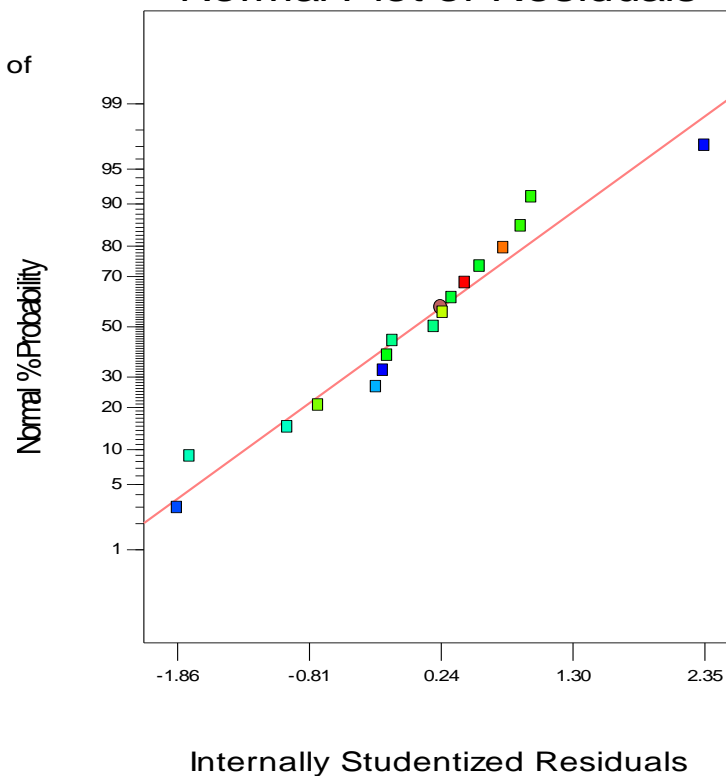
MS PARAMETERS
Hydroxy-tyrosol

Monitored response: Peak area

Hydroxytyrosol Area

Color points by value of
Hydroxytyrosol Area:
- 3.1133E+008
- 4.94076E+008
- 6.76823E+008

Normal Plot of Residuals

Hydroxytyrosol Area

Color points by value of
Hydroxytyrosol Area:
- 3.1133E+008
- 4.94076E+008
- 6.76823E+008

Residuals vs. Predicted

«ΕΡΕΥΝΗΤΙΚΑ ΣΧΕΔΙΑ ΚΑΙΝΟΤΟΜΙΑΣ»
Ε.Π. ΠΕΡΙΦΕΡΕΙΑΣ ΚΕΝΤΡΙΚΗΣ
ΜΑΚΕΔΟΝΙΑΣ
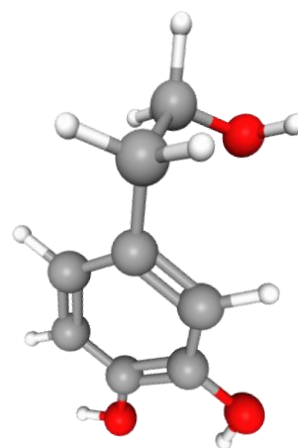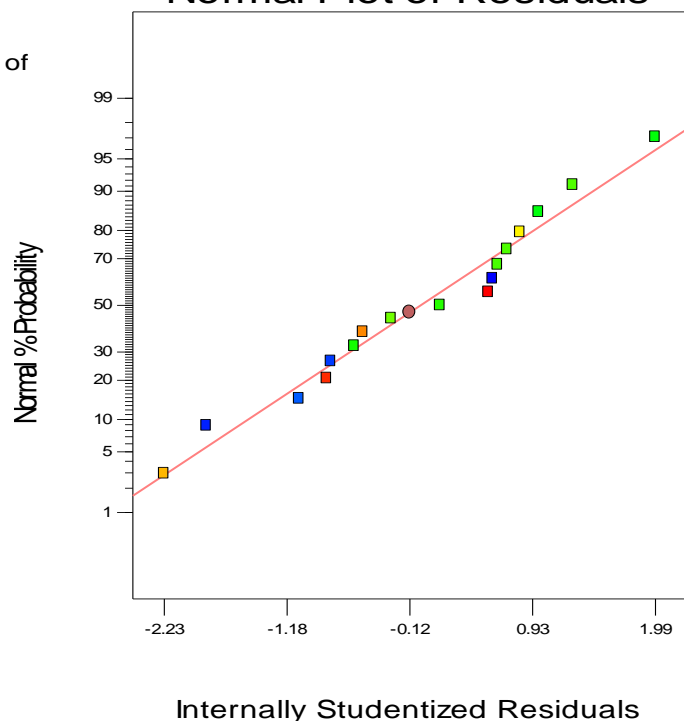
MS PARAMETERS
Oleocanthal

Oleocanthal Area

Color points by value of Oleocanthal Area:
- 9.62866E+006
- 1.76658E+007
- 2.57029E+007

Normal Plot of Residuals

Internally Studentized Residuals
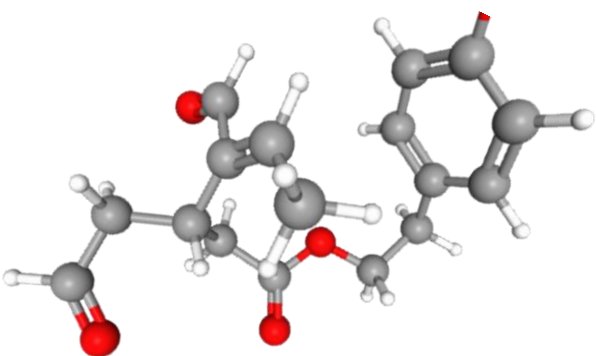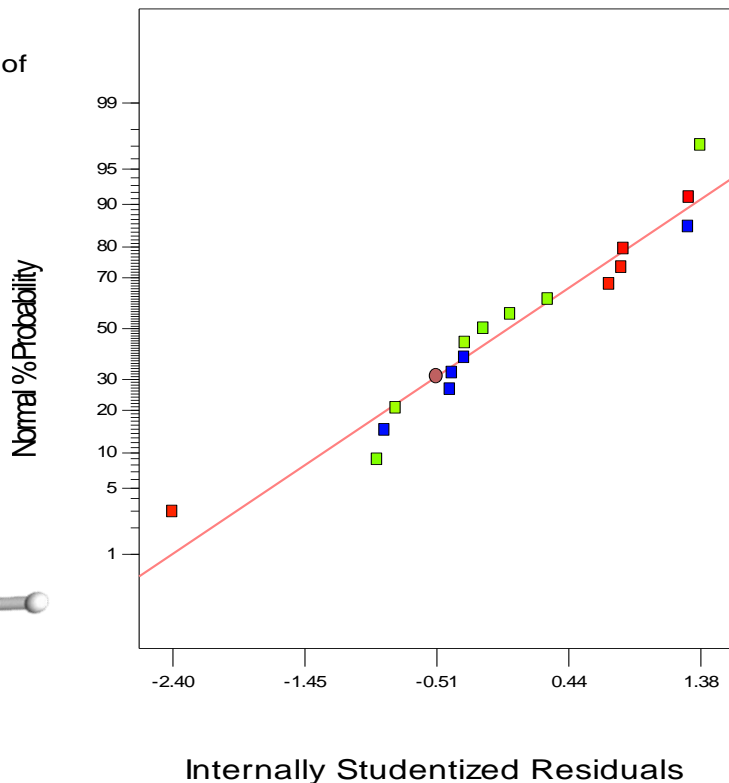
Design-Expert® Software
Oleocanthal Area

Color points by value of Oleocanthal Area:
- 9.62866E+006
- 1.76658E+007
- 2.57029E+007

Monitored response: Peak area

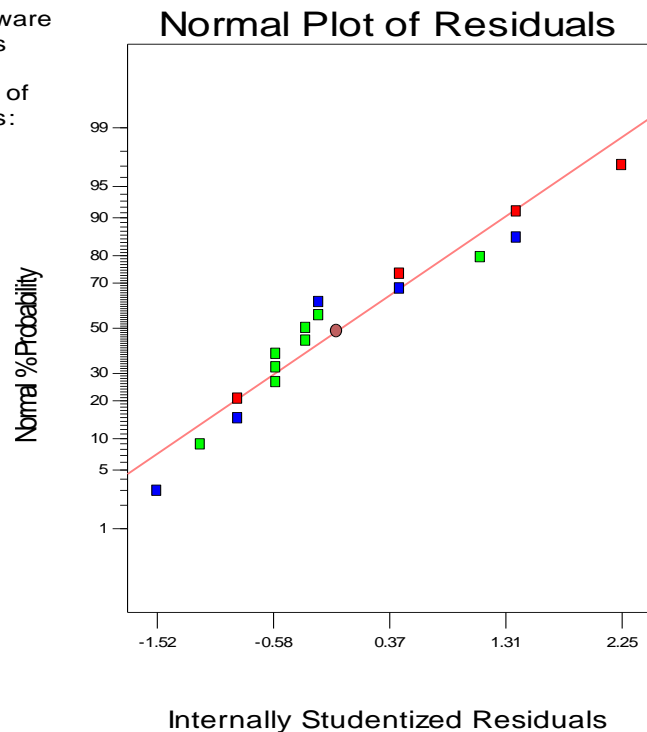### Residuals vs. Predicted

Predicted
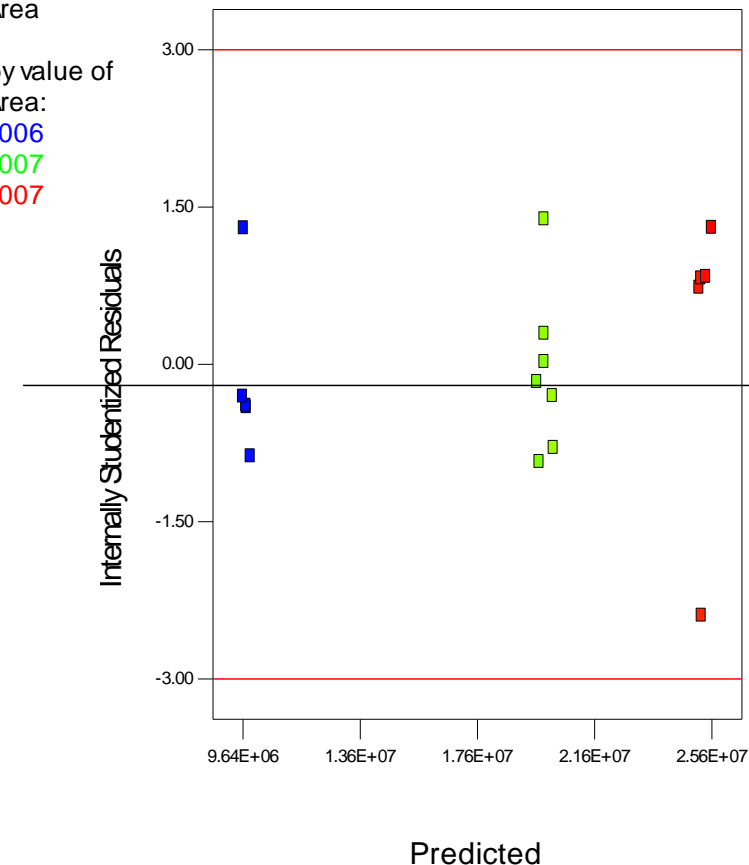
Apigenin

Monitored response: Peak area

Design-Expert® Software
Apigenine Fragments

Color points by value of
Apigenine Fragments:
- 6
- 7
- 8

Normal Plot of Residuals

Internally Studentized Residuals
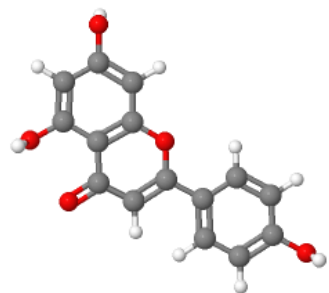
Design-Expert® Software
Apigenine Fragments

Color points by value of
Isolation window:
- 1.0
- 1.5
- 2.0

Residuals vs. Predicted

Predicted

«ΕΡΕΥΝΗΤΙΚΑ ΣΧΕΔΙΑ ΚΑΙΝΟΤΟΜΙΑΣ»
Ε.Π. ΠΕΡΙΦΕΡΕΙΑΣ ΚΕΝΤΡΙΚΗΣ
ΜΑΚΕΔΟΝΙΑΣ

Orbitrap: Design of experiments

Improving response on the complex matrix of EVOO by implementing a design of experiments (DoE)
Two major bioactive compounds, namely **oleuropein** and **hydroxytyrosol** were selected as model compounds, as well as **apigenin** and **oleocanthal**

Response surface modellin... designs (CCD) were select... and MS/MS parameters
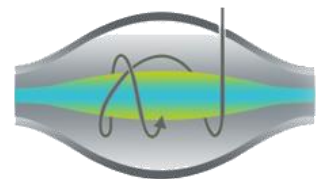Area and number of fragm... function (DF) approach

Aux...
CID...
AG...
MS...

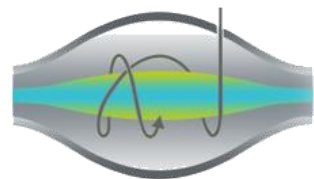|	Parameters	Levels
MS	Auxiliary heater temperature	200, 300, **400** °C
	AGC target	**2e5**, 5e5, 1e6
	in-source CID	**5**, 10, 15 eV
MS/MS	AGC target	5e4, 1e5, **2e5**
	in-source CID	5, 10, **15** eV
	isolation window	1, 1.5, **2** m/z

Orbitrap: Design of experiments

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Response surface modellin
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and MS/MS parameters
Area and number of fragm
function (DF) approach

Aux
CID
AG
MS,

	Parameters	Levels
MS	Auxiliary heater temperature	200, 300, **400** °C
	AGC target	**2e5**, 5e5, 1e6
	in-source CID	**5**, 10, 15 eV
MS/MS	AGC target	5e4, 1e5, **2e5**
	in-source CID	5, 10, **15** eV
	isolation window	1, 1.5, **2** m/z

Orbitrap: Design of experiments

Improving response on the complex matrix of EVOO by implementing a design of experiments (DoE)
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Response surface modelling designs (CCD) were select and MS/MS parameters
Area and number of fragm function (DF) approach

Aux
CID
AG
MS,

	Parameters	Levels
MS	Auxiliary heater temperature	200, 300, **400** °C
	AGC target	**2e5**, 5e5, 1e6
	in-source CID	**5**, 10, 15 eV
MS/MS	AGC target	5e4, 1e5, **2e5**
	in-source CID	5, 10, **15** eV
	isolation window	1, 1.5, **2** m/z

Chemical fingerprint

Greek EVOO Samples

➤ **300 samples**

from different areas around Greece
(Chalkidiki, Creta, Thessaly, Peloponnese, Katerini)
(> 8 cultivars)

Target and non-target analysis

Non-target workflow in CD

Non-target workflow in CD

Food Research w Stats Unknown ID w Online and Local Database Searches

Selected databases:

Acros Organics

BIONET-Key Organics

Carotenoids Database

DISMA, Department of Agri-Food Molecular Sciences, FDA UNII – NLM

Food and Agriculture Organization of the United Nations

FooDB

Phenol-Explorer

Fill Gaps

Search mzCloud

Predict Compositio...

...e Mass Defect

Apply mzLogic

Apply Spectral Distance

Align Retention Times

Chemical fingerprint

Target Analysis
15 compounds
Isotopic profile
Retention time
MS2 Fragmentation
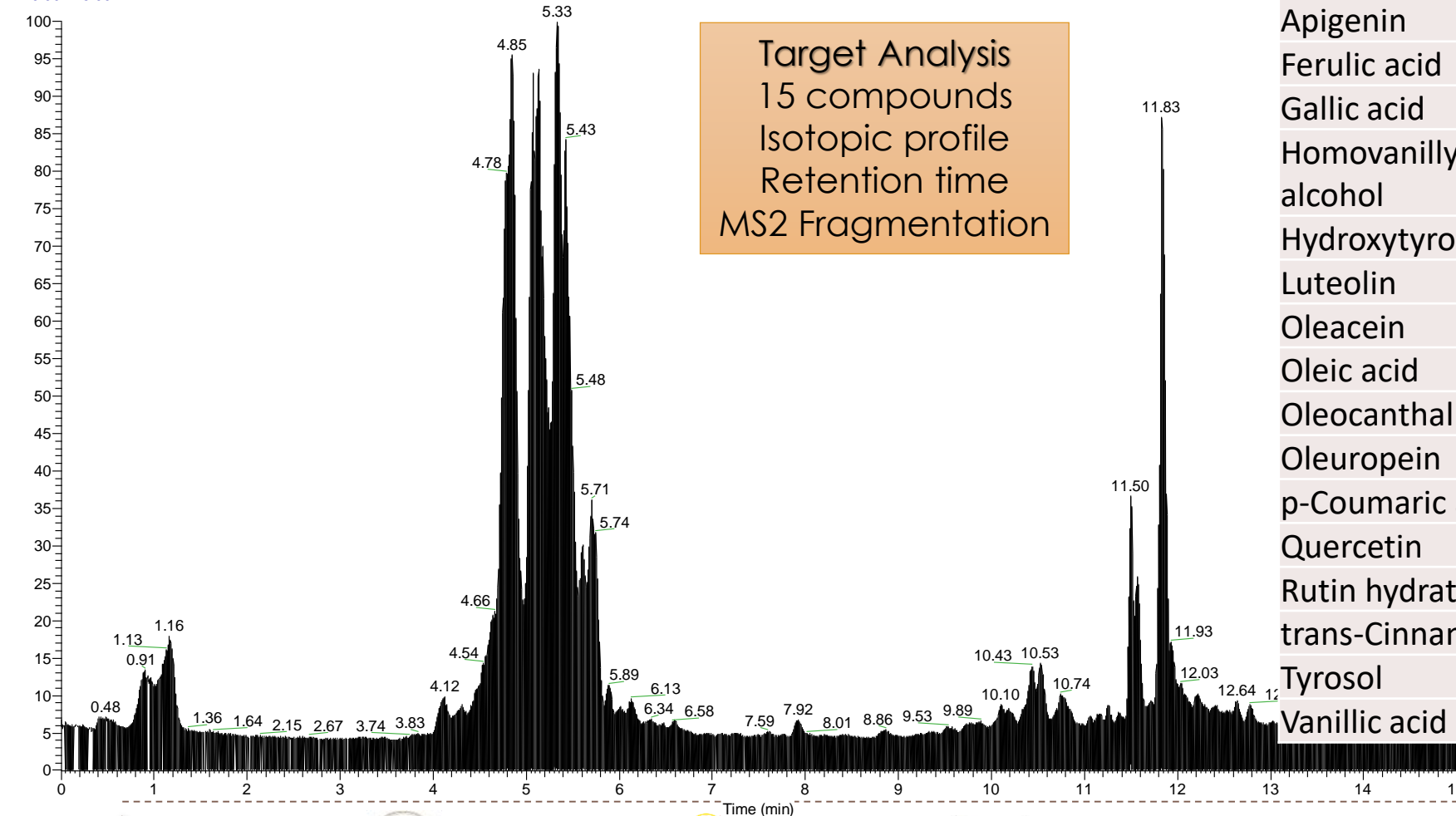
| Compounds	Mass [m/z]	Chemical formula
Apigenin	269.04555	C15H10O5
Ferulic acid	193.05063	C10H10O4
Gallic acid	169.01425	C7H6O5
Homovanillyl alcohol	167.07137	C9H12O3
Hydroxytyrosol	153.05572	C8H10O3
Luteolin	285.04046	C15H10O6
Oleacein	319.11871	C17H20O6
Oleic acid	281.2486	C18H34O2
Oleocanthal	303.1238	C17H20O5
Oleuropein	539.17701	C25H32O13
p-Coumaric acid	163.04007	C9H8O3
Quercetin	301.03538	C15H10O7
Rutin hydrate	627.15667	C27H32O17
trans-Cinnamic acid	147.04515	C9H8O2
Tyrosol	137.0608	C8H10O2
Vanillic acid	167.03498	C8H8O4

Chemical fingerprint

Chemical fingerprint

Filtered results

Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Ma▾	MS2
Oleic acid	C18 H34 O2	▪▪▪▪	-1.02	282.25559	14.049	17805406373	99.9	▪
Palmitic acid	C16 H32 O2	▪▪▪▪	-1.53	256.23984	14.078	953280713	99.9	▪
Oleic acid alkyne	C18 H30 O2	▪▪□□	-0.86	278.22434	13.397	573845671	99.8	▪
Oleanolic acid	C30 H48 O3	▪▪▪□	-0.77	456.36000	13.694	2788078101	99.8	▪
9(Z),11(E)-Conjugated linoleic acid	C18 H32 O2	▪▪▪□	-1.16	280.23990	13.690	2808585191	99.6	▪
Labdanolic acid	C20 H36 O3	▪▪▪□	-1.01	324.26612	13.143	19340575	89.0	▪
NP-011548	C18 H34 O3	▪▪□□	-1.56	298.25033	12.718	3576464646	88.7	▪
Myristyl sulfate	C14 H30 O4 S	░▪▪░	-0.74	294.18626	12.757	23980219	86.7	▪
Syringic acid	C9 H10 O5	▪▪▪□	-4.20	198.05199	1.567	46161941	85.5	▪
Corchorifatty acid F	C18 H32 O5	▪▪□▪	-0.88	328.22468	8.512	44281796	81.5	▪
16-Hydroxyhexadecanoic acid	C16 H32 O3	▪▪▪░	-0.71	272.23495	12.827	48663667	76.7	▪
(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	C18 H34 O5	▪▪□□	-0.62	330.24042	9.101	85845062	73.9	▪
(+/-)9-HpODE	C18 H32 O4	▪▪▪□	-1.11	312.22971	11.487	364499306	72.7	▪
Genistein	C15 H10 O5	▪▪□□	-0.46	270.05270	8.643	2105812282	66.1	▪
Luteolin	C15 H10 O6	▪▪▪▪	-0.73	286.04753	7.936	1187746042	64.7	▪
(3β,5ξ,9ξ)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic aci	C30 H46 O5	▪▪□□	-0.89	486.33409	11.939	38344605	64.4	▪

Chemical fingerprint

Filtered results

Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Ma ▼	MS2
Oleic acid	C18 H34 O2	🟩🟩🟩	-1.02	282.25559	14.049	17805406373	99.9	🟩
Palmitic acid	C16 H32 O2	🟩🟩🟩	-1.53	256.23984	14.078	953280713	99.9	🟩
Oleic acid alkyne	C18 H30 O2	🟩🟩🟧	-0.86	278.22434	13.397	573845671	99.8	🟩
Oleanolic acid	C30 H48 O3	🟩🟩						
9(Z),11(E)-Conjugated linoleic acid	C18 H32 O2	🟩🟩🟧						
Labdanolic acid	C20 H36 O3	🟩🟩🟧	-1.01	324.26612	13.143	19340575	89.0	🟩
NP-011548	C18 H34 O3	🟩🟩🟧	-1.56		13.710			🟩
Myristyl sulfate	C14 H30 O4 S							
Syringic acid	C9 H10 O5							
Corchorifatty acid F	C18 H32 O5							
16-Hydroxyhexadecanoic acid	C16 H32 O3							
(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	C18 H34 O5							
(+/-)9-HpODE	C18 H32 O4							
Genistein	C15 H10 O5							
Luteolin	C15 H10 O6							
(3β,5ξ,9ξ)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic aci	C30 H46 O5							

XIC for the 5 compounds with the highest annotation score

Oleic acid
Palmitic acid
Oleic acid alkyne
Oleanonic acid
Conjugated linoleic acid
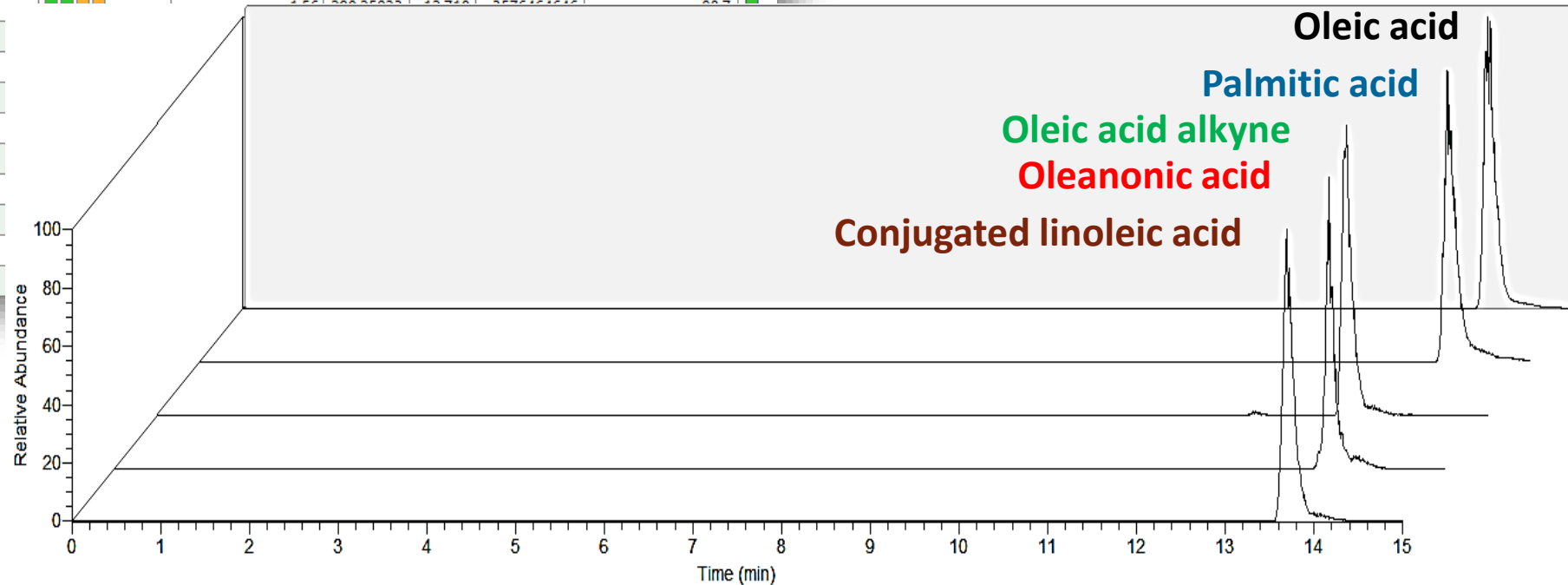
Chemical fingerprint

Preliminary results

150 samples
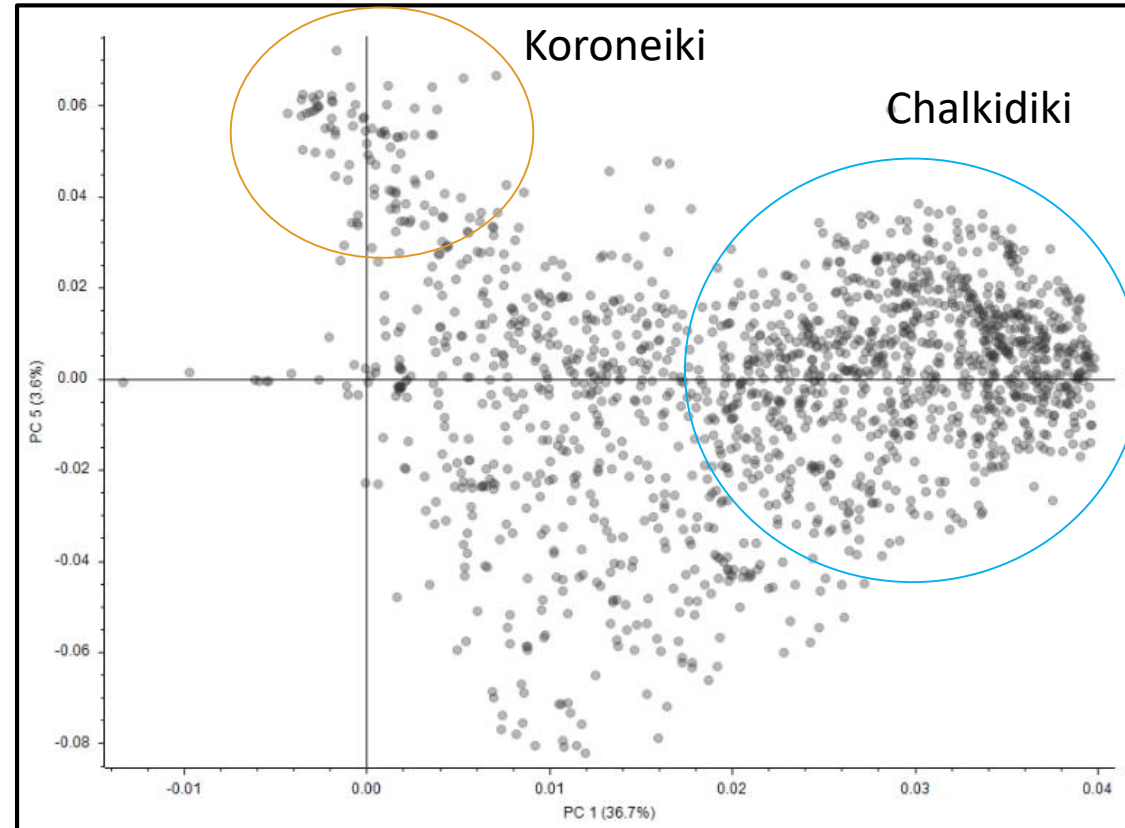
Treatment of data in progress

ArcGIS Mapping: Geographical origin

❑ **Olive oil authenticity assessment involves the detection of potential olive oil adulteration** (e.g. olive oil mixed with cheaper edible oils) and **the verification of geographical origin**, botanical variety and organoleptic profile of olive oil.

❑ **The metabolic profile of olive oil is strongly affected by different agronomic variables** such as the agricultural and harvesting methods, the environmental conditions, the olive fruit cultivar and ripening stage, as well as different technological variables including the milling and malaxation process.

❑ **The processing time, temperature and storage conditions also constitute important parameters** effecting the metabolic fingerprint of olive oil.

ArcGIS: Geographical origin

❑ **The verification of the geographical origin of extra virgin (EVOO) is crucial to protect consumers from misleading information**

❑ **However, despite the large number of studies performed, specific markers are still not available**

To develop efficient tools for the geographical authentication of EVOO, it is necessary to identify the most robust markers and analytical approaches

ArcGIS: Building an Interactive Geodatabase

On-line ArcGIS Tools

✓ **Exploitation and visualization of analytical results from different varieties of extra virgin olive oil (EVOO) is of high importance**

✓ **Employment of Environmental Systems Research Institute (ESRI) ArcGIS tools** was investigated, enabling their correlation with potential sources and chemical analysis in a single interface

Survey123

Dashboards

ESRI ArcGIS

Desktop Web Device

Web GIS

Portal

Apps

Portal

GIS Servers

Ready-to-Use Content

Server

Online Content and Services

ARCGIS ONLINE

AGOL

1 DATA Find authoritative data sources

2 APPS Build mobile and field apps

3 MAPS Design web maps and scenes

4 SHARE Collaborate with your organization

Survey123 at a glance

Ask Questions
(Create & Share)

Get Answers
(Capture Data)
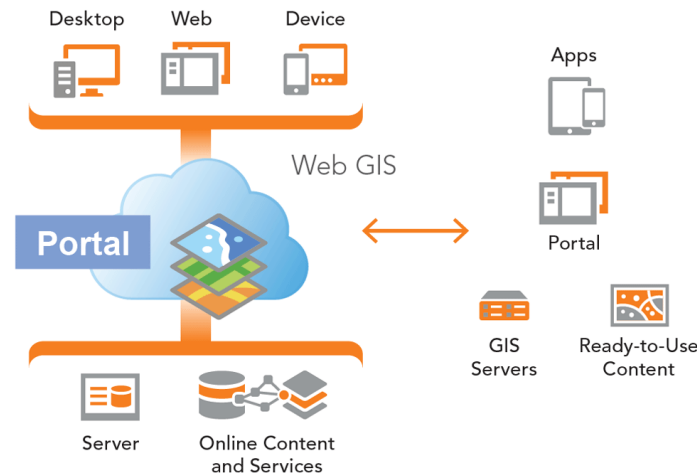
Make Decisions
(View & Analyse)

Survey123 is a simple 1-2-3 process

ArcGIS: Building an Interactive Geodatabase

Selection of the critical factors

Employing ArcGIS Survey123 for data collection

ArcGIS Dashboards for visualization as interactive graphs, maps, and tables of data acquired

ArcGIS: Building an Interactive Geodatabase

ArcGIS: Building an Interactive Geodatabase

ArcGIS: Building an Interactive Geodatabase

ArcGIS: Building an Interactive Geodatabase

ArcGIS: Building an Interactive Geodatabase

Ερωτηματολόγιο EVOO

Πληροφορίες καλλιέργιας και παραγωγής ελαιολάδου

Ποια/-ες ποικιλία/-ες καλλιεργείτε?*

- ☐ Κορωνέικη
- ☐ Αθηνολιά (Τσουνάτη)
- ☐ Λαδολιά
- ☐ Καλαμών
- ☐ Αμφίσσης
- ☐ Χαλκιδικής
- ☐ Μανάκι
- ☐ Κέρκυρας
- ☐ Άλλη:

Εφαρμόζετε πρακτικές βιολογικής καλλιέργειας?*

- ○ Ναι
- ○ Όχι

Καταχώρηση φυτοφαρμάκων με την εμπορική ονομασία

Αποποίηση ευθυνών: Η κάτωθι λίστα με τις εμπορικές ονομασίες φυτοπροστατευτικών σκευασμάτων παρατίθεται μόνο βοηθητικά στην συμπλήρωση του ερωτηματολογίου. Οι συμμετέχοντες του προγράμματος δεν συνεργάζονται με τις εταιρίες παραγωγής των κάτωθι προιόντων. Το παρόν δεν συνιστά διαφήμιση, προώθηση ή σύσταση για την χρήση συγκεκριμένων προιόντων.

- ☐ Insignia
- ☐ Mavita
- ☐ Χαλκούχα σκευάσματα
- ☐ Stroby
- ☐ Imidan
- ☐ Bulldock
- ☐ Admiral
- ☐ Rogor (έχει καταργηθεί)

Ποιές είναι οι πιο συνήθεις ασθένειες και έντομα που αντιμετωπίζετε?*

- ☐ Δάκος
- ☐ Ασπιδιωτός ή λευκή ψώρα εσπεριδοειδών
- ☐ Πυρηνοτρήτης
- ☐ Βαμβακάδα
- ☐ Μύκητες και βακτήρια
- ☐ Ρυγχίτης
- ☐ Ακάρεα
- ☐ Λεκάνιο, Φιλλίπια και άλλα κοκκοειδή
- ☐ Γλοιοσπόριο (Ανθράκωση, Παστέλλα)
- ☐ Παραμορφωτικό ακάρι
- ☐ Φλοιοφάγος
- ☐ Φλοιοτρίβης

ArcGIS: Building an Interactive Geodatabase

Εκτιμώμενη απαιτούμενη ποσότητα νερού για την επεξεργασία στο ελαιοτριβείο (L)

1 2³

Απευθύνεστε σε τοπικό ελαιοτριβείο?*

○ Ναι

○ Όχι

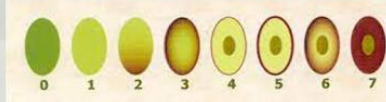**Φιλτράρετε το ελαιόλαδο πριν την τυποποίηση/διάθεση στον καταναλωτή?**

○ Ναι

○ Όχι

Μέσο αποθήκευσης ελαιολάδου μετά το ελαιοτριβείο*

○ Ανοξείδωτο μεταλλικό δοχείο

○ Πλαστικό δοχείο

○ Άλλο:

Μήνες ωρίμανσης ελαιοκαρπού*

1 2³

Ωρίμανση ελαιοκαρπού

○——○——○——○——○——○——○
1   2   3   4   5   6   7

Η αποθήκευση/μεταφορά ελαιόκαρπου από ελαιώνα σε ελαιοτριβείο γίνεται με

○ Σακιά γιούτας

○ Σακιά πλαστικά

○ Σακιά υφασμάτινα

○ Διάτρητα δίχτυα

○ Πλαστικά διάτρητα τελάρα

Τι χρόνος μεσολάβησε από συγκομιδή σε ελαιοποίηση (εβδομάδες)
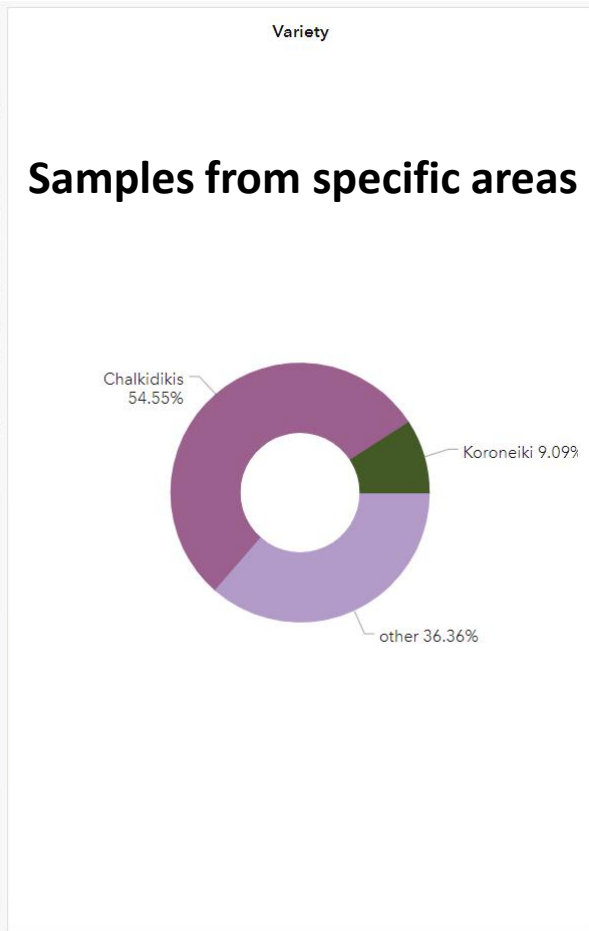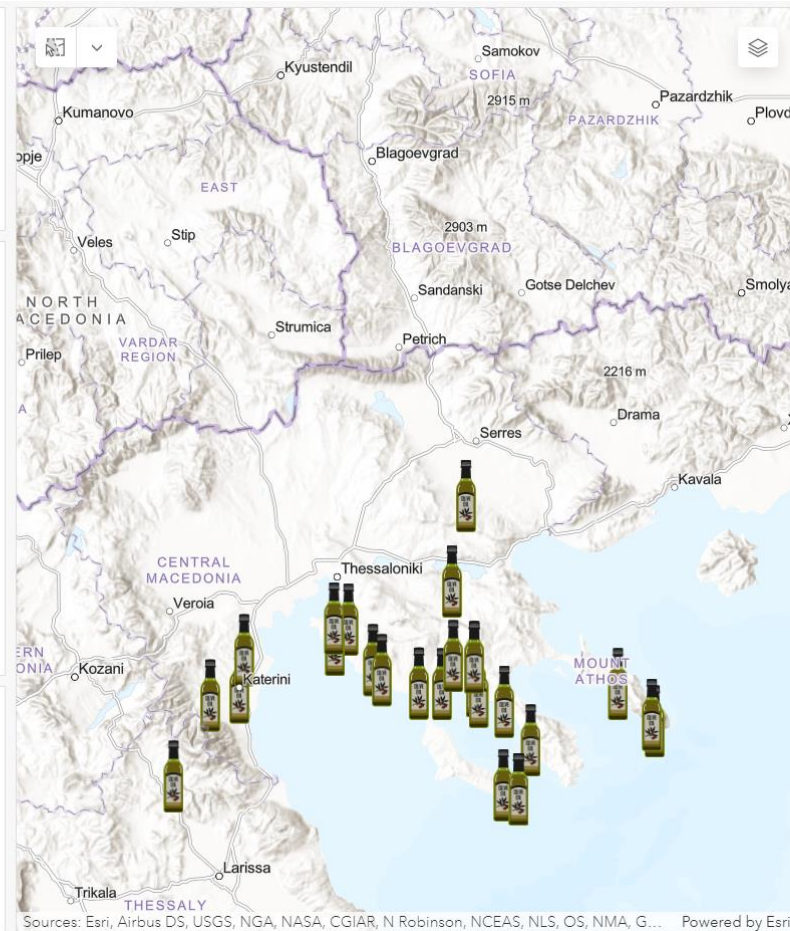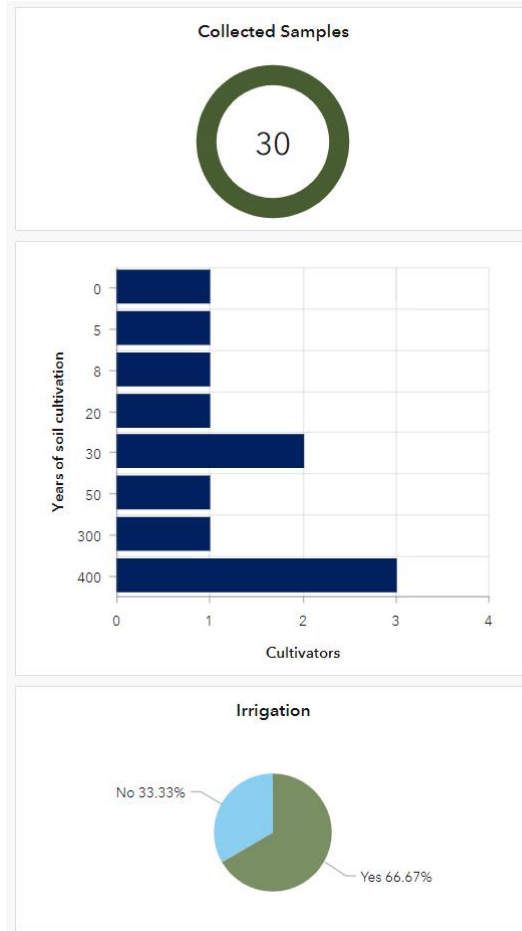
1 2³

Ποιά μέθοδο επεξεργασίας λαδιού εφαρμόζετε στο ελαιοτριβείο?*

☐ Ψυχρή έκθλιψη

☐ Μη Ψυχρή έκθλιψη

☐ Άλλη:

ArcGIS: Building an Interactive Geodatabase
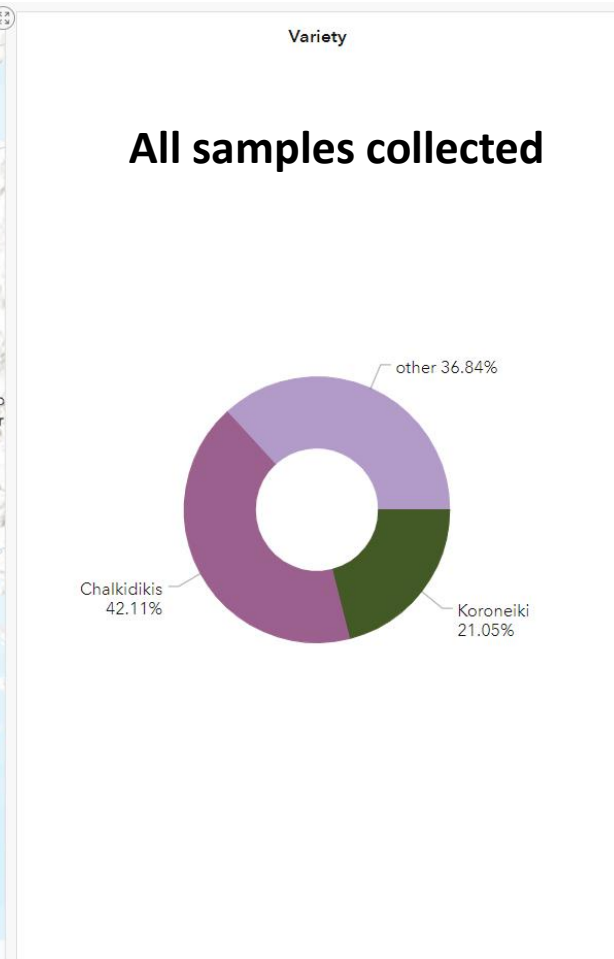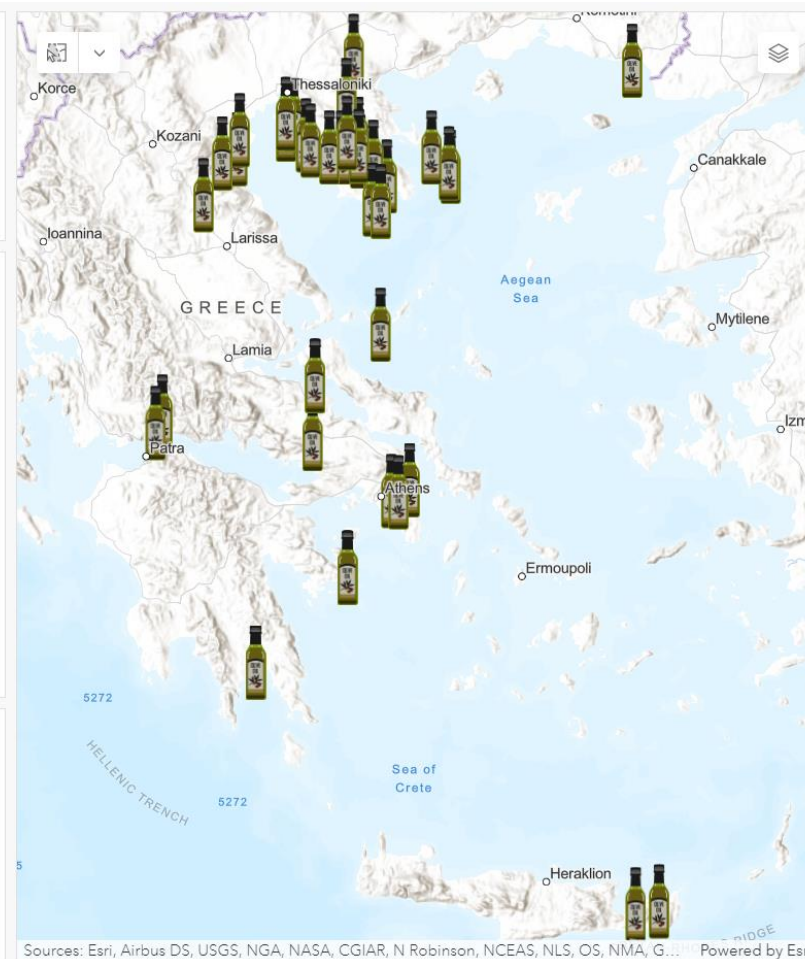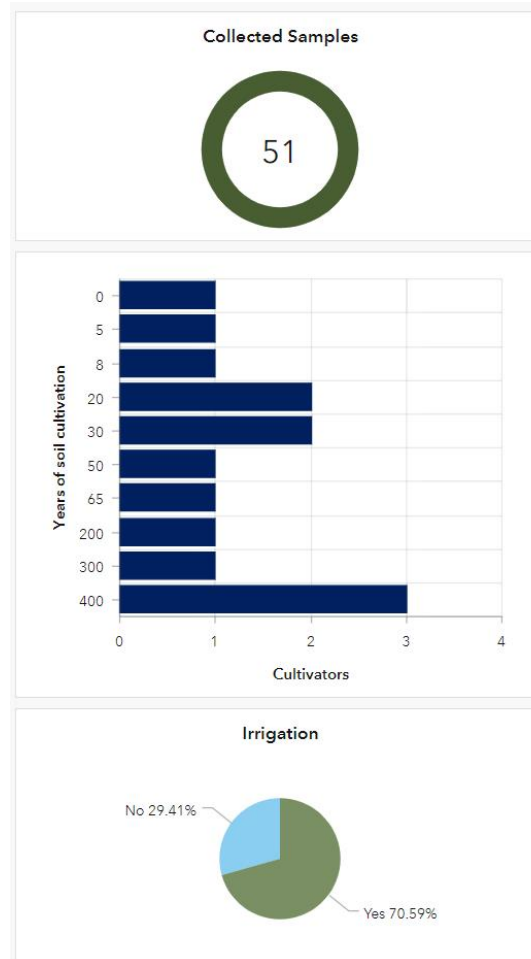
ArcGIS: Building an Interactive Geodatabase

Conclusions

- ✓ **HRMS analysis is proved a powerful tool for olive oil authenticity evaluation.** However, the challenge in HRMS methodologies is to export and evaluate the massive quantities of the data generated.

- ✓ **Central composite designed improved the efficiency of MS and MS/MS analysis** of the complex matrix of EVOO

- ✓ **Chemometrics data processing and feature selection are essential to propose markers and guarantee the authenticity of food products.**

Conclusions

- ✓ Preliminary results suggest that levels **of apigenin, vanillic acid, luteolin and oleocanthal play a crucial role in categorizing samples** among different locations and cultivars

- ✓ PCA and cluster analysis was performed in order to classify the cultivars of EVOO (in progress)

- ✓ The primary objective will be to Identify **key-factors that differentiate between cultivars and provide insight on how location and cultivation practices affect levels of key-bioactive components in EVOO**

Conclusions

✓ **ArcGIS proved as useful tool** for comprising a geodatabase and combining geodata and chemical analysis data

✓ **ArcGIS Survey123 enabled the participation of local cultivators**

✓ **Visualise data** in a public easy-to-use interactive platform **in Dashboards, engaging local cultivators**

✓ Finalisation of geodatabase is in progress

Next steps

- ✓ **The mapping of the NIR spectroscopic profile of various types of Greek olive oil** in order to create a representative Library for the areas of interest.

- ✓ **The use of chemometric techniques to correlate the NIR spectroscopic profile** with the qualitative/quantitative characteristics of olive oil, resulting in faster identification and identification of bioactive ingredients.

- ✓ Chemical fingerprint by coupling NIR spectroscopic profile and HRMS data

- ✓ The development of an innovative HRMS method using specialized metabolomics and chemometric tools to identify the geographical origin of an EVOO
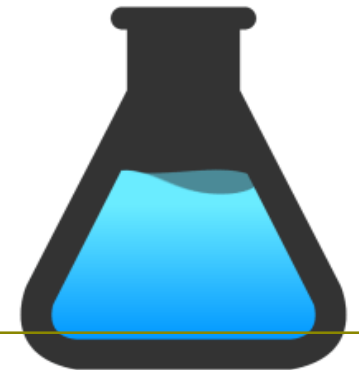
Thank You!

- *Ανάπτυξη "Έξυπνων Εργαλείων" για τον Έλεγχο της Ποιότητας, της Αυθεντικότητας και της Γεωγραφικής Προέλευσης του Εξαιρετικά Παρθένου Ελαιολάδου*

2nd ISO-FOOD Symposium
Portorož, Slovenia
April 24 - 26, 2023

European Union
European Regional
Development Fund

ΕΣΠΑ
2014-202
ανάπτυξη - εργασία - αλληλεγγύη

ENVMS Group
High-Resolution Mass Spec

Aristotle University of Thessaloniki
Environmental Pollution Control Laboratory (EPCL)
ENVMS Group

ΚΕΔΕΚ
ΚΕΝΤΡΟ ΔΙΕΠΙΣΤΗΜΟΝΙΚΗΣ ΕΡΕΥΝΑΣ ΚΑΙ ΚΑΙΝΟΤΟΜΙΑΣ ΑΠΘ

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