

**2018 AOCS Annual
Meeting & Expo
May 6-9th, Minneapolis, USA**



Oleum

Better solutions to
protect olive oil quality
and authenticity

Hot Topic 7

**Olive Oil: Innovative Analytical
Strategies to Guarantee Quality
and Fight Fraud.
Focus on the Advancements of
the EU H2020 Project OLEUM**

The OLEUM Project: Analytical Solutions Addressing Olive Oil Quality and Authenticity Issues

Start date: 1st September 2016

Now: 21/48 months

Prof. Tullia Gallina Toschi

Department of Agricultural and Food Science – University of Bologna

Scientific Coordinator EU H2020 OLEUM

Minneapolis, 7th May 2018



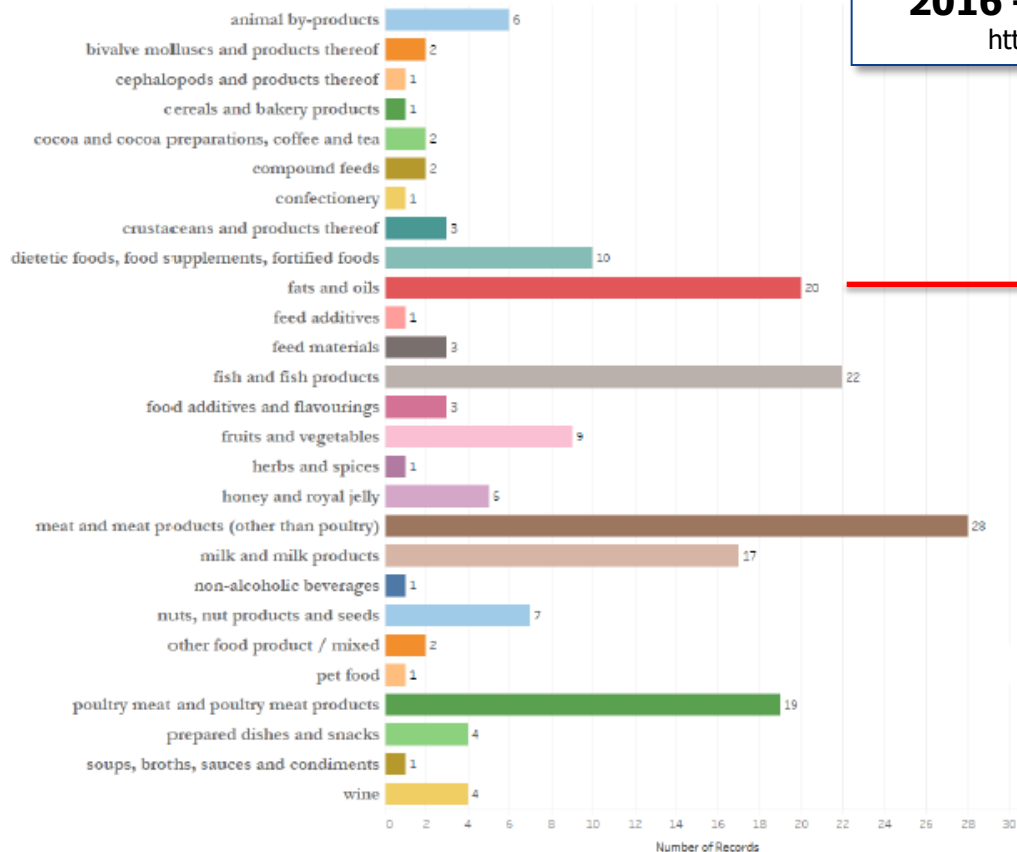
This project has received funding from the European
Union's Horizon 2020 research and innovation
programme under grant agreement No 635690.

Main concepts and assumptions

Olive oil (OO) is in the top of foods subjected to fraudulent activities

EU Parliament resolution of 14 January 2014 on food crisis, fraud in the food chain and the control thereof
[Document reference 2013/2091(INI); Johnson, 2014]

Food fraud cases for product categories



2016 – Food Fraud Network Activity Report

https://ec.europa.eu/food/safety/food-fraud/reports_en



Fats and oils

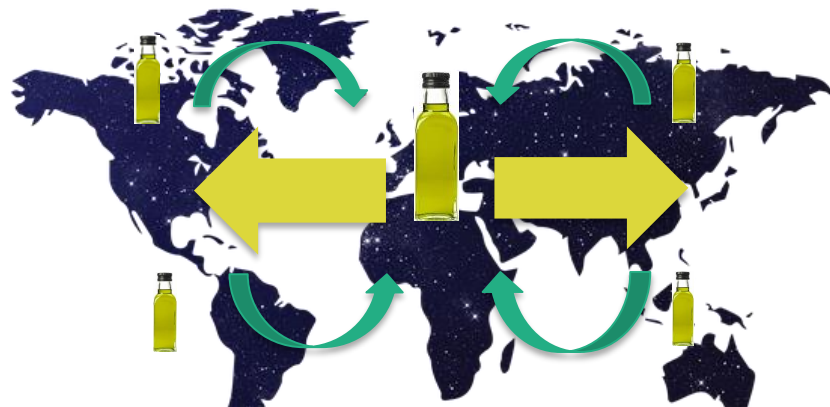
Cases for non compliances

Documents (17)

Mislabeling composition (21)

Unapproved treatment and/or processes (23)

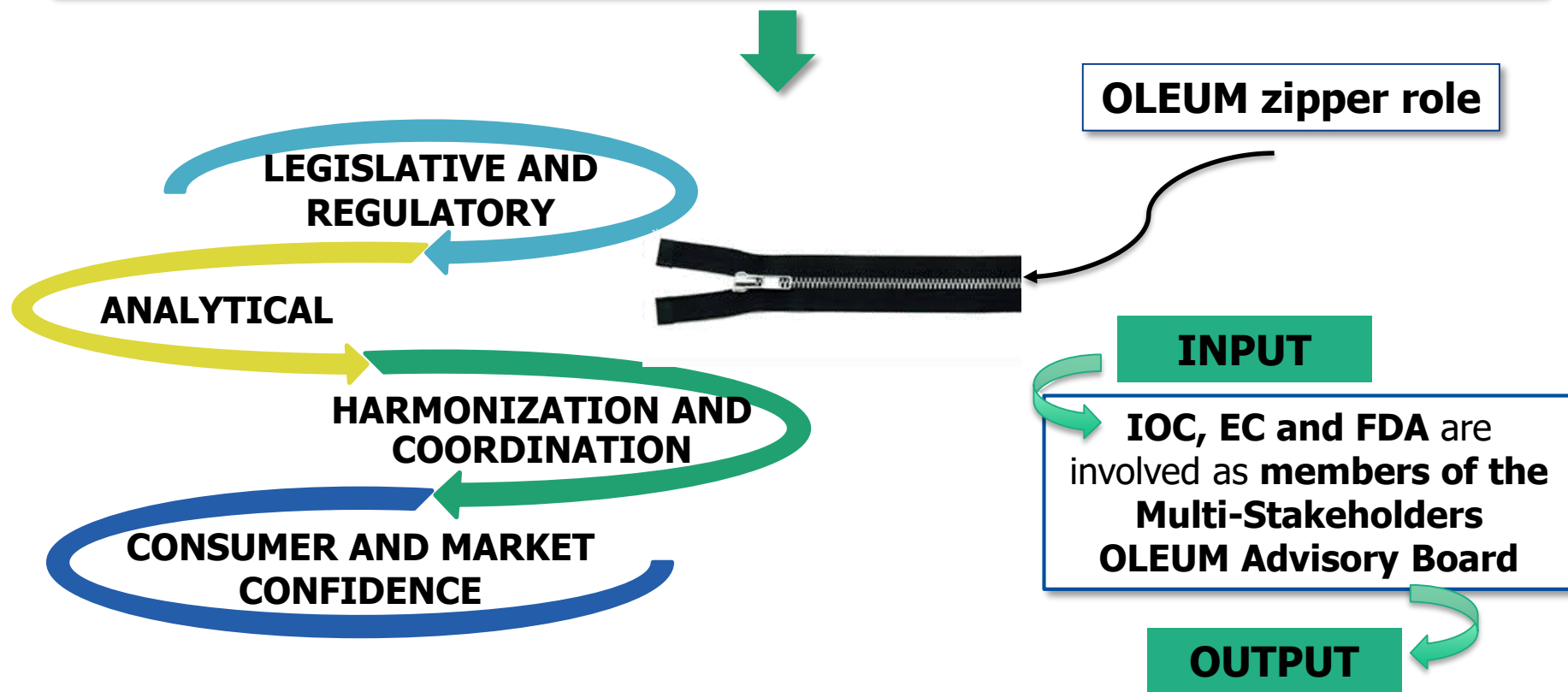
The asymmetry of the olive oil market



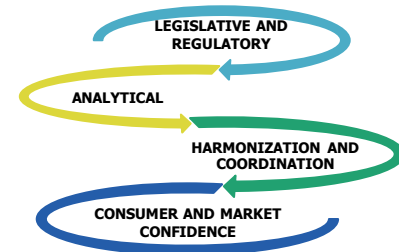
- **Europe is the largest producer of OO (62.4%) and non-EU countries are expanding their domestic production** (IOC stats on OO production – last OO crop 2017-18).
- The International Olive Council (**IOC**) member countries account for **92.6% of world OO supply**, but **79.7% of OO demand comes from non-member countries** (e.g. USA, Brazil, Japan, China, Russia, Australia and Canada) (IOC stats on olive oil production and importations– prev. olive oil crop oil 2017-18).
- **Increasing competitiveness, expanding markets in non-OO producing countries, a lack of a centralised databank of validated methods and a lack of harmonization** have led to significant weaknesses and vulnerability that can be exploited by counterfeiters.
- **New shared approaches and analytical tools** to check the quality and authenticity of OO are **timely and urgent**, targeting also the **most promising export markets (USA, Brazil, Canada, Australia and Japan)**, **new markets (China, Russia and India)** and **non-producer countries in the EU**.

Gap levels

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



Specific objectives



LEGISLATIVE AND
REGULATORY

To enable EU and international regulators and policy makers with an array of potential solutions that can contribute to the improvement of **REGULATORY STANDARDS** or **NORMATIVES** (WP2), on the basis of an analysis of **FAILURES** (lack of methods for a specific fraud identification, e.g. deodorization) and/or **INAPPROPRIATENESS** (e.g. Commission Regulation EU No 432/2012 reporting an “OO polyphenols” health claim, but not defining an harmonized methods of quantification).

ANALYTICAL

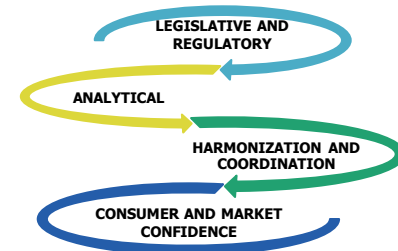
To revise **EXISTING METHODS**, to verify OO quality and to detect fraud by: identifying the **DRAWBACKS** and improving methods in terms of **performance and efficiency**.

To enhance methodology for **organoleptic assessment** improving reproducibility and developing a quantitative equivalent procedure (**Quantitative Panel Test**).

To identify **NOVEL ANALYTICAL MARKERS** with the aim of developing and validating **INNOVATIVE ANALYTICAL SOLUTIONS** to:

- Detect **illegal blends** between EVOO and **soft deodorized OO**.
- Reveal **illegal mixtures** between **OO** and **other vegetable oils**.
- Measure the **OO conservation state** in terms of **freshness** and **best before quality** establishment.
- To monitor **compliance with the labelled geographical origin**.

Specific objectives



HARMONIZATION AND COORDINATION

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 (e.g. analytical discussions, needs of ring tests).

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 (e.g. calibration for not targeted methods) and others from reliable sites, to be used for the standardization and make downloadable data and spectra.

CONSUMER AND MARKET CONFIDENCE

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 a sustainable source of **reliable information on the methodology for authenticating OO that will be available to the international user community and to the public**.

OVERALL OBJECTIVE

To **better guarantee OO quality** and **authenticity** empowering the **detection** and fostering the **prevention** of OO fraud.



Strategic objectives

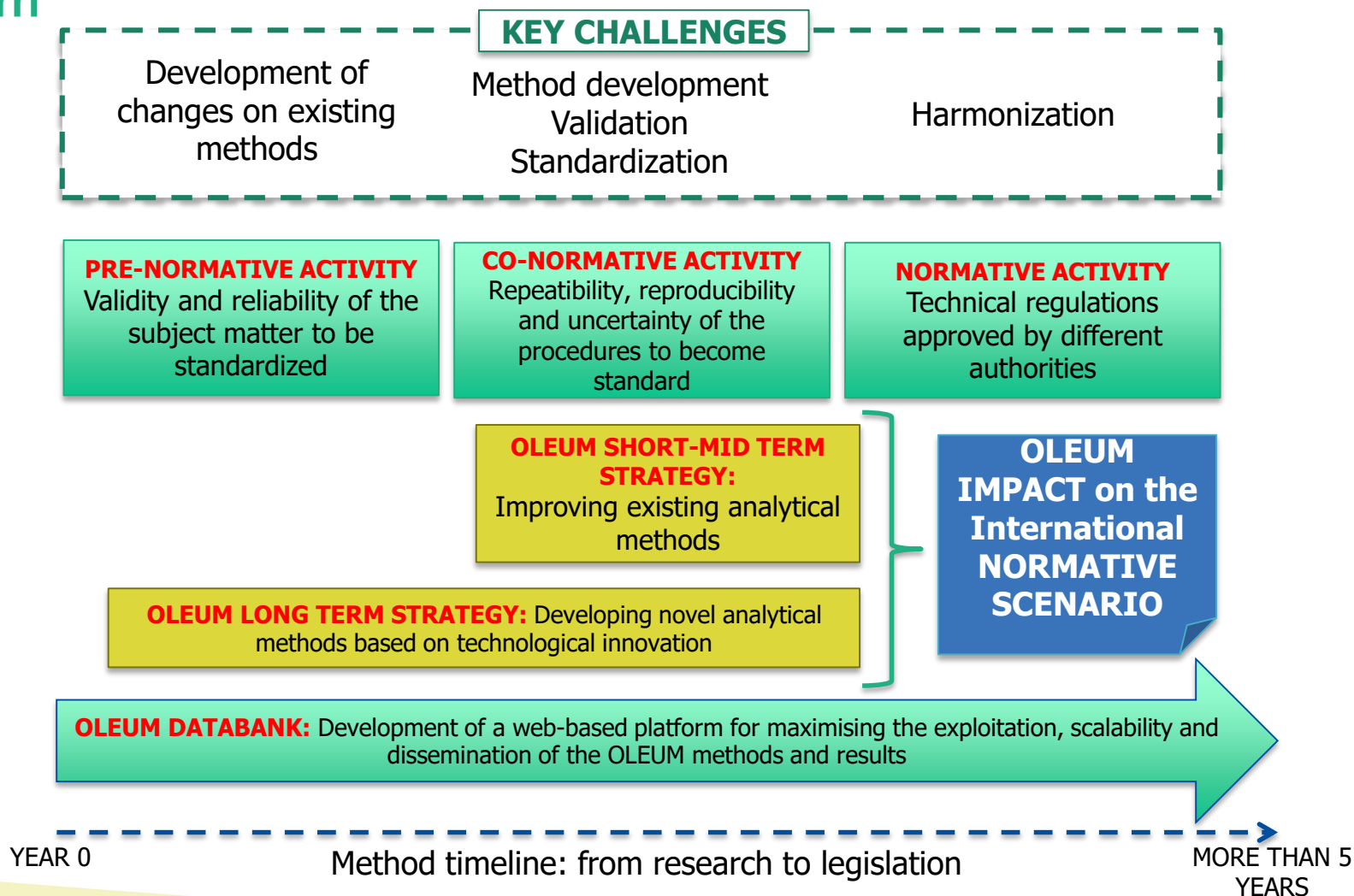


- To develop **new/improved methods** for assuring the **quality** and **authenticity** of OOs.
- To develop an **integrated quality assurance infrastructure** for **methods** of analysis (**reference materials, downloadable library of analytical methods** and **compositions** collected in a **databank**).
- To develop and give a technical support at a **worldwide community of analytical laboratories** involved in the analysis.



Oleum

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**.



Strategy and assumption

Four main streams of work:

- 1. Improving existing analytical methods** that are officially recognized (EU, IOC, CODEX) to evaluate the quality and authenticity of OO. Critical points in these protocols will be evaluated in terms of: sensitivity; reproducibility; analysis time (including sample preparation); environmental impact; usability.
- 2. Setting up of novel analytical methods based on technological innovation** with the objectives: to perform **rapid qualitative screening of OOs**; to identify **new markers of poor quality** (e.g. volatile compounds responsible for the main sensory defects); to detect **undesired processing** (e.g. soft deodorization); to detect fraudulent activities (e.g. illegal blends with other vegetable oils).
- 3. Developing an OO Databank** to ensure that the improved and the newly developed OLEUM methods applying novel technological advancements, are readily available for the implementation by quality control labs on a global scale.
- 4. Establishing of a** wide community of laboratories involved in the OO quality control and detection of frauds, the **OLEUM Network**, to foster laboratories proficiency and to disseminate and harmonize methods, limits and ranges, at global scale.

Strategy and assumption

Improving existing analytical methods that are officially recognized (EU, IOC, CODEX) to evaluate the quality and authenticity of OO.

Envisioned improvement of the existing analytical methods

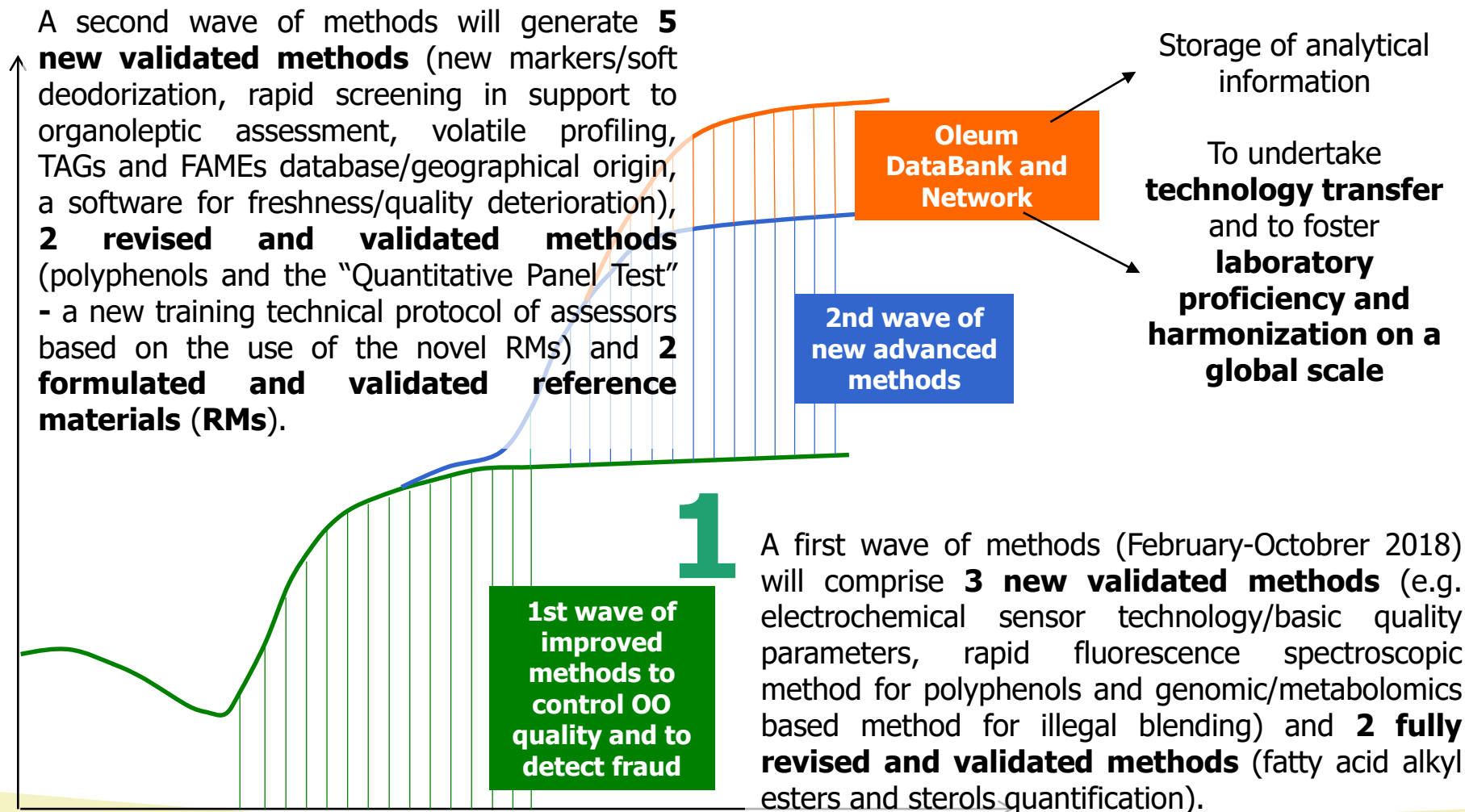
Scope	Method	<i>Sensitivity</i>	<i>Reproducibility</i>	<i>Analysis time</i>	<i>Environmental protection</i>	<i>Usability</i>
Quality (commercial categories)	Sensory panel test (EU Reg. 1227/2016)	X	X	X		
Quality (health claim added value)	Biophenols by HPLC (COI T20 Doc. 29/2009 EU Reg. 432/2012)				X	X
Quality-Authenticity (illegal blends with soft deodorized OO)	Fatty acids ethyl esters by GC (EU Reg. 1830/2015)			X	X	X
Authenticity (illegal blends with extraneous vegetable oils)	Global method: TGs by HPLC & FAMES by GC (COI T20 Doc. 25/2013; EU Reg. 1833/2015)	X	X	X	X	X
Authenticity (illegal blends with extraneous vegetable oils)	Sterols and triterpenic alcohols by TLC / GC (EU Reg. 1833/2015)	X	X	X		

Strategy and assumption

Setting up of novel analytical methods based on **technological innovation**.

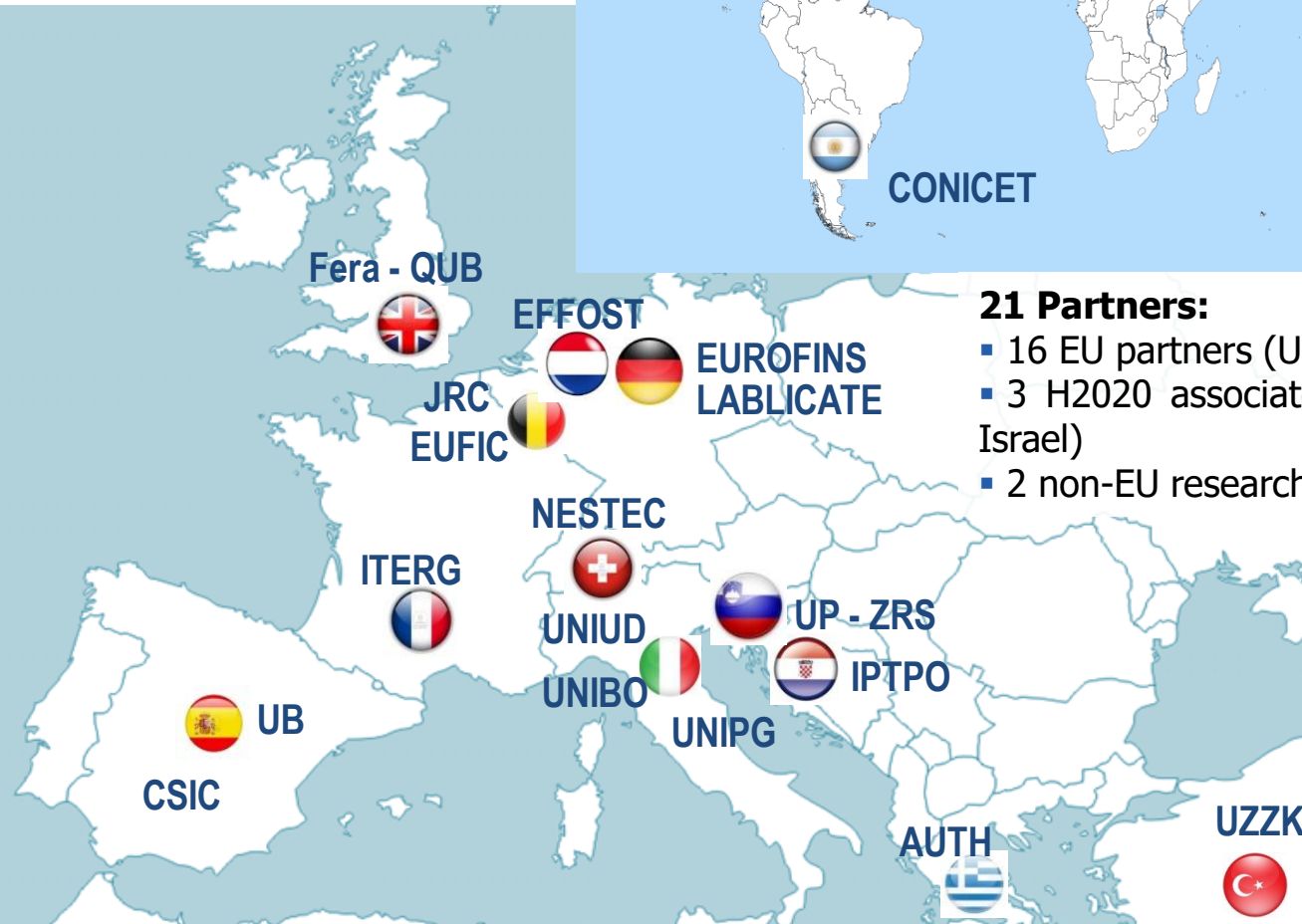
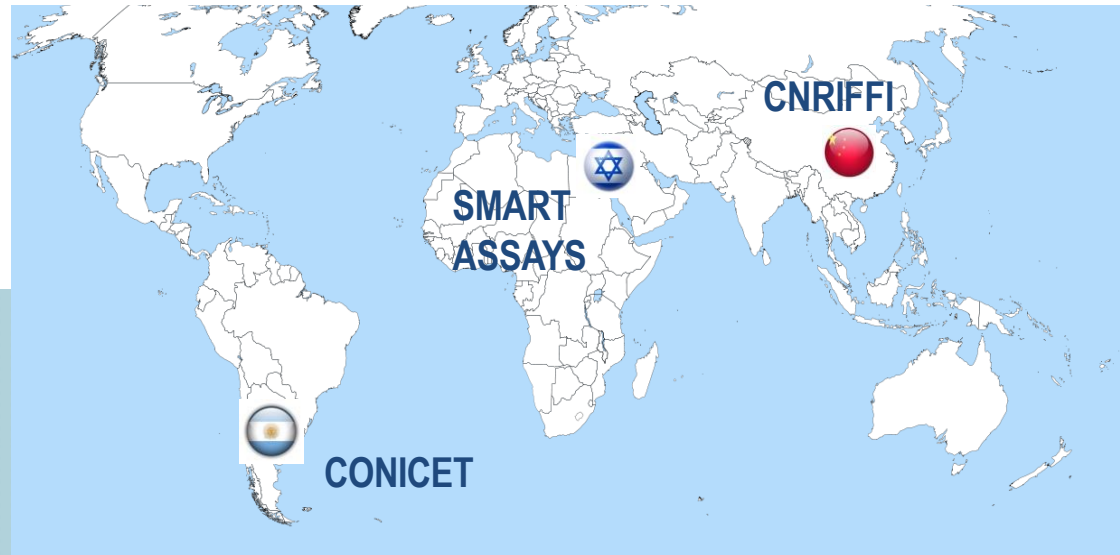
Development of new technological advances				
Method	<i>Quality (new markers)</i>	<i>Undesired processing (new markers)</i>	<i>Adulteration (new markers)</i>	<i>Rapid screening/procedure</i>
Volatile profiles by SPME-GC-MS NMR, FT-IR, GC-IMS, FGC-e-nose	X	X	X	X
DGs and TGs by FIA-UHRMS	X		X	X
Steryl esters by SPE and LC-MS-MS		X		
Conjugated fatty acids by GC/HPLC-TOF-MS		X		
Fatty acids ethyl esters by TDR, FT-IR		X		X
Next generation sequencing and DNA molecular markers by qRT-PCR, CE (non targeted MiSeq analysis and verification using Nanopore technology)			X	X
Polyphenols, chlorophylls and tocopherols by fluorescence spectroscopy method	X		X	X
Free acidity and peroxide value by electrochemical sensors	X			X
Fingerprint by ^1H NMR, FT-IR, mass spectrometry and isotopic analysis	X	X	X	X

Expected results





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



1 large food industry



Research

1 private research company



3 non-profit organizations



14 universities and public research centers



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS



ARISTOTLE
UNIVERSITY OF
THESSALONIKI



UNIVERSITÀ DEL
LIGURIA
UNIVERZA NA PRINOKEM



ZRS
CENTRO DI RICERCHE SCIENTIFICHE
ZNASTVENO-RAZISKOVALNISCHE



CONICET



Joint Research Centre



ITERG
Expertise Corps Gras



UNIVERSITAT DE
BARCELONA



Institute of Agriculture and
Tourism



中国食品发酵工业研究院

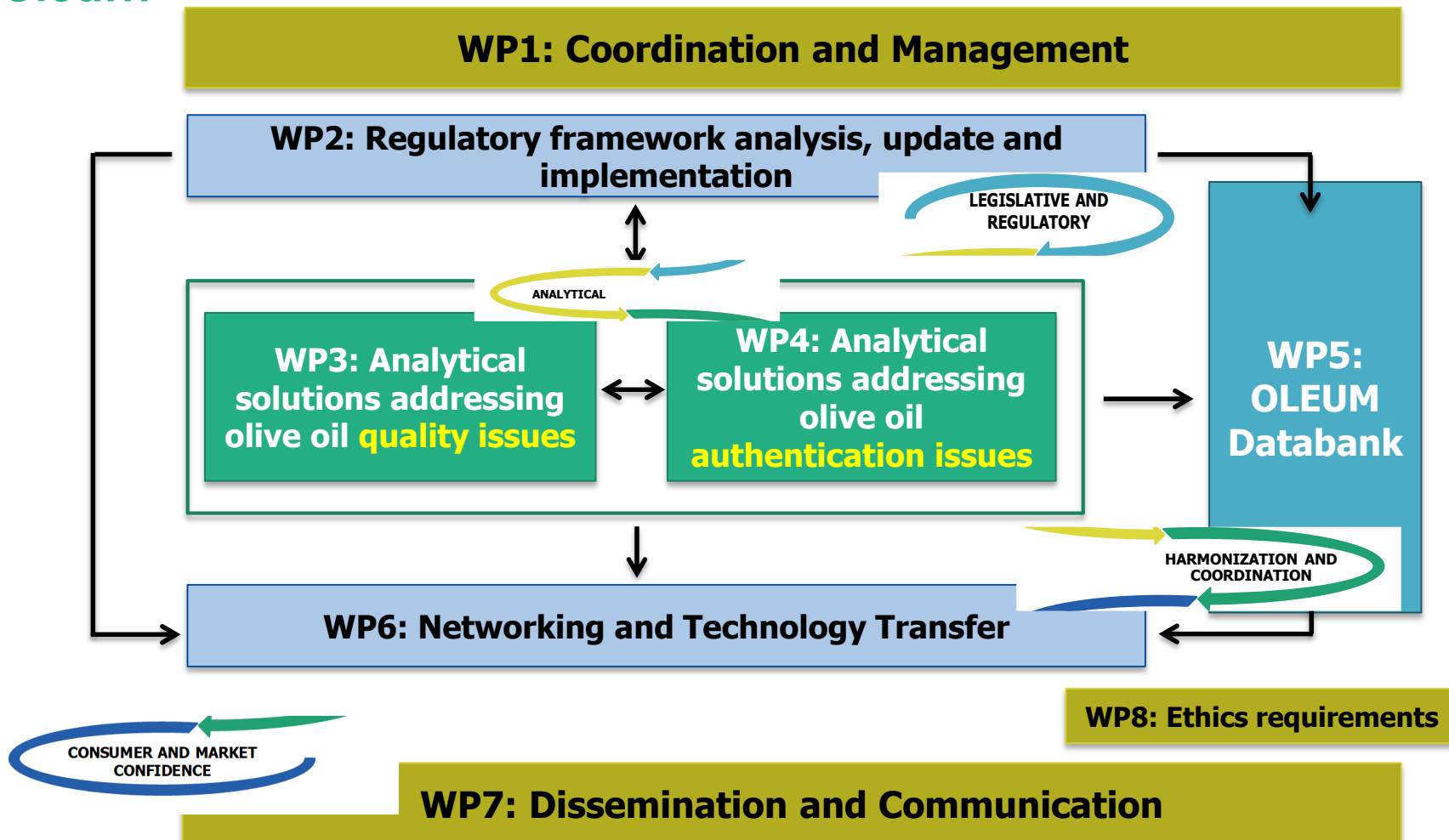
CHINA NATIONAL RESEARCH INSTITUTE OF FOOD & FERMENTATION INDUSTRIES



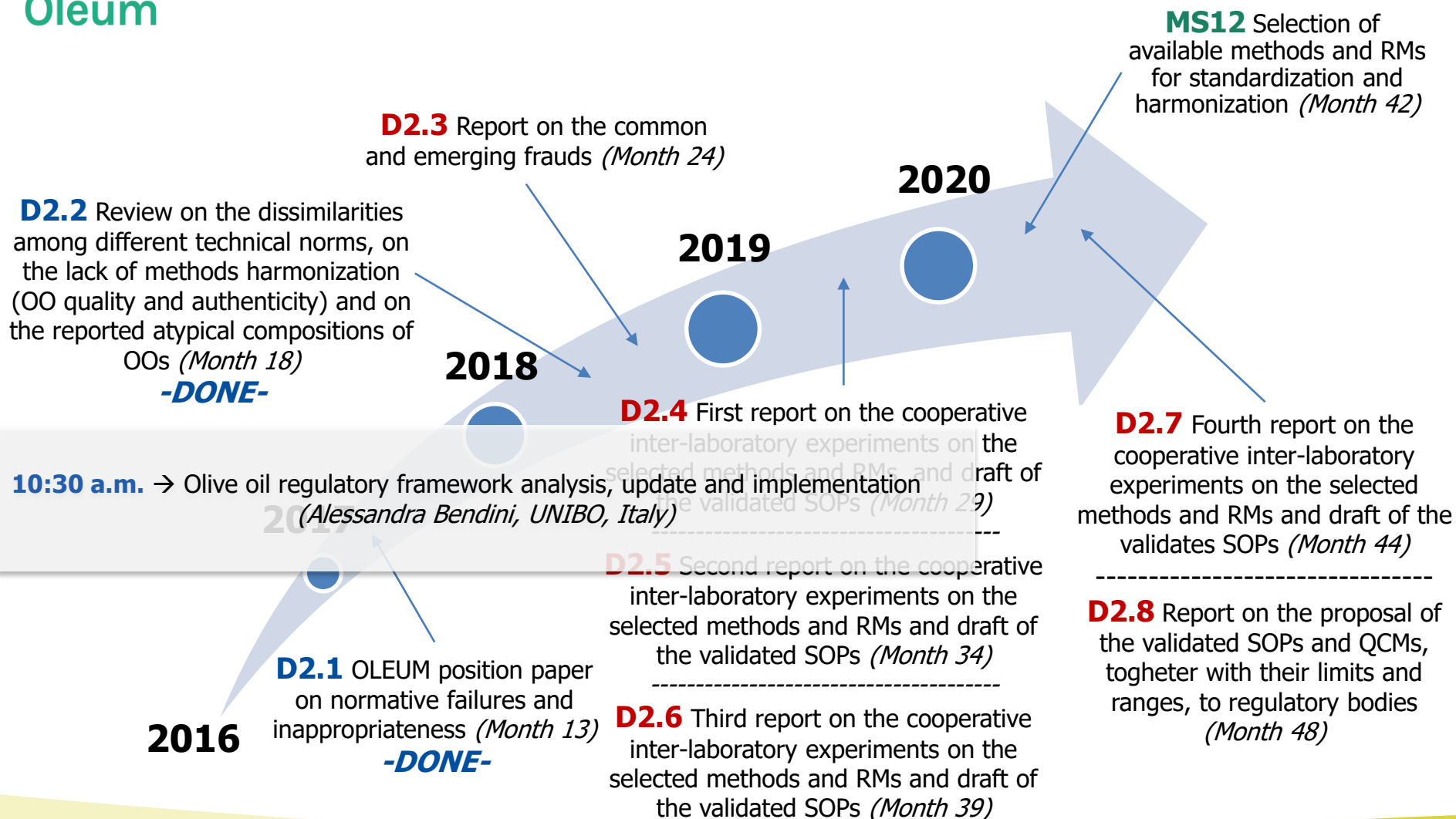
The OLEUM Multi-Stakeholder Advisory Board

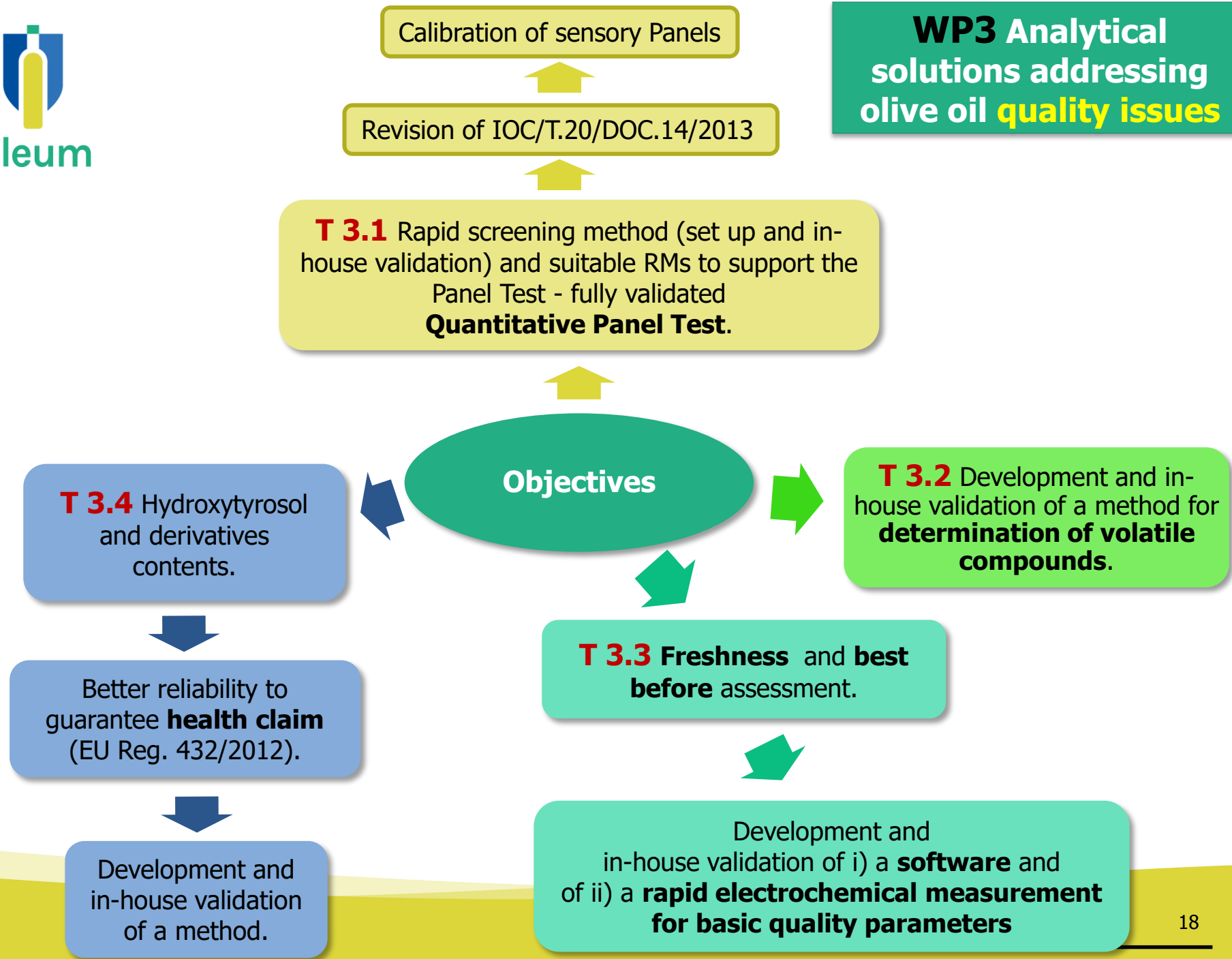
- ❖ Caroline Jeandin, **European Commission DG Agriculture and Rural Development - Unit G.4 (Arable Crops and Olive Oil)**, Belgium
- ❖ Abdellatif Ghedira, Executive Director, **International Olive Council (IOC)**, Chemical & Standardization Unit, Spain
- ❖ Bruno Di Simone, **Italian Ministry of Agricultural, Food and Forestry Policies**, ICQRF-Laboratorio di Perugia, Italy
- ❖ Roland Poms, Secretary General, **MoniQA Association**, Austria
- ❖ Breda O'Dwyre, Research Centre Manager of Centre for Entrepreneurship and Enterprise Development (CEED) at the Institute of Technology, **TRADEIT Network**, Ireland
- ❖ Fernando José Burgaz Moreno, General Director of Food Industry, **Ministry of Agriculture and Fisheries, Food and Environment**, Spain
- ❖ Richard Cantrill, Chief Science Officer, **American Oil Chemist's Society (AOCS)**, USA
- ❖ Dan Flynn, Executive Director, and Selina Wang, Research Director, **UC Davis Olive Center**, USA
- ❖ Pierluigi Delmonte, Researcher, **U.S. Food and Drug Administration (USFDA)**, USA
- ❖ Massimo Vicenzini, President, **Tuscan Food Quality Center**, Italy

PERT Scheme – Work Plan

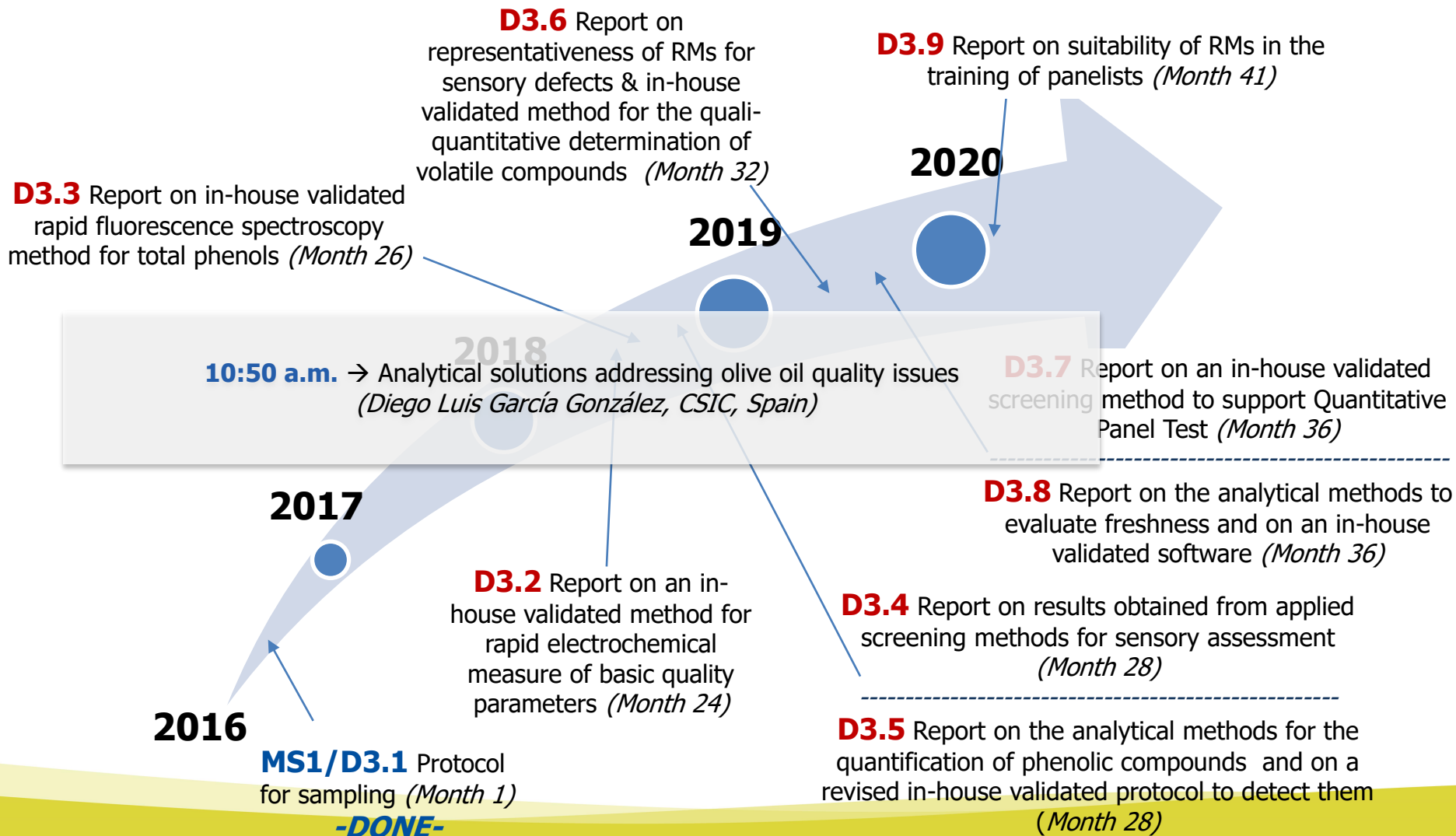


WP2. Regulatory framework analysis, update and implementation





WP3. Analytical solutions addressing olive oil quality issues





Oleum

Sample set (EVOO, VOO, LOO)
Reference standards

**In-house validation*

**Fully-validation*

**Organoleptic
assessment**
(6 panels)

Instrumental approaches
(Flash-GC E-nose,
SPME-GC-MS, NMR)

**High resolution volatile
profiling methods**
(SPME-GC-FID, GCXGC-MS,
TDU-GC-MS, SPME-GC-O)
- on a significant selection of
samples -

Classification models

SESSION → Olive Oil, including Sensory Analysis

Tuesday 10:40 a.m. → The profitable relation between sensory and analytics in virgin olive oil quality detection

(Tullia Gallina Toschi, Sara Barbieri, Chiara Cevoli, Ole Winkelmann, Karolina Brkić Bubola, Florence Lacoste, Milena Bučar-Miklavčič, Ummuhan Tibet, Ramón Aparicio-Ruiz, Diego L. García González and Alessandra Bendini)

***Rapid screening method(s)**
(setting-up and validation)

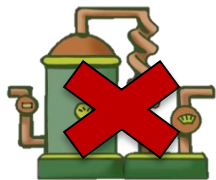
*** Validated method
for **volatiles**
determination**

*** Formulated and
validated **reference
materials (RMs)****

OBJECTIVE 1:
Reducing the number of samples
assessed by the sensory panels

OBJECTIVE 2:
Improving the panel performance
(sensitivity, discriminant capacity etc.)

WP4. Analytical solutions addressing olive oil authentication issues



T 4.1 Set up of a real olive oil "deodorization scenario"

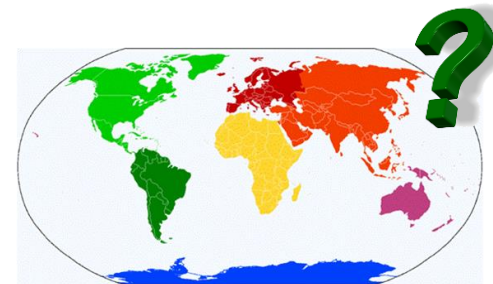
Soft-deodorized OOs

Lab scale production

Pilot plant production

Development of a method to verify the **authenticity of label-declared geographical origin**

T 4.4 Assessment of the **geographical origin** (EU, extra-EU EVOOs)



Objectives

T 4.2 Detection of **illegal blends with soft-deodorized OOs**

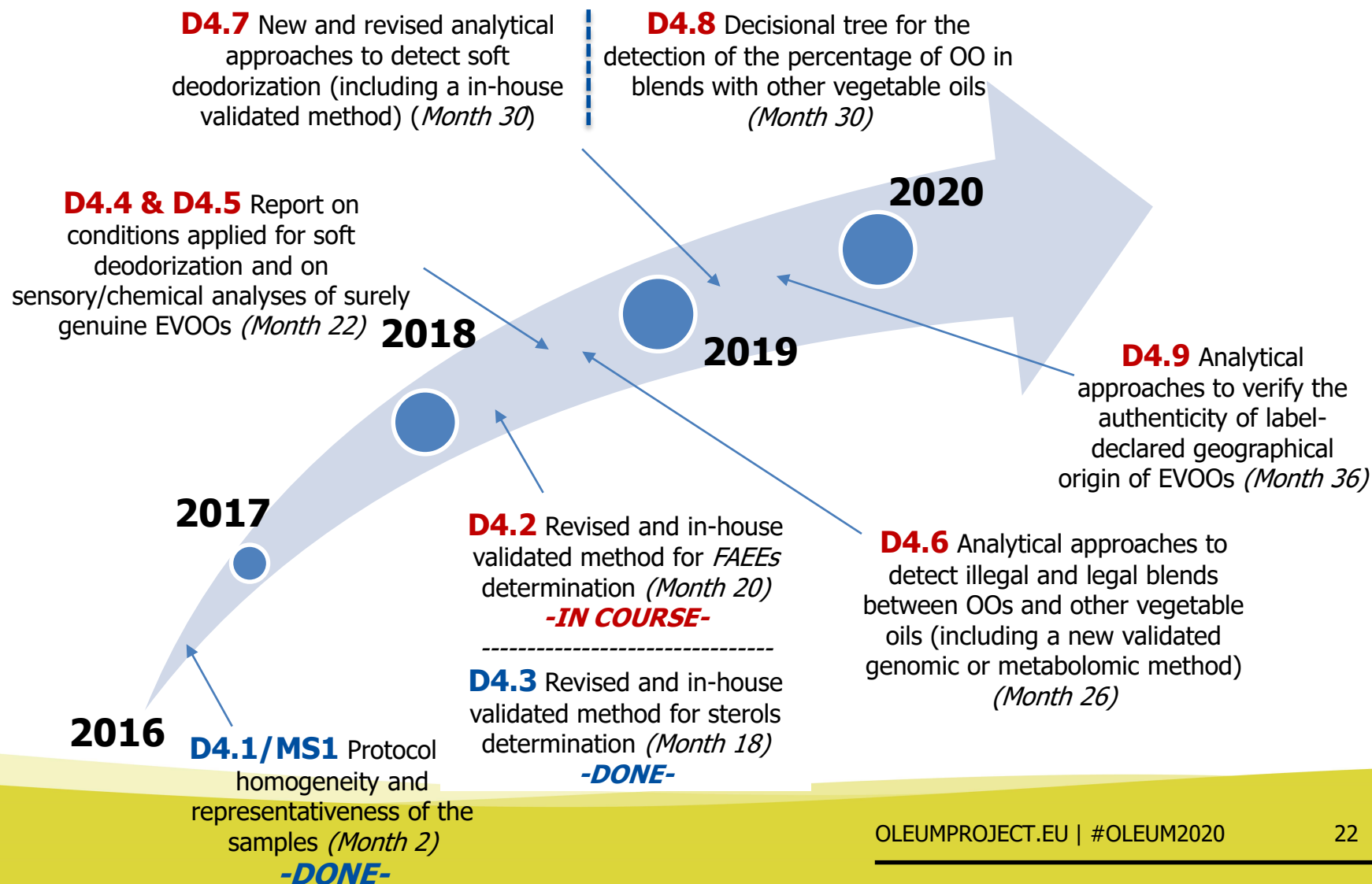
Revision and in-house validation of current EU **FAEE method**
Development and full validation of a new or revised method

T 4.3 Detection of **illegal blends between OOs and other vegetable oils**

Revision and in-house validation of current EU **sterols method**
Development and full validation of a **new genomic or metabolomic method**

PRC


WP4. Analytical solutions addressing olive oil authentication issues



T4.1 - Soft deodorized OOs production: pilot-plant and blends

Activities of the first year

Conditions applied for the short-path distillation: 140°C, 1L/h and vacuum below 1 mbar (which seems to be the best compromise between discarding of the defect and formation of stigmastadienes).

- 
- Disappearance/strong decrease in the intensity of the **most perceived defect**
 - Appearance of a **butter like** sensory note
 - Disappearance/decrease in the intensity of **fruity**
 - Slight decrease of the **free acidity**
 - Slight increase of the **peroxide value**
 - No effects on the **UV extinction coefficients**
 - No evident decrease of the **FAEEs** content
 - Increase of **stigmastadienes** content, but below the EVOO EU legal limit (0.05 mg/kg)
 - No formation of **trans fatty acids**

T4.2 - Detection of illegal processing (deodorization): analytical tools and markers

Analytical determinations performed on all the samples

UNIBO

- FAEEs determination using Time Domain Reflectometry (TDR)
- Analysis of FAEEs by chromatographic methods (HPLC/GC-FID)

CSIC

- Analysis of the DAG and pyropheophytin by chromatographic methods
- Analysis of FAEEs by chromatographic methods (SPE/GC-FID)
- Analysis of the volatile headspace profile by ion mobility gas chromatography

UB

- Analysis of TAGs profile by direct injection-heated electrospray ionization-ultra high resolution mass spectrometry (HESI-UHRMS)
- Analysis of volatile compounds by SPME-GC/MS

EUROFINS

- FAEEs determination by ^1H -NMR

ITERG

- GC-FID for fatty acid profile, LC-refractometry for TAG profile, NIR approach

T4.3- Illegal and legal blends between OOs and other vegetable oils: sampling and analytical tools

Illegal blends

- EVOO + virgin hazelnut (HV_1)
- EVOO + virgin avocado (EVAO_1)
- OO + refined palm olein (RPOO_1)
- OO + refined avocado (RAO_1)
- OO + refined hazelnut (HR_1)
- OO + desterolized HO sunflower (DOSO_1)*



Fera sent vegetable oils, EVOOs and OOs to the partners involved in the analysis. Each partner had to prepare its own blends.

Legal blends

- EVOO + refined NT (conventional) sunflower (RCS_1/NTSO)
- EVOO + refined HO sunflower (ROSO_1)
- OO + refined NT (conventional) sunflower (RCS_1/NTSO)
- OO + refined HO sunflower (ROSO_1)



Fera sent these **legal blends** (60:40, 50:50, 40:60) to the analytical partners, together with the related EVOO/OO and the vegetable oils.

* The desterolized high oleic sunflower oil was produced by ITERG



T4.3 - Illegal and legal blends between OOs and other vegetable oils: sampling and analytical tools

Analytical determinations performed by the partners

UNIUD

- Determination of TAGs by GC-FID
- Analysis of free and esterified sterols (SPE/GC-FID)

EUROFINS

- Analysis of fatty acid profiles by ^1H -NMR

CONICET

- ^1H -NMR fingerprinting of olive oil to detect the addition of seeds or vegetable oils in illegal and legal blends to olive oils

CSIC

- Analysis of TAG experimental and theoretical composition to check the coherence of composition in illegal blends
- Conventional techniques for detecting percentage of vegetable or seed oils in olive oils: TAGs, tocopherols, aliphatic saturated hydrocarbon, sterols
- Other advanced and cutting-edge solutions (a genomic targeted approach)

Fera

- Other advanced and cutting-edge solutions (DNA metabarcoding)

UB

- Determination of TAG by direct injection-heated electrospray ionization-ultra high resolution mass spectrometry (HESI-UHRMS)
- Analysis of volatile compounds by HS-SPME-GC/MS

Smart Assays

- Analysis of legal and illegal blends of olive oils with vegetable oils using fluorescence spectra scan

T4.4 - Increasing consumer's confidence on the geographical origin of EVOOs: development of analytical tools

Analytical determinations performed by the partners

CNRIFFI

- Isotopic analysis by stable isotope ratios $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$

EUROFINS

- Stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$ and D/H) by IRMS
- ^1H -NMR fingerprinting of olive oil

CONICET

- ^1H -NMR fingerprinting of olive oil

Fera

- Mass spectrometry fingerprinting by LC-TOF-MS

UNIBO

- Flash Gaschromatography Electronic Nose (volatile compounds)

UNIUD

- GC-FID and HPLC (TAGs)

NESTEC

- SPME-GC-MS (volatile compounds)

UB

- HS-SPME-GC-MS (sesquiterpene hydrocarbons)
- ESI-UHRMS (TAGs)

UNIPG

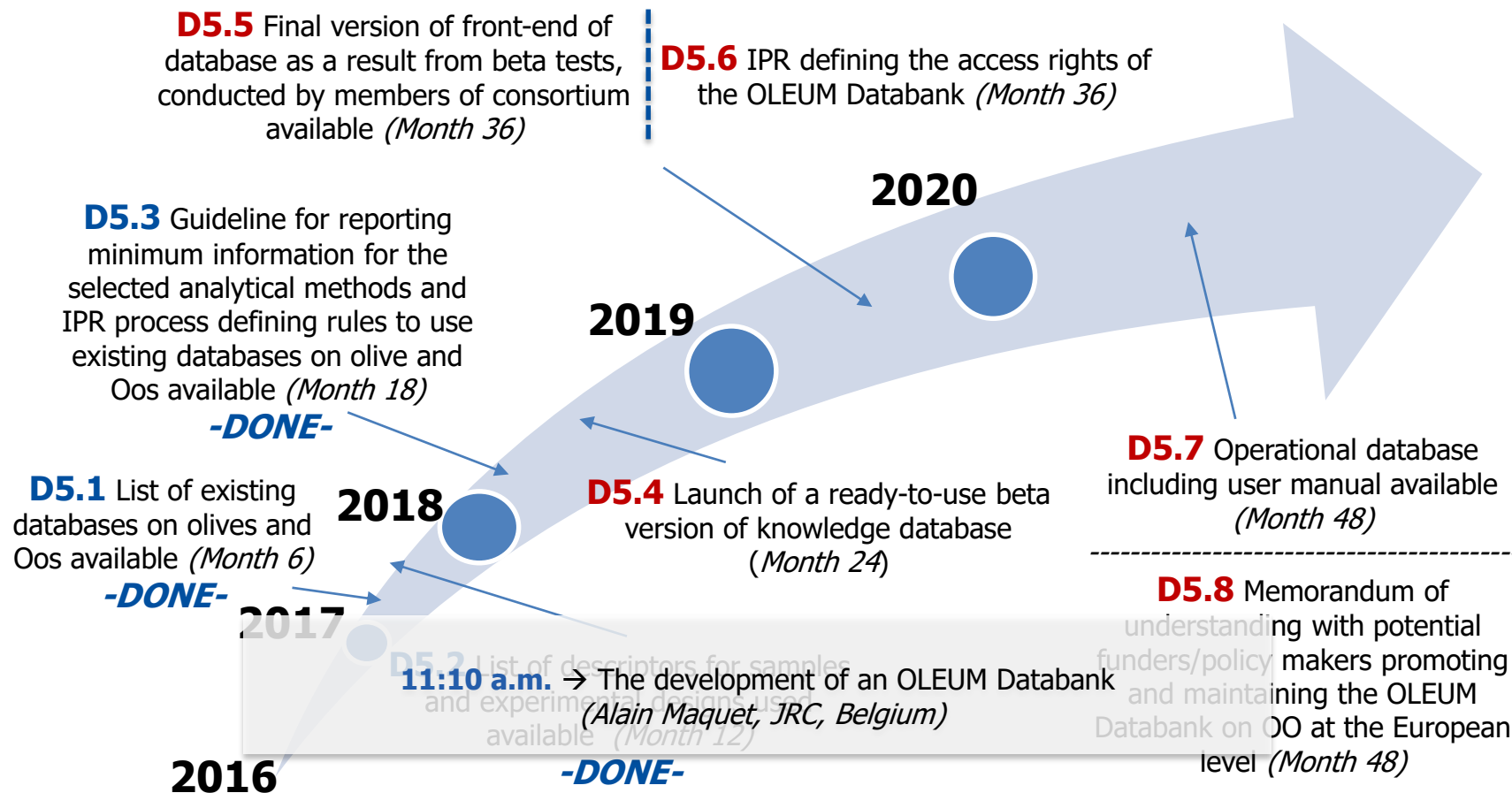
- UPLC-Q-Exactive Orbitrap MS (TAGs fingerprint)

ITERG

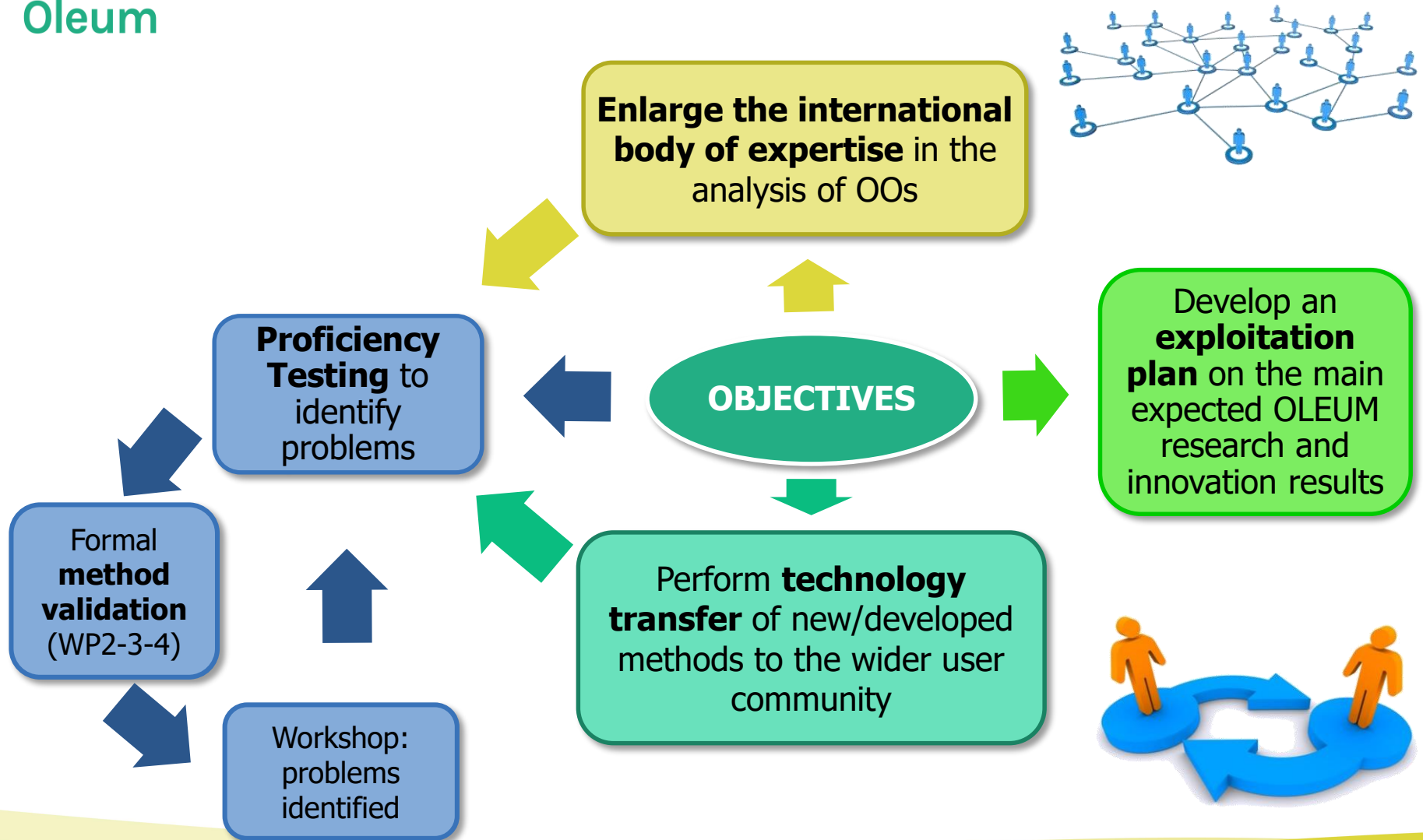
- HPLC-RID (TAGs fingerprint)
- GC-FID (FAME)

Smart Assays

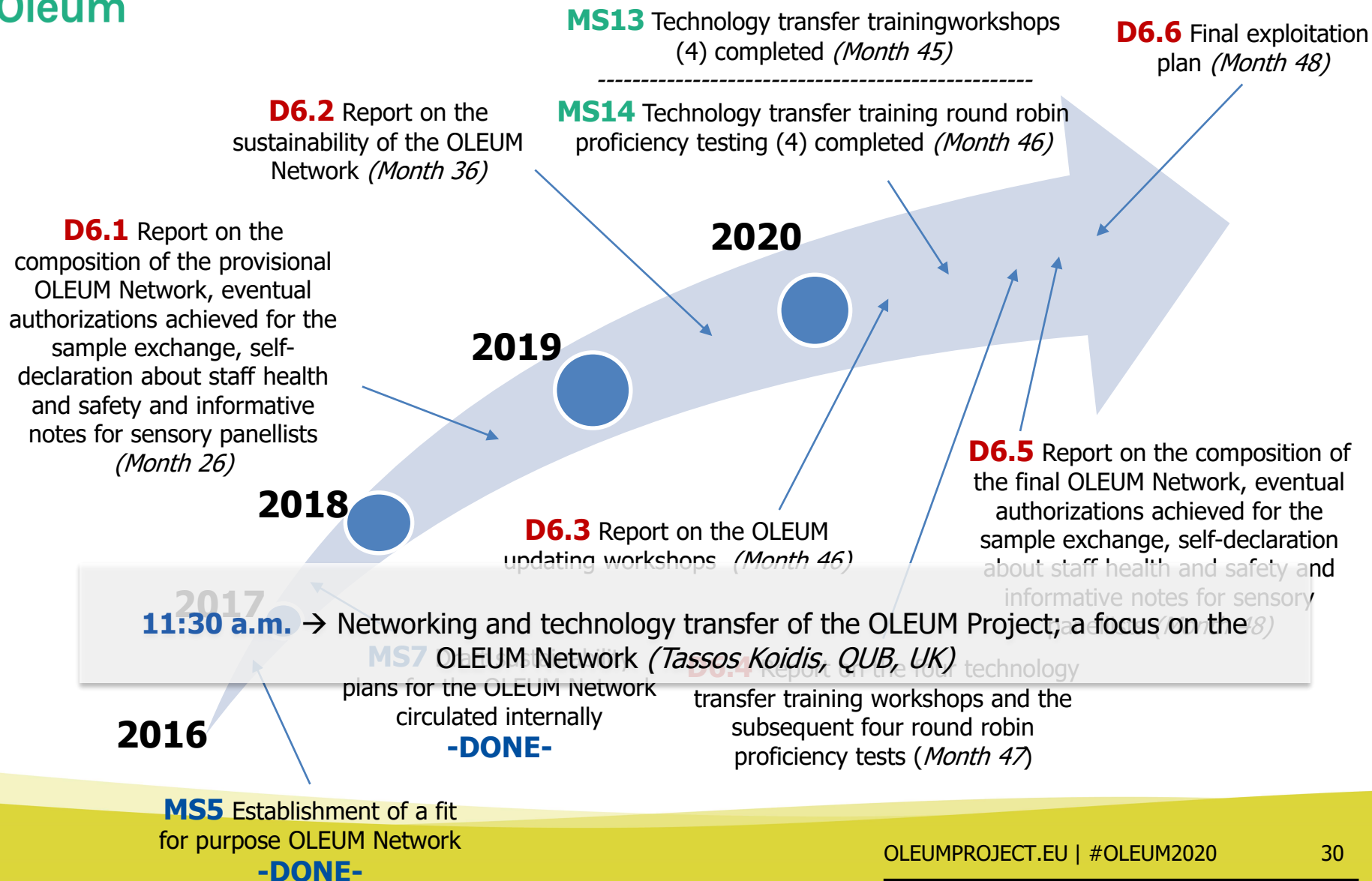
- Fluorescence spectroscopy



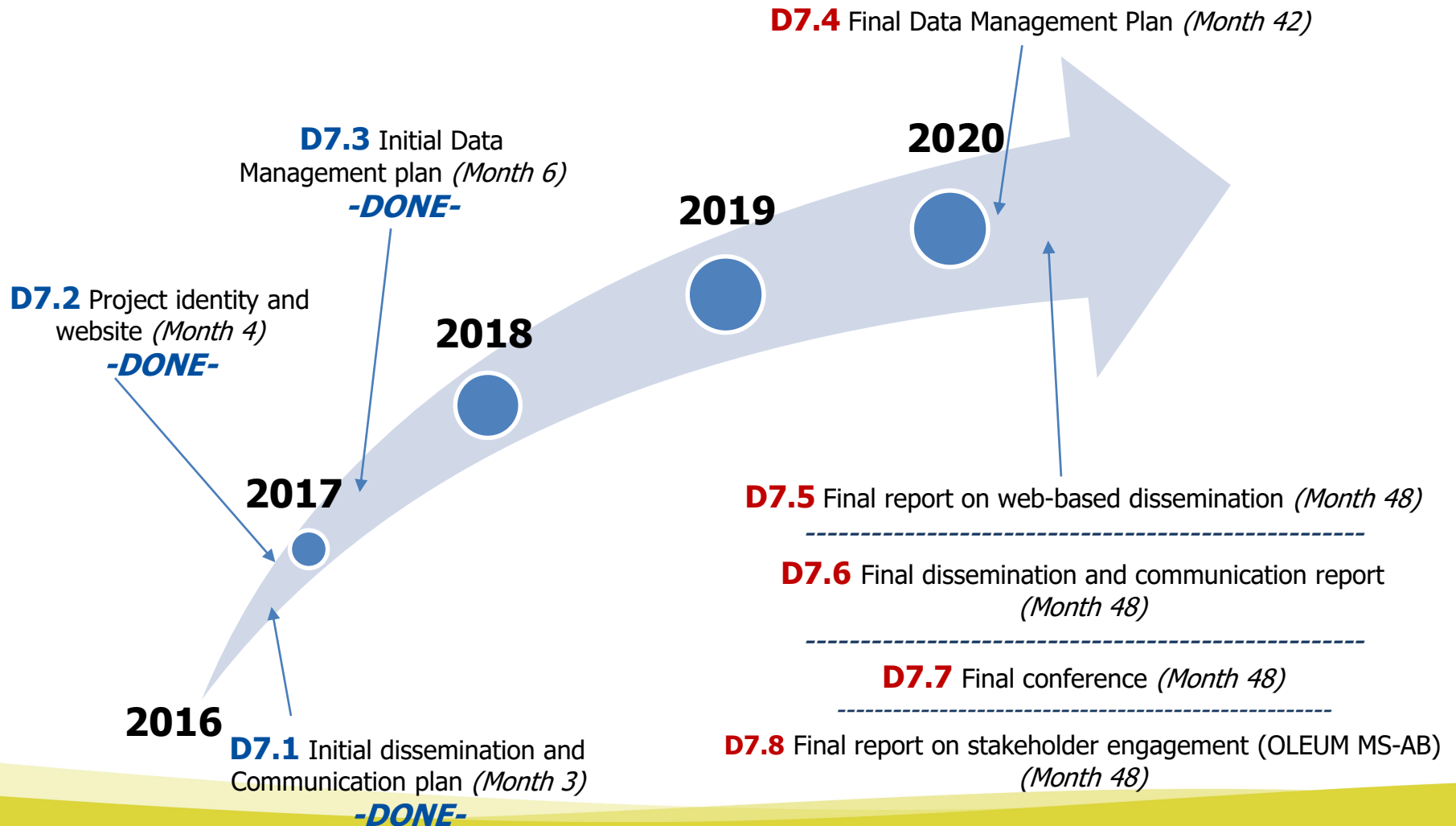
WP6. Networking and technology transfer



WP6. Networking and technology transfer



WP7. Dissemination and communication





WP7. Dissemination and communication

DONE

- ❖ **Dissemination & communication plan** outlines all activities (D7.1)
- ❖ **Project logo and graphic identity** developed (D7.2)
- ❖ Project **website** online at www.oleumproject.eu
- ❖ **Social media** presence on Twitter via @Oleum_EU and @SciFoodHealth (ca. 1500 followers)
- ❖ **Data management plan** in place
- ❖ OLEUM presented at **30 conferences/events**
- ❖ **Infographic** for public dissemination about the **production of olive oil** has been realized

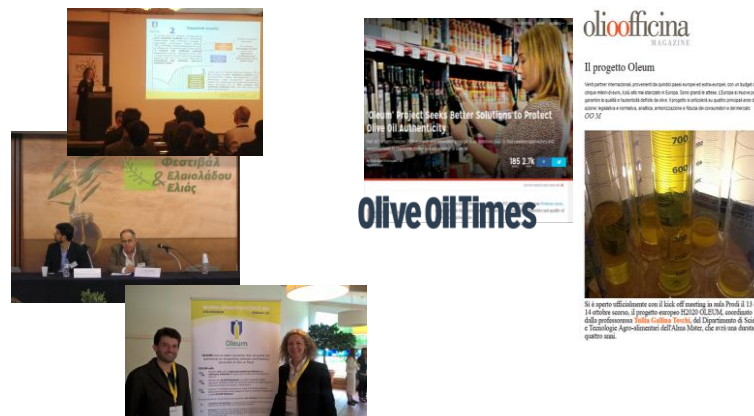


Bookmarks and posters



IN COURSE

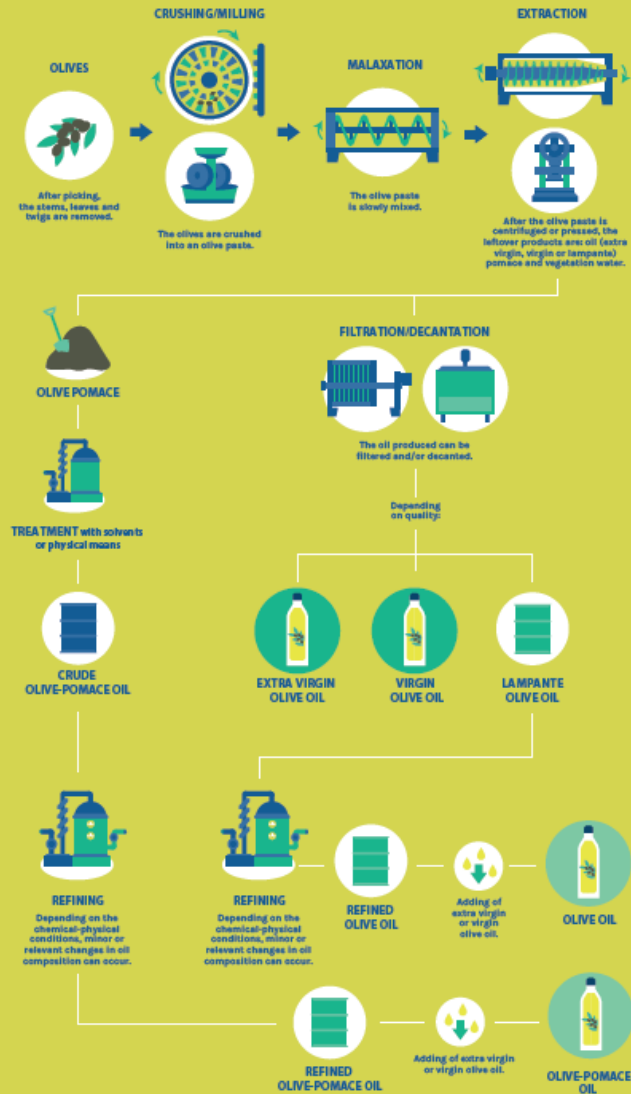
- Preparation of the **second project newsletter**. To sign up, visit oleumproject.eu
- Update of the **dissemination & communication plan**



NEXT STEPS

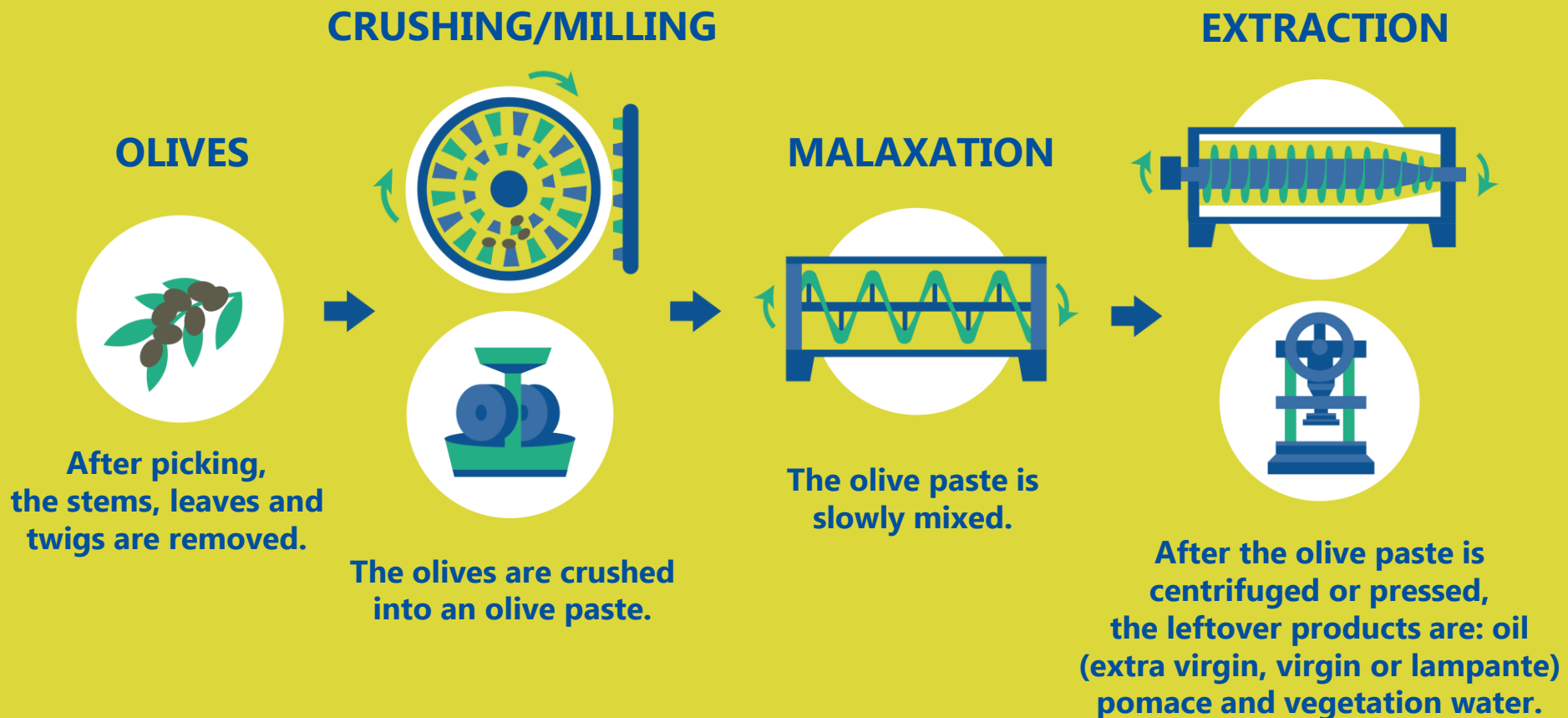
- ✓ Realization of **videos and other interactive** materials
- ✓ **Dissemination and communication** activities

HOW IS OLIVE OIL PRODUCED?



Infographic for public dissemination about the production of olive oil

HOW IS OLIVE OIL PRODUCED?



FILTRATION/DECANTATION



The oil produced can be filtered and/or decanted.

Depending on quality:



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

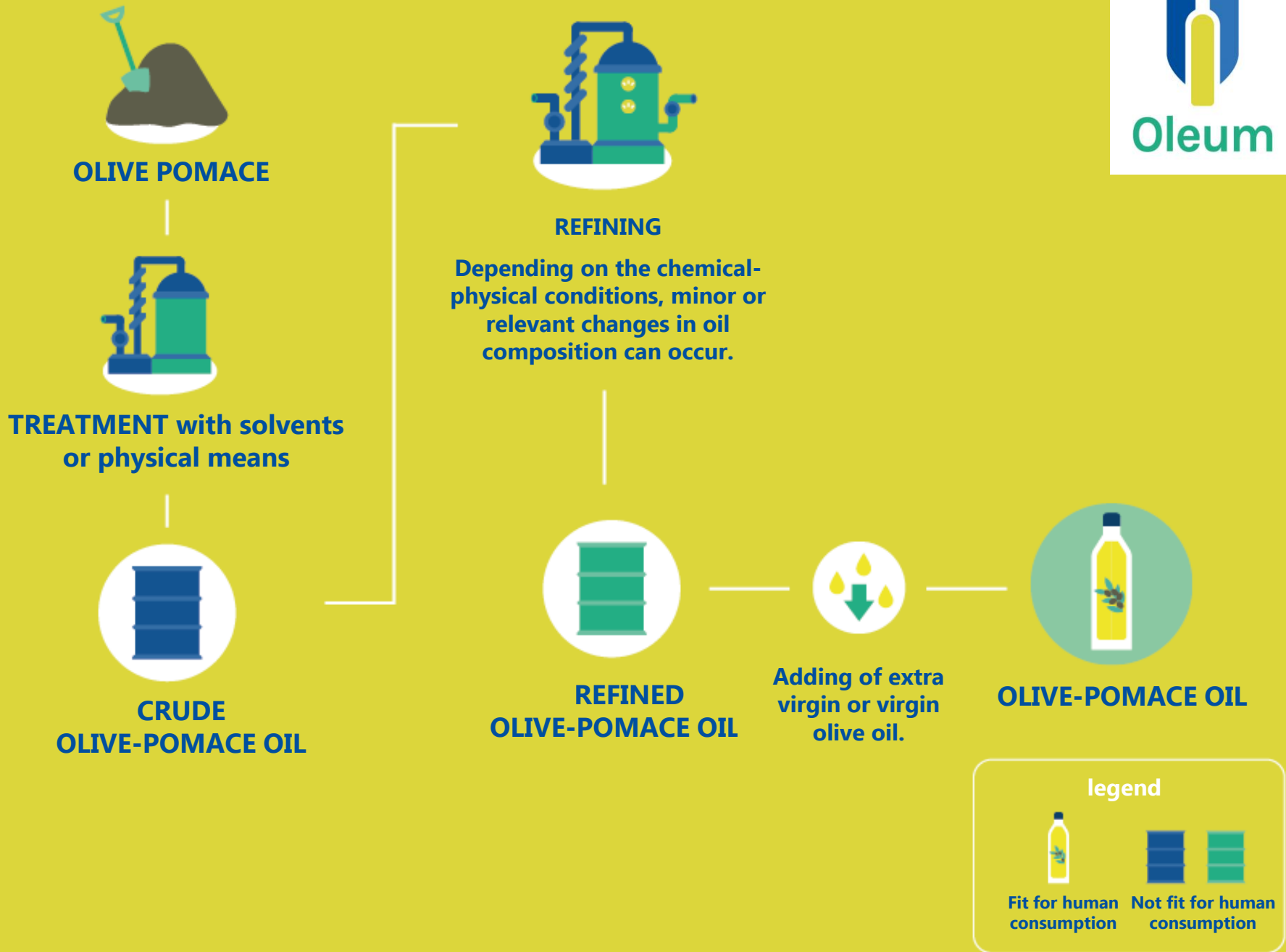
legend



Fit for human consumption

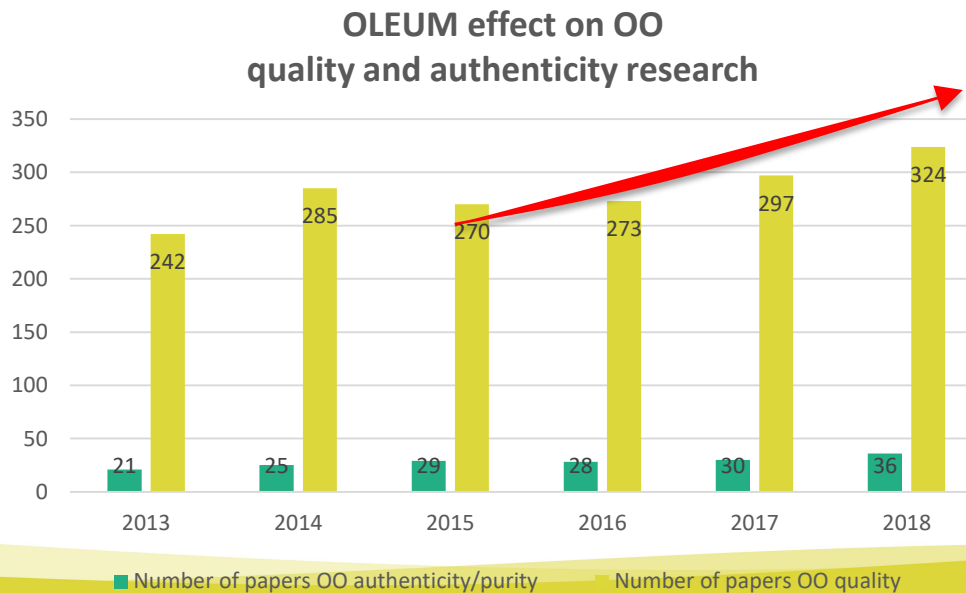


Not fit for human consumption



OLEUM MISSION

- **Promote directly and indirectly collaborative research to speed the control methods path: markers or level of compliance (not targeted) – validation – inclusion in the regulation.**
- **Open the collaboration at non OLEUM partners.**
- **Promote the maximum interaction with instruments' and analytics' producers and sellers.**



Thank you for your attention!



Scientific coordinator
Prof. Tullia Gallina Toschi, PhD

University of Bologna - Department of Agricultural and Food Sciences

tullia.gallinatoschi@unibo.it



Co-coordinator

**Dr. Alessandra Bendini,
PhD**

alessandra.bendini@unibo.it



Project manager

Dr. Andrea Gamberini, PhD

a.gamberini@unibo.it