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

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

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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?**

1. **Olive Crushing/Milling**
   - After picking, the stems, leaves and twigs are removed.
   - The olives are crushed into an olive paste.

2. **Malaxation**
   - The olive paste is slowly mixed.

3. **Extraction**
   - After the olive paste is centrifuged or pressed, the leftover products are:
     - oil (extra virgin, virgin or lampante)
     - pomace
     - vegetation water.
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 composition can occur.

- **REFINED OLIVE OIL**
- Adding of extra virgin or virgin olive oil.

**OLIVE OIL**

**Legend**

- Fit for human consumption
- Not fit for human consumption
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
...to different regulatory frameworks.

Codex Alimentarius

*Standard for olive oils and olive pomace oils*


1 Member Organization (EU)

188 Member Countries

International Olive Council

*International olive council. Trade standard for Olive oils and Olive Pomace Oils*


1 Member Organization (EU)

14 Member Countries

~94% of the OO world production

National standards

Argentina


Australia


California


Brazil


China


India

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

South Africa


USA


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
The Olive Oil Regulatory Framework

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

- Parameters
- Legal limits
- Analytical methods

QUALITY CONTROL

International and National Standards

Harmonization

ISO methods

AUTHENTICITY CONTROL

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

**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**

<table>
<thead>
<tr>
<th></th>
<th>FA (g oleic acid/100 g oil)</th>
<th>PV (meq O₂/Kg oil)</th>
<th>K₂₃₂</th>
<th>K₂₇₀</th>
<th>FAEEs (mg/kg oil)</th>
<th>Md</th>
<th>Mf</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>≤35</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>IOC</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>≤35</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>CODEX</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a. ***</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>Argentina</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>USDA</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>Australia</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>South Africa</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>California</td>
<td>≤0.5</td>
<td>≤15</td>
<td>≤2.40</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>China</td>
<td>≤1.6*</td>
<td>≤10**</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>Brazil</td>
<td>≤0.8</td>
<td>≤20</td>
<td>≤2.50</td>
<td>≤0.22</td>
<td>FAME + FAEE &lt; 75 mg/kg or &gt; 150 mg/kg if FAEE/FAME &gt; 1.5</td>
<td>0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>India</td>
<td>≤2.0*</td>
<td>≤20</td>
<td>n.a.</td>
<td>≤0.22</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

*Expressed as mg KOH/g: 1.6 corresponds to 0.8%.
**Expressed as mmol: 10 mmol correspond to 20 meq O₂/kg
*** Codex is evaluating to introduce 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

<table>
<thead>
<tr>
<th></th>
<th>Brassicasterol</th>
<th>Campesterol</th>
<th>Stigmasterol</th>
<th>Apparent β-sitosterol</th>
<th>Δ-7-stigmastenol</th>
<th>Sterol content mg/kg oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>≤0.1</td>
<td>≤4.0</td>
<td>&lt;Campest.</td>
<td>≥93.0</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>IOC</td>
<td>≤0.1</td>
<td>≤4.0</td>
<td>&lt;Campest.</td>
<td>≥93.0</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>CODEX</td>
<td>≤0.1</td>
<td>≤4.0</td>
<td>&lt;Campest.</td>
<td>≥93.0</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>Argentina</td>
<td>≤0.1</td>
<td>≤4.0</td>
<td>&lt;Campest.</td>
<td>≥93.0</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>USDA</td>
<td>≤0.1</td>
<td>≤4.5</td>
<td>&lt;Campest.</td>
<td>≥93.0</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>Australia</td>
<td>≤0.1</td>
<td>≤4.8</td>
<td>≤1.9</td>
<td>≥92.5</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>South Africa</td>
<td>≤0.1</td>
<td>≤4.8</td>
<td>≤1.9</td>
<td>≥92.5</td>
<td>≤0.5</td>
<td>≥1000</td>
</tr>
<tr>
<td>California</td>
<td>≤0.1</td>
<td>n.a.</td>
<td>≤1.9</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>China</td>
<td>n.a.</td>
<td>≤4.0</td>
<td>≤0.5</td>
<td>≥93.0</td>
<td>n.a.</td>
<td>≥1000</td>
</tr>
<tr>
<td>Brazil</td>
<td>≤0.1</td>
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<td>≥1000</td>
</tr>
<tr>
<td>India</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

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 to 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.

**KEY CHALLENGES**

- Development of changes on existing methods
- Method development
- Validation
- Standardization
- Harmonization

**PRE-NORMATIVE ACTIVITY**
Validity and reliability of the subject matter to be standardized

**CO-NORMATIVE ACTIVITY**
Repeatibility, reproducibility and uncertainty of the procedures to become standard

**NORMATIVE ACTIVITY**
Technical regulations approved by different authorities

**OLEUM SHORT-MID TERM STRATEGY:** Improving existing analytical methods

**OLEUM LONG TERM STRATEGY:** Developing novel analytical methods based on technological innovation

**OLEUM DATABANK:** Development of a web-based platform for maximising the exploitation, scalability and dissemination of the OLEUM methods and results

**OLEUM IMPACT on the International NORMATIVE SCENARIO**

**Method timeline: from research to legislation**

YEAR 0

MORE THAN 5 YEARS
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.
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**.
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.
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.
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**

**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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 350 mL</td>
<td>~ 40 mL</td>
<td>~ 20 mL</td>
<td></td>
</tr>
</tbody>
</table>

**Method A** does not require disposable material (SPE cartridges)

**Method B** requires less time and a less expensive equipment

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

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$_{\text{exp}}$) 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$_{\text{teo}}$) 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

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.
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 repetitiveness
- Low reproducibility

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

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.

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

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

Contact us: distal.oleum@unibo.it
WP1: Coordination and Management
WP2: Regulatory framework analysis, update and implementation
WP3: Analytical solutions addressing olive oil quality issues
WP4: Analytical solutions addressing olive oil authentication issues
WP5: OLEUM Databank
WP6: Networking and Technology Transfer
WP7: Dissemination and Communication
WP8: Ethics requirements

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

WP5: OLEUM Databank: A reference repository for olive oil quality and authenticity. Alain Maquet, JRC - Joint Research Centre, Belgium

WP8: Ethics requirements

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.

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

http://www.oleumproject.eu/