ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

2019 AOCS Annual Meeting
May 5-8th, St. Louis, USA

Session
The OLEUM project advancements for a global strategy to guarantee olive oil quality and fight fraud

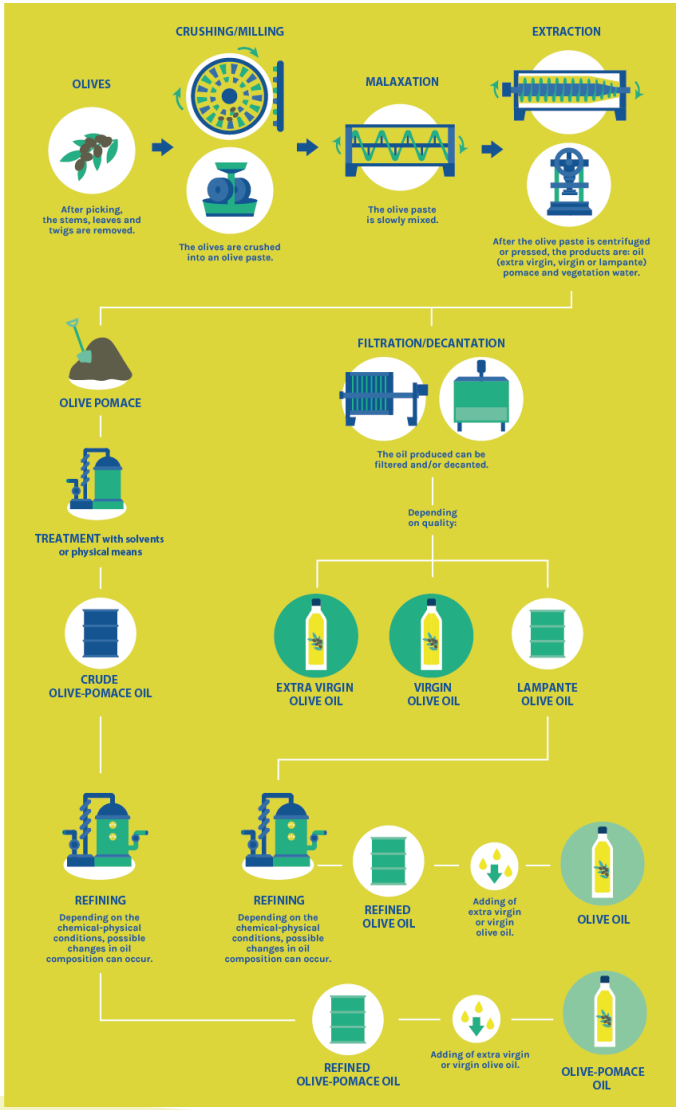
Oleum
Better solutions to protect olive oil quality and authenticity

Olive oil: from different processing to different regulatory frameworks. How to ensure its quality and authenticity at a global level? Challenges, gaps and improvements proposed by the OLEUM project

Prof. Tullia Gallina Toschi
Department of Agricultural and Food Science – University of Bologna
Scientific Coordinator EU H2020 OLEUM

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635690.

From different processing…



HOW ARE OLIVE OILS PRODUCED?

*OLEUM "Advanced solutions for assuring the authenticity and quality of olive oil at a global scale" has received funding from the European Commission within the Horizon 2020 Programme (2014–2020), grant agreement no. 635690. The information expressed in this infographic reflects the authors' views; the European Commission is not liable for the information contained therein. Definitions according to European Regulation. Created by **OLEUM Partners**, edited by **EUFIC** and designed by **Pouce-pied**.*

HOW ARE OLIVE OILS PRODUCED?

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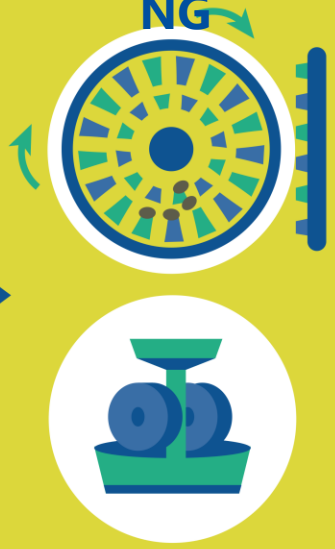
OLIVES

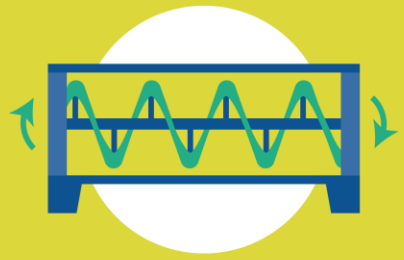
After picking, the stems, leaves and twigs are removed.

CRUSHING/MILLING



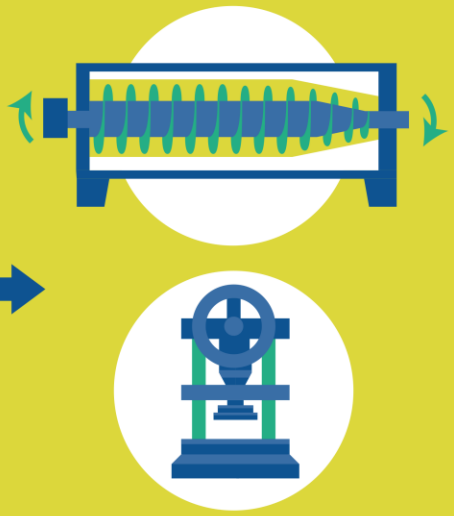
The olives are crushed into an olive paste.

MALAXATION



The olive paste is slowly mixed.

EXTRACTION



After the olive paste is centrifuged or pressed, the leftover products are:
oil (extra virgin, virgin or lampante) pomace and vegetation water.

FILTRATION/DECANTATION

The oil produced can be filtered and/or decanted.

Depending on quality it is possible to obtain:

EXTRA VIRGIN OLIVE OIL

VIRGIN OLIVE OIL

LAMPANTE OLIVE OIL

REFINING

Depending on the chemical-physical conditions, minor or relevant changes in oil

REFINED OLIVE OIL

Adding of extra virgin or virgin olive oil.

OLIVE OIL

Legend

Fit for human consumption

Not fit for human consumption

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OLIVE POMACE

TREATMENT with solvents or physical means

CRUDE OLIVE-POMACE OIL

REFINING

Depending on the chemical-physical conditions, minor or relevant changes in oil composition can occur.

REFINED OLIVE-POMACE OIL

Adding of extra virgin or virgin olive oil.

OLIVE-POMACE OIL

Legend

Fit for human consumption

Not fit for human consumption

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...to different regulatory frameworks.

Codex Alimentarius

Standard for olive oils and olive pomace oils
CODEX STAN 33-1981. Adopted in 1981.
Revisions: 1989, 2003, 2015, 2017.
Amendments: 2009, 2013.

1 Member Organization (EU)
188 Member Countries

International Olive Council

International olive council. Trade standard for Olive oils and Olive Pomace Oils
COI/T.15/NC No 3/Rev. 12. June 2018

1 Member Organization (EU)
14 Member Countries

~94% of the OO world production

The EU

European Commission, Reg. (CEE) 2568/91
European Communities Official Journal L 248 5.9.1991 and further amendments

27 Member Countries

~71% of the OO world production

National standards

Argentina
Código alimentario argentino. Capitulo VII. Alimentos grasos. Aceites alimenticios. Artículos 535 y 536.

Australia
Australian standard. Olive oils and olive-pomace oils. July 2011.

California
State of California. Department of food and agriculture. Grade and Labeling Standards for Olive Oil, Refined-Olive Oil and Olive-Pomace Oil. Effective September 2014. Amendment February 2015.

Brazil
Ministério da agricultura, pecuária e abastecimento. Gabinete do ministro. Instrução normativa nº 1, de 30 de Janeiro de 2012.

China
General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) National Standard of the People's Republic of China ICS 67.200.10.

India
Draft Indian Standard olive oil — specification ICS No. 67.200 Doc No.: FAD 13 (2505).

South Africa
South African national standard. Olive oils and olive-pomace oils. SANS 1377:2015

USA
United States Standards for grades of olive oil and olive-pomace oil. Effective October 25, 2010.

The Olive Oil Regulatory Framework

OOs have to comply with different rules and standards depending on where they are traded

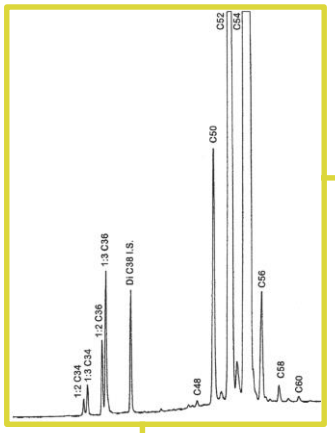
QUALITY CONTROL

International and National Standards

- *Parameters*
- *Legal limits*

Harmonization ISO methods → *Analytical methods*

AUTHENTICITY CONTROL



DRAFT INTERNATIONAL STANDARD
ISO/DIS 21846

ISO/TC **34**/SC **11** | Secretariat: **BSI**

Voting begins on: **2017-11-16** | Voting terminates on: **2018-02-08**

Vegetable fats and oils — Determination of composition of triacylglycerols and composition and content of diacylglycerols by capillary gas chromatography

The Olive Oil Regulatory Framework

Dissimilarities that involve different commercial categories

	Virgin oils					Non-virgin oils		Olive pomace oils		
	Edible oils				Non-edible oil	Edible oils		Non-edible oil	Edible oils	
	EVOO	**VOO**	**MGV**	**OOO***	**LOO**	**ROO**	**OO**	**COPO**	**ROPO**	**OPO**
EU	X	X	n.c.	n.c.	X	X	X	X	X	X
IOC	X	X	n.c.	X	X	X	X	X	X	X
CODEX	X	X	n.c.	X	n.c.	X	X	n.c.	X	X
Argentina	X	X	n.c.	X	X	X	X	n.c.	X	n.c.
USDA	X	X	n.c.	n.c.	X	X	X	X	X	X
Australia	X	X	n.c.	n.c.	X	X	X	X	X	X
South Africa	X	X	n.c.	n.c.	n.c.	X	X	n.c.	X	X
California	X	X	n.c.	n.c.	X**	X***	X	X	X°	X
China	X§	n.c.	X	n.c.	X	X	X	X	X	X
Brazil	X	X	n.c.	n.c.	X	X	X	n.c.	X	X
India	X	X	n.c.	X	X	X	n.c.	X	X	n.c.

EVOO = Extra Virgin Olive Oil, **VOO** = Virgin Olive Oil, **MGV** = Medium Grade Virgin Oil, **OOO** = Ordinary Olive Oil, **LOO** = Lampante Olive Oil, **ROO** = Refined Olive Oil, **OO** = Olive Oil, **CPOO** = Crude Olive Pomace Oil, **RPOO** = Refined Olive Pomace Oil, **OPO** = Olive Pomace Oil.

***The category ordinary olive oil is going to be deleted by IOC and, consequently, by Codex too.**

****is named Crude olive oil *****is named Refined olive oil blend °is named Refined olive-pomace blend oil §is named Premium virgin olive oil.**

Categories of olive oils and olive pomace oils in international and national standards (n.c., category not considered)

Table from D2.2 - Review on the dissimilarities among different technical norms, on the lack of methods harmonization (OO quality and authenticity) and on the reported atypical compositions of Oos.

The Olive Oil Regulatory Framework

Dissimilarities that involve quality parameters

EVOO category

	FA g oleic acid/100 g oil	PV meq O_2/Kg oil	K_{232}	K_{270}	FAEEs mg/kg oil	Md	Mf
EU	≤0.8	≤20	≤2.50	≤0.22	≤35	0	>0
IOC	≤0.8	≤20	≤2.50	≤0.22	≤35	0	>0
CODEX	≤0.8	≤20	≤2.50	≤0.22	n.a. ***	0	>0
Argentina	≤0.8	≤20	≤2.50	≤0.22	n.a.	n.a.	n.a.
USDA	≤0.8	≤20	≤2.50	≤0.22	n.a.	0	>0
Australia	≤0.8	≤20	≤2.50	≤0.22	n.a.	0	>0
South Africa	≤0.8	≤20	≤2.50	≤0.22	n.a.	0	>0
California	≤0.5	≤15	≤2.40	≤0.22	n.a.	0	>0
China	≤1.6*	≤10**	≤2.50	≤0.22	n.a.	0	>0
Brazil	≤0.8	≤20	≤2.50	≤0.22	FAME + FAEE< 75 mg/kg or > 150 mg/kg if FAEE/FAME > 1.5	0	>0
India	≤2.0*	≤20	n.a.	≤0.22	n.a.	n.a.	n.a.

*Expressed as mg KOH/g: 1.6 corresponds to 0.8%. **Expressed as mmol: 10 mmol correspond to 20 meq O_2/kg

*** Codex is evaluating to intoduce the FAEEs determination

Limits for quality parameters for EVOO category (n.a., not applied)

The Olive Oil Regulatory Framework

Dissimilarities that involve purity parameters

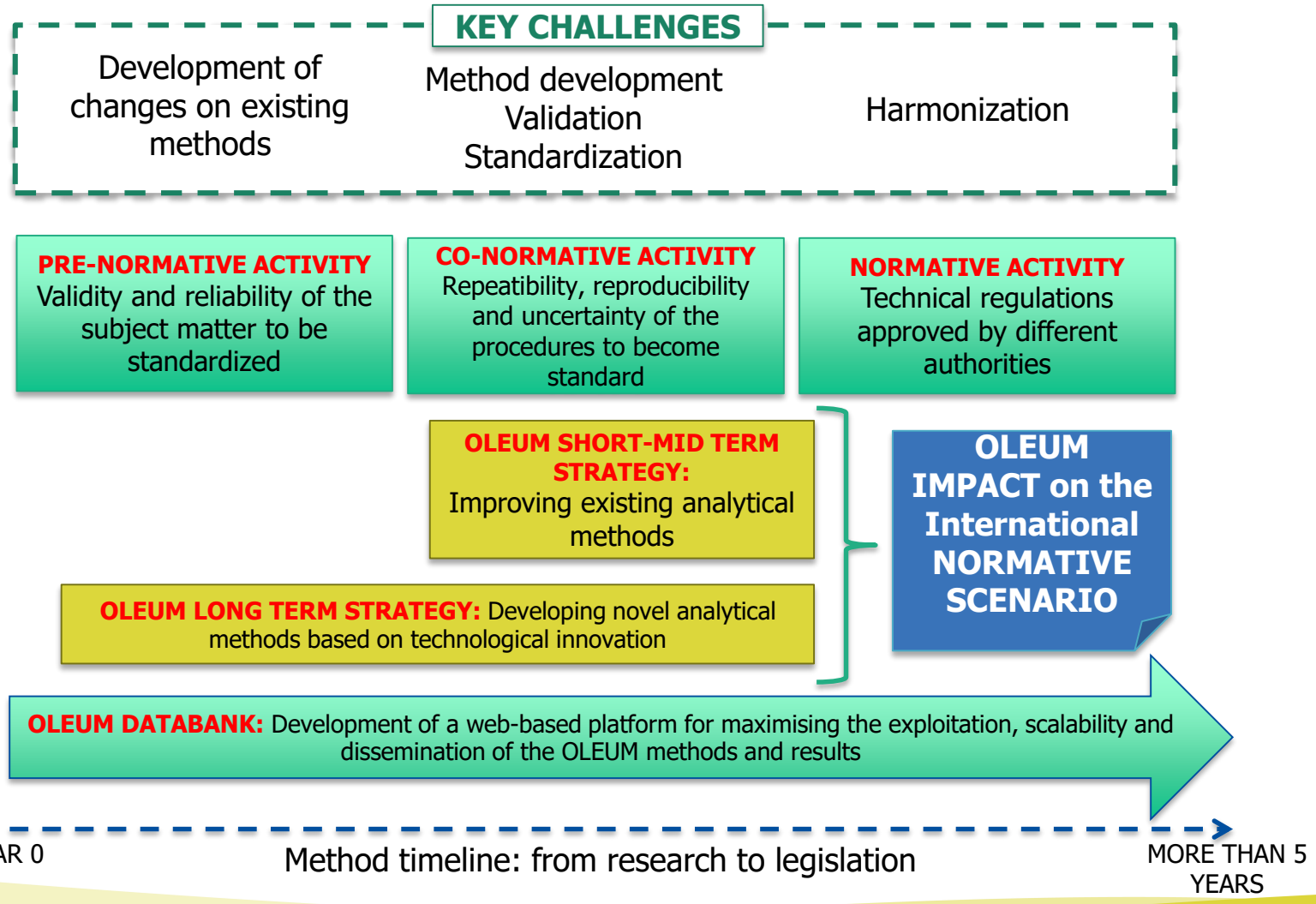
EVOO category

	Brassicasterol %	Campesterol %	Stigmasterol %	Apparent β-sitosterol %	Δ-7-stigmastenol %	Sterol content mg/kg oil
EU	≤0.1	≤4.0[a)]	<Campest.	≥93.0	≤0.5	≥1000
IOC	≤0.1	≤4.0[a)]	<Campest.	≥93.0	≤0.5	≥1000
CODEX	≤0.1	≤4.0[b)]	<Campest.	≥93.0	≤0.5	≥1000
Argentina	≤0.1	≤4.0[c)]	<Campest.	≥93.0	≤0.5	≥1000
USDA	≤0.1	≤4.5[d)]	<Campest.	≥93.0	≤0.5	≥1000
Australia	≤0.1	≤4.8	≤1.9	≥92.5	≤0.5	≥1000
South Africa	≤0.1	≤4.8	≤1.9	≥92.5	≤0.5	≥1000
California	≤0.1	n.a.	≤1.9	n.a.	n.a.	n.a.
China	n.a.	≤4.0	≤0.5	≥93.0	n.a.	≥1000
Brazil	≤0.1	≤4.0	<Campest.	≥93.0	n.a.	≥1000
India	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

a) When an authentic oil naturally has a campesterol level > 4.0 and ≤ 4.5, it is considered virgin or extra virgin olive oil if the stigmasterol level is ≤ 1.4%, the delta7 stigmastenol level is ≤ 0.3%. The other parameters shall meet the limits set out in the standard.
b) When an authentic oil naturally has a campesterol level > 4.0 and ≤ 4.5, it is considered virgin or extra virgin olive oil if the stigmasterol level Is ≤ 1.4%, the delta7 stigmastenol level is ≤ 0.3% and stigmastadienes is ≤ 0.05 mg/kg. The other parameters shall meet the limits set out in the standard.
c) When an authentic oil naturally has a campesterol level > 4.0 and ≤ 4.5, it is considered virgin or extra virgin olive oil if the delta7 stigmastenol level is ≤ 0,3% and the level of stigmasterol is ≤ 1.6%.
d) Campesterol values between 4.0 and 4.5 would be subject go further testing.

Limits for purity parameters for EVOO category (n.a., not applied)

Timeline of an OO analytical method *from its inception, validation, standardization (by Standard Developing Organization SDO) and regulation approval and the synergistic OLEUM strategy to maximize the impact on the* ***international normative scenario****.*



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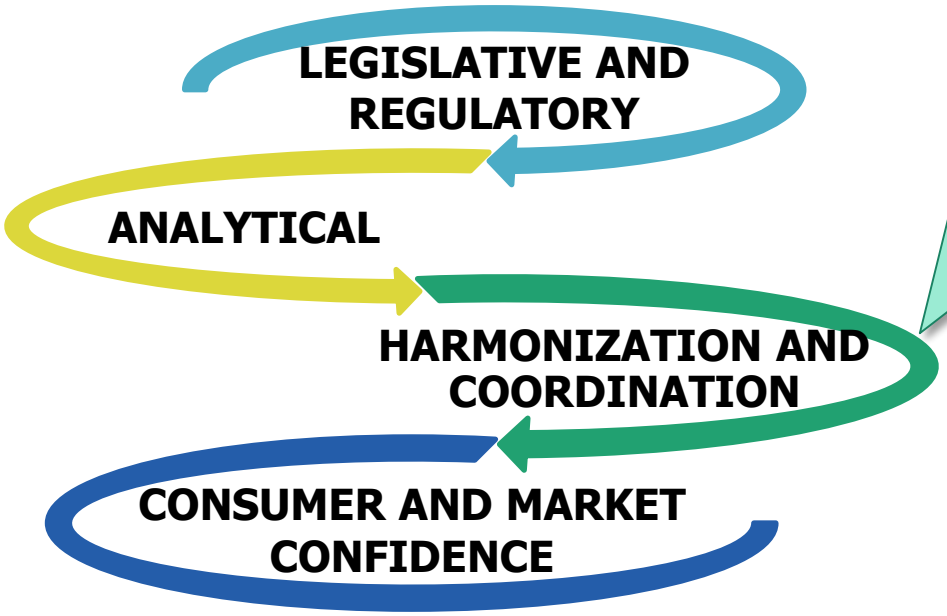
OLEUM project identified **four main gap levels** that need to be addressed through the **research & development** in the OO sector.



- To suggest improvements to **INTERNATIONAL REGULATIONS** and **RECOGNISED PROCEDURES** (EU, IOC, CODEX, ISO) including potential adoption of **new methods** and **reference materials**.
- To undertake technology transfer of new methods and procedures to the **WIDER ANALYTICAL COMMUNITY** and assess its **PROFICIENCY** by specific fit-for-purpose actions.
- To compile an **INVENTORY** of **EXISTING** and **EMERGING FRAUDULENT PRACTICES**.
- To promote **OPEN-ACCESS KNOWLEDGE GENERATION AND DISSEMINATION** by making **globally available** all the information coming from OLEUM research and others from reliable sites, to be used for the standardization and make downloadable data and spectra.

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WP2 - Regulatory framework analysis, update and implementation

Improve the guarantee of **OO quality** and **authenticity** by:

- **Suggesting updates of international norms** and recognized procedures (EU, IOC, CODEX, ISO) and proposing the adoption of the new or improved OLEUM methods and RMs (developed in WP3 and WP4).
- **Updating** and surveying the appearance of common and emerging **frauds.**

The objectives will be reached by **revising the regulatory framework** to propose solutions for the:

1) Normative failures: lack of methods for a specific fraud identification (e.g. soft deodorization);

2) Normative inappropriateness: lack of an appropriate method for a specific cited marker (e.g. EU Reg. 432/2012, olive oil polyphenols health claim);

3) Analytical method drawbacks: review of the main drawbacks of existing procedures to control OO quality and authenticity and delivery of the solutions to the international technical scientific community.

4) Lack of methods harmonization: dissimilarity between regulations approved by different authorities, lack of interchangeability of methods, or lack of mutual understanding of the provided results;

5) Atypical compositions of Oos.

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future

Lanfranco Conte[a], Alessandra Bendini[b,*], Enrico Valli[b], Paolo Lucci[a], Sabrina Moret[a], Alain Maquet[c], Florence Lacoste[d], Paul Brereton[e], Diego Luis García-González[f], Wenceslao Moreda[f], Tullia Gallina Toschi[b]

Scope and approach: This review will **identify current gaps in EU legislation** and **discuss drawbacks of existing analytical methods** with respect to OO. **Suggestions** for replacement of specific steps within the present EU methods with more efficient analytical solutions to reduce time and/or solvent consumption will be proposed.

Key findings and conclusions: This review **critiques existing regulatory methods and standards**, highlights weaknesses and **proposes possible solutions** to safeguard the consumer and protect the OO market.

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WP2 - Regulatory framework analysis, update and implementation

Task 2.3 Standardization and harmonization

Sub-task 2.3.1 Selection of methods for standardization

At least 4 analytical methods, developed or revised in WP3 and WP4 and 2 formulated RMs will be selected, with the contribution of a discussion groups composed by EU, IOC, CODEX, ISO, other competent authorities or international bodies for the subsequent phases of standardization.

Sub-task 2.3.2 Cooperative inter-laboratory experiments

Pre-trial with one or two samples sent to laboratories for an early indication of method performance.

Sub-task 2.3.3 Standardization of the validated SOP and harmonization

The result will be the production of validated SOPs and QCMs, the latter to be further respectively standardized and certified by a Standard Developing Organization (SDO). The validated SOPs and QCMs sent for the standardization to a SDO (e.g. IOC, AOCS, ISO) will be also proposed, together with their limits and ranges, to the competent authorities and international bodies for their inclusion in official regulations.

Methods to be full validated

1) New or revised method to detect **blends of EVOOs with soft-deodorized OOs**.

2) Method **to be selected during the OLEUM project development (free-choice**, but not focused on the objectives of 1, 3 or 4).

3) New/revised genomic or metabolomic based method to detect **illegal blends of OOs with other vegetable oils**.

4) Method for the assessment of the organoleptic characteristics of OO **(Quantitative Panel Test) including two RMs** → including also a screening method and a volatile compound method.

1) New or revised method to detect **blends of EVOOs with soft-deodorized OOs**

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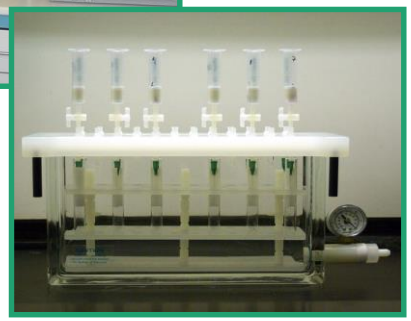
CSIC Consejo Superior de Investigaciones Científicas

DELIVERABLE 4.2

Title: Report on a revised and validated method for FAAEs determination

Fractionation step by:

- ✓ HPLC (silica column)
- ✓ SPE (1 g of silica gel cartridges)
  - lower solvent volumes requested
  - possibility to mechanize the fractionation step
  - less time-consuming
- ✓ PTV (Programmed Temperature Vaporization) injector for GC
- ✓ Split mode injection



	Official method *(EU Reg. 61/2011)*	**Method A** *(HPLC-UV-Vis/GC-FID)*	**Method B** *(SPE/GC-FID)*
Volume of solvents *(for each determination)*	~ 350 mL	~ 40 mL	~ 20 mL
Time *(for each determination)*	~ 6 hours	~ 2.5 hours	~ 1.5 hours

- Method A does not require disposable material (SPE cartridges)
- Method B requires less time and a less expensive equipment

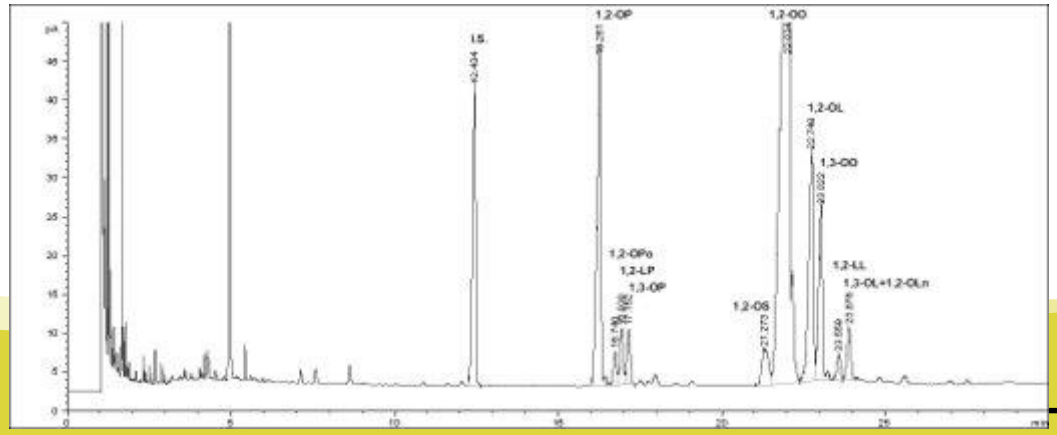
Official method for FAEEs was adopted by the EU Reg. 61/2011, legal limit was revised by the EU Reg. 1348/2013 and then by the EU Reg. 2095/2016

Determination of **diacylglycerols (DAG)** by SPE (diol phase)-GC-FID

The **DAG** content can give an idea of the **hydrolytic quality** of the oil. The parameter is not included in the legislation because it can be attributed to several reasons.

The **absolute amount of DAG** (DAG_{exp}) is related to the acidity, because all DAG, apart from the ones coming from biosynthesis, come from the hydrolyzed TAG.

From the acidity we can calculate the **theoretical DAG content** (DAG_{teo}) following a specific formula. From the experimental and theoretical value, it is possible the **calculation of DAG that can give an idea if the oil contain soft deodorized oil**. In the same manner, the **ratio between free acidity and DAG content** can confirm the presence of soft deodorized oil.



2) Method **to be selected during the OLEUM project development**

molecules

MDPI

Article

In House Validated UHPLC Protocol for the Determination of the Total Hydroxytyrosol and Tyrosol Content in Virgin Olive Oil Fit for the Purpose of the Health Claim Introduced by the EC Regulation 432/2012 for "Olive Oil Polyphenols"

Maria Z. Tsimidou [1,*], Michaela Sotiroglou [1], Aspasia Mastralexi [1], Nikolaos Nenadis [1], Diego L. García-González [2] and Tullia Gallina Toschi [3]

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The liquid chromatographic profile of the extracted polar fraction before and after acid hydrolysis is recorded by means of **diode array detection**.

The **acid hydrolysis** of the polar fraction gives rise only to free Htyr and Tyr, the content of which can then be accurately quantified using commercially available standards and expressed as total Htyr and Tyr in (mg/20 g of oil) after correction for molecular weight differences between free and bound forms. **UHPLC** conditions speed up the overall elution procedure increasing usability and reducing the environmental impact.

The method performance upon **in-house validation** is satisfactory according to established criteria. Hydroxytyrosol seems to be sensitive during hydrolysis leading frequently to the formation of two additional peaks, which, when taken into account improve significantly recovery.

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3) New/revised genomic or–metabolomic based method to detect **illegal blends of OOs with other vegetable oils**.

Advantages and disadvantages of DNA based methods for the authentication of vegetable oils

- Advantages:
  - Fast and economic analytic tools
  - High specificity and sensitivity
  - Not influenced by environmental conditions

- Disadvantages:
  - Low yield and quality of extracted DNA
  - Low repetitivity
  - Low reproducibility

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3) New/revised genomic or–metabolomic based method to detect **illegal blends of OOs with other vegetable oils**.

Determination of **sterols** in free and esterified forms by **SPE/GC-FID**

The analytical evaluation of the composition of **sterols** is a well established tool for assessing of purity of olive oils, as it depends on the botanical origin of oils. The method that is available is suitable to determine the total composition of sterols, not depending on being in the free or in the esterified form.

In different vegetable oils, sterols can be differently distributed between **free or the esterified form**, this ratio can be utilized a screening tool to detect adulteration of olive oil with seed oils.

In this revised method, free sterols are converted into silyl derivatives, in such a way, their polarity became the same of esterified sterols. Oil is then fractioned by **SPE** and the fraction with free and esterified sterols is analysed by capillary GC with on column injection.

Method had been **in house validated** by evaluating repeatability on three different oils (EVOO, Olive pomace oil and High oleic Sunflower oil). Toxic n-hexane had been substituted with less healthy risk isooctane and a significance reduction in solvent volume was obtained in the SPE elution (just 20 mL).

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4) Method for the assessment of the organoleptic characteristics of OO **(Quantitative Panel Test) including two RMs (T3.1)** → including also a screening method and a volatile compound method.

Screening methods

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Screening method based on **Head Space - Solid Phase Micro Extraction – Gas-chromatography - Mass Spectrometry (HS-SPME-GC/MS) untargeted approach**. The validated model made with virgin olive oil volatile fraction fingerprint is able to predict the commercial category of samples successfully. Thus, it can be an excellent supportive tool for sensory panels because it would allow reducing the number of samples to be assessed.

The **FGC-E-Nose** allows the head-space analysis of volatile compounds in VOOs samples. Its application aims to support the organoleptic assessment by a rapid screening methods based on volatile markers, able to decrease the daily work of the Sensory Panels. With this purpose, a classification model to verify the quality grade of virgin olive oils using a fingerprinting approach on the volatile profile, was also developed for the data analysis.

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In the **HS/GC-IMS**, volatile compounds present in the sample head space are pre-separated by gas chromatography then inserted in the atmospheric ionization region by a low radiation Tritium source. The GC-IMS permits a twofold separation of analytes by GC runtime and by IMS signal. HS-GC-IMS is a promising non-targeted approach to realize a fast screening of samples for supporting the sensory analysis. PLS-DA seems permit higher percentages of correct classification for EVOOs and secondly for LOOs.

Timeline of the full validation

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Methods	Availability of in-house validated method(s) (WP3 and WP4)	Selection (WP2, ST2.3.1)	Commencement of pre-trials (WP2, ST2.3.2)	Training workshops (WP6, ST6.2.1)	Draft of SOPs to be fully validated (WP2, ST2.3.2)	Commencement of trials proper (WP6, ST6.2.2)	Proposal of the 4 validated SOPs and the 2 QCMs to regulatory bodies (WP2, ST2.3.3)
New/revised genomic or metabolomic based method to detect illegal blends of OOs with other vegetable oils (T4.3)	May 19	June 19	Sept. 19	Dec. 19	Jan. 20	Jan. 20	July 20
New or revised method to detect blends of EVOOs with soft-deodorized OOs	Feb 19	March 19	June 19	Sept. 19	Oct. 19	Oct. 19	April 20
Method to be selected during the OLEUM project development	Feb 19	March 19	June 19	Sept. 19	Oct. 19	Oct. 19	April 20
Method for the assessment of the organoleptic characteristics of OO (Quantitative Panel Test) including two RMs (T3.1)	May 19	June 19	Sept. 19	Dec. 19	Jan. 20	Jan. 20	July 20

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ARE YOU INTERESTED IN PARTICIPATING TO THE FULL VALIDATION OF SOMEONE OF THESE METHODS?

Contact us: distal.oleum@unibo.it

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– Work Plan

Wednesday – 10.45 a.m. Flash Gas-Chromatography in tandem with chemometrics: a screening tool to discriminate the olive oil quality.
Alessandra Bendini, University of Bologna

9:00 a.m. - The OLEUM Databank: A reference repository for olive oil quality and authenticity.
Alain Maquet, JRC - Joint Research Centre, Belgium

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alysis, update and

WP3: Analytical solutions addressing olive oil quality issues

WP4: Analytical solutions addressing olive oil authentication issues

WP5: OLEUM Databank

echnology Tr

8:20 a.m. - Volatile compounds as useful markers for the quality assessment of virgin olive oils.
Diego Luis García González, Instituto de la Grasa, Spain.

8:40 a.m. - Use of NMR technique in the OLEUM project.
Torben Kuechler, Eurofins, Germany.

WP8: Ethics requirements

WP7: Dissemination and Communication

9:20 a.m. – The US experience on olive oil production and quality.
Juan Polari, UC Davis Olive Center, USA.

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Thank you for your attention

http://www.oleumproject.eu/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635690.