The OLEUM project:  
Advanced solutions for assuring the overall authenticity and quality of olive oil

Prof. Tullia Gallina Toschi
Department of Agricultural and Food Sciences, Alma Mater Studiorum – Università di Bologna
EU H2020 OLEUM project Coordinator

28 September 2020
OLEUM project identified **four main gap levels** that need to be addressed through the **research & development** in the OO sector.

**Gap levels**

- **Legislative and Regulatory**
- **Analytical**
- **Harmonization and Coordination**
- **Consumer and Market Confidence**

IOC, EC and FDA are involved as members of the Multi-Stakeholders OLEUM Advisory Board.

**OLEUM zipper role**

**INPUT**

**OUTPUT**
Specific objectives

➢ Analysis of FAILURES (need of new markers/strategies for a specific fraud identification, e.g. illegal mixtures between olive oil and other vegetable oils) and/or INAPPROPRIATENESS (e.g. lack of an harmonized method of quantification addressed to verify the “OO polyphenols” health claim according to the possibility given by EU Reg. No 432/2012) of the actual regulatory framework.

➢ Proposal to international regulators and policy makers of an array of potential solutions that can contribute to the improvement of REGULATORY STANDARDS or NORMATIVES.
Specific objectives

➢ To revise EXISTING METHODS, to improve them in terms of performance and efficiency in verifying OO quality and authenticity.
➢ To enhance methodology for sensory evaluation improving its reproducibility (e.g. by the use of artificial Reference Materials for training of assessors) and developing instrumental tools as a support to the Panel Test (e.g. targeted and untargeted approaches based on volatile compound analysis).
➢ To identify NOVEL ANALYTICAL MARKERS and/or to develop and validate INNOVATIVE ANALYTICAL SOLUTIONS addressed to:
  ✓ Measure the OO conservation state (e.g. freshness/storage time/shelf-life)
  ✓ Detect illegal blends between EVOO and soft deodorized OO
  ✓ Reveal illegal mixtures between OO and other vegetable oils
  ✓ Monitor compliance with the information reported in the label (e.g. health claim, geographical origin).
Specific objectives

➢ 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 validated technical protocols to a WIDER ANALYTICAL COMMUNITY and assess its PROFICIENCY by specific fit-for-purpose actions (e.g. analytical discussions, needs of ring tests inside OLEUM Network).

➢ To promote a user-friendly OPEN-ACCESS OLEUM Databank for storing consolidate information coming from OLEUM research and other fragmented sources and for sharing data to be used for the standardization of procedures.
Specific objectives

➢ To engage the widest range of STAKEHOLDERS (opinion leaders/regulators, food and drink industries including SMEs, the media, the scientific community, consumers) in the dissemination, exploitation and knowledge exchange.

➢ To establish an updated source of reliable information on the methodology for authenticating OO, available to the international user community and to the public.
The OLEUM Consortium

21 Partners:
- 16 EU partners (Univ., SME, Associations)
- 3 H2020 associated countries (Switzerland, Turkey, Israel)
- 2 non-EU research centres (Argentina, China)
The OLEUM Consortium

- 2 analytic and service providers SMEs
  - SMART ASSAYS TECHNOLOGIES LTD
  - Lablicate

- 1 large food industry
  - Nestlé Research

- 1 private research company
  - eurofins

- 3 non-profit organizations
  - EFFoST
  - Eufic
  - UZMK

- 14 universities and public research centers
  - Alma Mater Studiorum Università di Bologna
  - CSIC
  - Fera
  - Aristotle University of Thessaloniki
  - UPV
  - UP
  - University of Primorska
  - University of Sfax
  - University of Barcelona
  - Institute of Agriculture and Tourism
  - 中国食品发酵工业研究院
  - European Commission
  - Joint Research Centre
  - Expertise Corps Gras
  - China National Research Institute of Food & Fermentation Industries
PERT Scheme – Work Plan

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
Another review that highlights the most common and the emerging cases of fraud in the olive oil sector, thus defining the scenario in which analytical and regulatory efforts should be addressed to ensure olive oil authenticity, is under preparation.

The answers received to an on-line survey sent to EU and non-EU stakeholders of the olive oil sector and to a questionnaire addressed to the EU Food Fraud Network National Contact Points are also presented and discussed.

Hypothesis for an enforced control in the future scenario are formulated: from the validation of multivariate - robust and easy to use models - to the adoption of mixed strategies (quality control + traceability trough blockchain).
Some WP3 activities

**DELIVERABLE D3.4**
Title: Report on results obtained from the applied screening methods for sensory analysis

Date: 15/1/2019, revised on 19th February 2020

**DELIVERABLE D3.7**
Title: Report on a validated screening method to support the Quantitative Panel Test

Date: 9/08/2019, revised on 20th of February 2020

Full validation of an untargeted methods is in course
Some WP3 activities

DELIVERABLE 3.5

D3.5: Report on the analytical methods for the quantification of phenolic compounds (health claim) and on a revised and validated protocol to detect and quantify them

Date: 31/12/2018, revisions completed on 20/2/2020

DELIVERABLE 3.8

Title: Report on the analytical methods to evaluate the freshness/quality deterioration of OOs and on the validation of a software for their estimation

Date: 3rd September 2019, revisions completed on 17th March 2020
Some WP3 activities

**DELIVERABLE 3.6**

Date: 13/02/2020, revisions completed on 14/02/2020

**DELIVERABLE D3.9**
Title: Report on the suitability of Reference Materials in the training and monitoring of panellists according to the “IOC Guide for the selection, training and monitoring of skilled virgin olive oil tasters”

Date: 15/01/2020

Full validation of the use of sensory RMs is in course
Hypothesis of use of targeted approaches to determine volatiles for the classification (Panel disagreement)

COI/T20/Doc.15 Rev. 10-2018

§10.6

Should the panel not confirm the declared category as regards the organoleptic characteristics, the interested party may request the national authorities or their representatives to have carried out without any delay two independent counter-assessments by two other panels recognised by the IOC or approved by the competent authorities at national level. The characteristics concerned shall be deemed consistent with the characteristics declared if both counter-assessments confirm the declared category. If that is not the case, the interested party shall be responsible for the cost of the two counter-assessments.

![Diagram]

1. **Panel 1**
   - Classification Panel 1

2. **Panel 2**
   - Classification Panel 2

3. **Diamond 1**
   - Panel 1 = Panel 2
   - Panel 1 <> Panel 2

4. **Diamond 2**
   - Classification confirmed
   - Instrumental approach

5. **Model**
   - Classification not confirmed
Development of a Prediction Model for the Quality Deterioration of Extra Virgin Olive Oils According to the Storage Conditions.

Servili M.\textsuperscript{a}, Taticchi A.\textsuperscript{a}, Esposto S.\textsuperscript{a}, Urbani S.\textsuperscript{a}, Selvaggini R.\textsuperscript{a}, Veneziani G.\textsuperscript{a}, Sordini B.\textsuperscript{a}, García-González D.L.\textsuperscript{b}, Cruciani G.\textsuperscript{a}, Gallina Toschi T.\textsuperscript{c}

\textit{Department of Agricultural, Food and Environmental Sciences, a University of Perugia, Italy; b Instituto De La Grasa Spain; c University of Bologna, Italy.}
HOME PAGE OF THE SOFTWARE

VITA OLEI

SOFTWARE «USER FRIENDLY»

Shelf-life prediction software prototype (version 1.0)
SOFTWARE INPUT

SCREENSHOT SHOWING THE VALUES OF THE PARAMETERS OF SAMPLE USED FOR PREDICTING THE “BEST BEFORE DATE” SUITABLE FOR CONSUMPTION.

The data for each parameter, together with the sample name and the EVOO production date, can be entered manually or imported via a csv file containing one or more samples.
SOFTWARE OUTPUT

SCREENSHOT OF A PLOT SHOWING THE PREDICTED DATE AND DAYS NECESSARY TO SAMPLE TO REACH THE THRESHOLD VALUE (LIGHT).
SOFTWARE OUTPUT

SCREENSHOT OF A PLOT SHOWING THAT IN THE PERIOD (24 MONTHS) THE SAMPLE HAS NOT REACHED THE THRESHOLD VALUE (DARK).
SOFTWARE OUTPUT

SCREENSHOT OF THE TABLE WITH THE PREDICTED DAYS AND DATES NECESSARY TO SAMPLE TO REACH THE THRESHOLD VALUES.

LIGHT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>deltaK</td>
<td>348</td>
<td>sabato 28 novembre 2020</td>
</tr>
<tr>
<td>k270</td>
<td>204</td>
<td>martedì 7 luglio 2020</td>
</tr>
<tr>
<td>(E)-2-Decenal</td>
<td>176</td>
<td>martedì 9 giugno 2020</td>
</tr>
<tr>
<td>(E,E)-2,4-decadienal</td>
<td>174</td>
<td>domenica 7 giugno 2020</td>
</tr>
<tr>
<td>Total phenols</td>
<td>274</td>
<td>martedì 15 settembre 2020</td>
</tr>
</tbody>
</table>

More accurate and precise parameters for the prediction of the "best before date" at the light.
**SOFTWARE OUTPUT**

SCREENSHOT OF THE TABLE WITH THE PREDICTED DAYS AND DATES NECESSARY TO SAMPLE TO REACH THE THRESHOLD VALUES.

**DARK**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>deltaK</td>
<td>1609</td>
<td>domenica 12 maggio 2024</td>
</tr>
<tr>
<td>k270</td>
<td>988</td>
<td>martedì 30 agosto 2022</td>
</tr>
<tr>
<td>(E)-2-Decenal</td>
<td>794</td>
<td>giovedì 17 febbraio 2022</td>
</tr>
<tr>
<td>(E,E)-2,4-decadienal</td>
<td>407</td>
<td>martedì 26 gennaio 2021</td>
</tr>
<tr>
<td>Total phenols</td>
<td>766</td>
<td>giovedì 20 gennaio 2022</td>
</tr>
</tbody>
</table>

More accurate and precise parameter for the prediction of the "best before date" in the dark.
Why Tyrosol Derivatives Have to Be Quantified in the Calculation of “Olive Oil Polyphenols” Content to Support the Health Claim Provisioned in the EC Reg. 432/2012

Maria Z. Tsimidou,* Nikolaos Nenadis, Maurizio Servili, Diego Luis García-González, and Tullia Gallina Toschi

The health claim on “olive oil polyphenol” refers to both tyrosol and hydroxytyrosol, free or in bound forms. The consensus among scientists, the European food authorities, IOC, and the olive industry on which compounds should be determined to support the health claim on olive oil polyphenols (EC Reg. 432/2012) is of utmost importance and can be supported by the evidence provided in this viewpoint article.
The determination of the total hydroxytyrosol (Htyr) and tyrosol (Tyr) content of virgin olive oil is of utmost interest after the issuing of a health claim that "olive oil polyphenols contribute to the protection of blood lipids from oxidative stress."

The systematic work presented in this paper proved to be useful toward harmonization and standardization of an extraction protocol for the polar fraction of olive oil, which then can be used for the determination of total polar phenols or of the total content of Htyr and Tyr.
An ongoing challenge in olive oil analytics is the development of a reliable procedure that can draw the consensus of all interested parties regarding the quantification of concentrations above the required minimum value of 5 mg of bioactive "olive oil polyphenols" per 20 g of the oil, to fulfill the health claim introduced by the European Commission (EC) Regulation 432/2012.

In this paper, a UHPLC-DAD method fit for the purpose of the health claim has been developed and in-house validated for the first time.
The pros of a recently published in house validated UHPLC protocol are discussed comparatively with those of other procedures that determine directly or indirectly the compounds hosted under the health claim on “olive oil polyphenols”. Findings point out the simplicity of sample preparation, fast elution time that increase the number of samples analyzed per day and integration of well-resolved peaks with the aid of only two commercially available external standards.
Sensory quality, assessed following a standardized method, is one of the parameters defining the commercial category of virgin olive oil. Considering the difficulties linked to the organoleptic evaluation, especially the high number of samples to be assessed, the setting up instrumental methods to support sensory panels becomes a need for the olive oil sector. This paper explores the use of **Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry** as a tool to evaluate the volatile fraction responsible for virgin olive oil sensory attributes.

Virgin olive oil volatile fingerprint and chemometrics: Towards an instrumental screening tool to grade the sensory quality

Beatriz Quintanilla-Casas\textsuperscript{a,b}, Julen Bustamante\textsuperscript{a,b}, Francesc Guardiola\textsuperscript{a,b}, Diego Luís García-González\textsuperscript{e}, Sara Barbieri\textsuperscript{d}, Alessandra Bendini\textsuperscript{d}, Tullia Gallina Toschi\textsuperscript{d}, Stefania Vichi\textsuperscript{a,b,*}, Alba Tres\textsuperscript{a,b}
Results obtained

The sensory methodology for virgin olive oils (VOOs) known as the “panel test” represents the most valuable approach to assess sensory characteristics and quality for consumer and producer protection.

Sensory data obtained by 6 panels were elaborated with two main objectives:

(i) to classify and characterize samples in order to use them for possible correlations with physical–chemical data

(ii) to monitor and improve the performance of panels.

This paper highlights the possibilities for improvement the reliability of panel test results by the application of specific formative training.

At the end, 329 of 334 VOOs (98.5%) were classified.
A headspace gas chromatography-ion mobility spectrometer (HS-GC-IMS) was used to analyze 198 commercial VOOs (EVOOs, VOOs, LOOs) by a semi-targeted approach. Different partial least squares-discriminant analysis (PLS-DA) chemometric models were then built by data matrices composed of 15 volatile compounds, which were previously selected as markers: a first approach was proposed to classify samples according to their quality grade (77% average of correctly classified samples) and a second based on the presence of three sensory defects (64% average of correct prediction for fusty-muddy, mouldy and rancid).
This research aims to develop a classification model based on untargeted elaboration of volatile fraction fingerprints of virgin olive oils (n = 331) analyzed by flash gas chromatography to predict the commercial category of samples. Data matrix was elaborated using the linear technique partial least squares discriminant analysis (PLS-DA), applying, in sequence, two sequential classification models with two categories (EVOO vs. no-EVOO followed by VOO vs. LOO and LOO vs. no-LOO followed by VOO vs. EVOO). The results from this large set of samples provide satisfactory percentages of correctly classified samples, ranging from 72% to 85%, in external validation.
Some WP4 activities

DELIVERABLE 4.4
Title: Final report on the OLEUM analytical conditions applied for soft deodorization (T4.1, M22)
Date: 28 January 2020, revised on 20th February 2020

DELIVERABLE D4.7
Title: Report on the OLEUM new and revised analytical approaches to detect soft deodorization (including a validated method)
Date: 19/8/2019, revised on 24th February 2020

DELIVERABLE 4.2
Title: Report on a revised and validated method for FAAEs determination
Date: 14 November 2018, revised on 27th January 2020

Full validation of the method is in course
Some WP4 activities

DELIVERABLE D4.6
Title: Report on the Oleum Analytical approaches to detect illegal and legal blends between olive oils and other vegetable oils (including a new or revised in-house validated genomic or metabolomic based method)
Date: 5/8/2019

Full validation of the method is in course

DELIVERABLE 4.3
Title: Report on a revised and validated method for sterols determination
Date: 26/04/2018, revised on 21th February 2020

DELIVERABLE D4.8
Title: Decisional tree for the detection of the percentage of OO in blends with other vegetable oils
Date: 25/6/2019
The verification of the geographical origin of extra virgin (EVOO) and virgin olive oil (VOO) is crucial to protect consumers from misleading information. Despite the large number of studies performed, specific markers are still not available. The present study aims to evaluate sesquiterpene hydrocarbons (SHs) as markers of EVOO geographical origin and to compare the discrimination efficiency of targeted profiling and fingerprinting approaches (both by SPME-GC-MS).
Results obtained

Compliance with EU vs. extra-EU labelled geographical provenance in virgin olive oils: A rapid untargeted chromatographic approach based on volatile compounds

Rosa Palagano, Enrico Valli, Chiara Cevoli, Alessandra Bendini*, Tullia Gallina Toschi

**WP4_T4.4**

A flash gas chromatography (FGC) untargeted approach based on volatile compounds, followed by a chemometric data analysis, is proposed for discrimination of EVOOs and VOOs according to their geographical origin (EU and extra-EU).

A set of 210 samples was analyzed and two different classification techniques were used, one linear (Partial Least Square-Discriminant Analysis, PLS-DA) and one non-linear (Artificial Neural Network, ANN). The two models were also validated using an external data set. Satisfactory results were obtained for both chemometric approaches: with PLS-DA, 89% and 81% of EU and extra-EU samples, respectively, were correctly classified; for ANN, the percentages were 92% and 88%.
The commercialization of declared blends of olive oil and seed oil is allowed by the European Union. For olive oil, the percentage must be at least 50% if the producer aims to advertise its presence on the front label. However, there is currently no designated method to verify such proportion.

In this article, OLEUM researchers propose the use of decisional trees based on the greatest differences between olive and seed oils for a variety of parameters including triacylglycerols, saturated hydrocarbons, sterols, and tocopherols.
In this paper, researchers propose **two factors to confirm the absence of sof-deodorized** oils in EVOO: \( R_1 \) \((10 \times \text{free acidity/DAG}_{\text{exp}}) \geq 0.23 \) and \( R_2 \) \((\text{DAG}_{\text{exp}} - \text{DAG}_{\text{theor}}) < 0 \), in genuine EVOO. They demonstrate that such approach is **useful to detect the presence of soft deodorized** olive oil when this is **at least at 30% in the mixture**.
In this study, an o-line HPLC-GC-FID method for determination of FAEEs is presented, revising the preparative step and the GC injector required by the official method. The main advantages of this revised protocol are: (i) significant reduction in time and solvents needed for each analytical determination; (ii) application of HPLC as an alternative to traditional LC, carried with manually packed glass columns, thus simplifying the separation step.

**Fatty Acid Ethyl Esters in Virgin Olive Oils: In-House Validation of a Revised Method**

Rosa Palagano¹, Enrico Valli¹✉, Matilde Tura¹, Chiara Cevoli¹, María del Carmen Pérez-Camino², Wenceslao Moreda², Alessandra Bendini¹,✉ and Tullia Gallina Toschi¹✉
Create an **open-access web platform** for the collection of information and validated data of some analytical methods generated by OLEUM.

Gather, query, retrieve ad **export the data** in a standard format.

Develop a strategy to ensure the long-term **maintenance of the OLEUM Databank** and its **availability** to authorised bodies through the European Commission.
The WP6 activities

❖ Analytical methods in-house validated
❖ Analytical methods selected for full validation process
❖ Activation of the OLEUM Network
❖ Development of full validation process

Who is the OLEUM Network?

OLEUM Network
• 90 members from 20 different countries.

2 deliverables finalized and 4 in progress
OLEUM Network, two complementary approaches

**LinkedIn group** for general aspects relating to the quality and authenticity of olive oil

(135 members up to now

~ 45% industry, 40% researchers, 15% other)

A secure network (**Basecamp**) for those involved in the laboratory analysis of olive oil

(86 members up to now)

If you want to join the OLEUM Network or if you have any questions or comments about, please feel free to contact us (oleum@unibo.it)
Actions completed and in progress

OLEUM technology transfer...up to the OLEUM Databank

Objectives

- Enlarge the international body of expertise
- Develop an exploitation plan on the results
- Technology transfer of new/developed methods

Selected datasets, SOPs, documents...

Agreements with companies

Pre-trials to identify problems

A new timeline is defined due to the Covid-19 pandemic.

Try trial proper (formal method validation)

Problems identified

Already done

Training workshops

Already done

Technology transfer of new/developed methods

OLEUM technology transfer...up to the OLEUM Databank
The WP7 activities

Communication and dissemination

➢ 16 papers have been already published in scientific journals, in open access, and deposited in appropriate repositories together with the relative dataset (when available).

➢ OLEUM results were also presented in many national and international scientific conferences.

➢ Production of dissemination materials for a lay audience (infographics, game, roll-up, video..).
The WP7 activities

Infographics and Bookmarks for general public
The final project meeting and the final conference are expected for 17th-19th February 2021 (by videoconference).

http://www.oleumproject.eu/: sign up for the OLEUM newsletter
http://www.oleumproject.eu