European Commission

Newsletter

Workshop on olive oil authentication

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10 & 11 June 2013

Organised by
European Commission
Directorate General
Agriculture and Rural Development
and the
European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

with the participation of the
International Olive Council

Introduction

This newsletter presents the outcome of the workshop on olive oil (OO) authentication, which was held in Madrid on 10 and 11 June 2013.

Olive oil quality is regulated at international level by the International Olive Council (IOC) trade standard (COI/T.15/NC n° 3) and organoleptic assessment methods (COI/T.20/DOC.15, DOC.14, DOC. 4, DOC. 5 and DOC. 6) , Codex Alimentarius (CODEX STAN 33-1981), and accordingly at EU level by Regulation (EEC) n° 2568/91 establishing a list of chemical and organoleptic characteristics, as well as methods for their analysis, which are regularly updated to include new scientific findings thus improving control of quality and authenticity of OO. Despite this regular revision certain problems did not find appropriate solutions yet. In particular: i) the blend of extra virgin olive oil (EVOO) or virgin olive oil (VOO) with soft deodorized OO or ii) with other adulterant oils; iii) the evaluation of quality parameters related to “freshness”.

The EU, member of IOC, is worldwide by far the biggest producer (74%), consumer (66%) and exporter (62%) of OO. OO is an expensive product and fraudulent activities are very tempting. To preserve the image of OO it is necessary to ensure its quality. Moreover, on the occasion of the Agriculture Council meeting of 18 June 2012, Commissioner Cioloş presented an action plan for strengthening the European OO sector, which includes as main action item promoting the quality of OO and its control to preserve and promote the image of European OO and to protect / inform consumers better.

Despite the progress realised so far, scientific knowledge concerning OO chemistry and technology lags behind the inventiveness of certain operators. Therefore the development of efficient anti-fraud methods is necessary in order to avoid disturbance of the market and the deterioration of the image of the OO.

Twenty-two worldwide-known experts working in the field of OO authentication were invited by the Directorate General for Agriculture and Rural Development (DG AGRI) and the Joint Research Centre (JRC) at IOC headquarters to brainstorm on the current most challenging issues related to OO quality and authentication and to work out a proposal on the priorities of actions for solving them. This workshop will enable the preparation of a call text for a research project on OO authentication, which will be included in the Horizon 2020 research programme of the European Union.

All these main points were introduced in detail during the opening session by Mr Jean-Louis Barjol (Executive Director of IOC), by Mr José Manuel Silva Rodriguez (Hors Class Adviser at DG AGRI) and by Ms Elke Anklam (Director of the JRC – Institute for Reference Materials and Measurement).

Session 1 - Trade standards, regulations, and fraud cases

In this session chaired by Lanfranco Conte (University of Udine, Italy), four presentations introduced the market of OO, its regulation and examples of fraud cases the control agencies have to fight.

J.-L. Barjol reviewed the **world market trends in terms of quality and product categories**. While IOC member countries account for 98% of world OO supply, the reverse occurs for demand of which 80% comes from non-member countries

(e.g. U.S.A., Brazil, Japan, China, Australia, and Canada). He highlighted an obvious lack of harmonised classification of OO. The only international harmonised statistics available are those of the World Customs Organisation, which only makes a distinction between three categories: VOO, OO, and olive-pomace oil (OPO) while the IOC recognises four categories for VOO, two for OO and three for OPO. However, in a nutshell, VOO is gaining prominence over the other categories on the world market and it is highly probable according to data from trade associations that in some markets EVOO dominates in the VOO category.

Sandrine Valentin from DG AGRI reported **differences among OO trade standards** (issued by IOC, EU, Codex Alimentarius, USA and AUST/NZ). Differences among these standards were related to the level of campesterol, or the inclusion of the level of pyropheophytin a (PPP a) and 1,2-diacylglycerols (DAGs) as quality parameters to the voluntary AUST/NZ standard. Some differences in the provisions may be explained by the spread of OO production beyond its historical geographical area. On the other hand, provisions of standards are regularly revised to adapt changes in OO composition and to technical progress.

Angela Sheridan from the Canadian Food Inspection Agency summarised the results of laboratory tests applying the **IOC prescribed analytical methods** and finding that a number of samples taken from the Canadian market were not compliant in 2012-2013. Technical issues encountered by the laboratory testing programme were also discussed, highlighting the length and cost of methods of analysis (e.g. sterols). She also recommended the use of faster as well as the application of newer methods of analysis.

Juan Ramón Izquierdo from the Ministry of Agriculture, Food and Environment in charge of the coordination of inspections of fraud in Spain also mentioned that a number of samples analysed in 2012 were **non-compliant with the provisions of the EU standards**. The violations found were related in 47% of cases with quality and purity, 33% with labelling, 4% with traceability and 16% with other reasons. The non-compliance related to quality was basically due to poor organoleptic quality of VOO. Generally speaking, the most common violation was the declaration of low quality oils as EVOO. He also highlighted that the more common fraud cases can be detected by the currently available analytical methods, however frauds using deodorised oils are more challenging to detect.

Types /Categories of OO	IOC	Codex	EU	AUST/NZ	US
Extra virgin olive oil (EVOO)	*	*	*	*	*
Virgin olive oil (VOO)	*	*	*	*	*
Ordinary virgin olive oil	*	*	no	no	no
Lampante virgin olive oil	*	no	*	*	*
Refined olive oil	*	*	*	*	*
Olive oil (blend of refined olive oil & virgin olive oils)	*	*	*	*	*

Session 2 - State of the art & challenges in olive oil authentication

In this session, chaired by Franz Ulberth (JRC), seven presentations gave an overview on the state-of-the-art and challenges in OO authentication.

Christian Gertz from the German Society for Fat Science (DGF) presented his results on the possibility of using **PPP and DAGs to estimate the quality of VOO**. According to his studies a low content of 1,2-DAGs is indicative of poor quality oils and its further decrease during storage depends among other factors also on the initial free fatty acids (FFAs) level. The evolution of 1,2-DAGs values is predictable if storage conditions are known. The second part of his presentation was related to the potential of near infra-red (NIR) spectroscopy to determine the official parameters used for describing the quality and authenticity of OO. He considers that this simple and cheap method could become an important analytical tool for routine quality controls.

Diego García-González from the Spanish National Research Council (CSIC) explained the progress made in the **interpretation of the composition of volatiles and phenolic substances of VOO** to complement the results produced by a sensory panel. They studied not only the etiology of the defects but also the chemical markers of VOO sensory defects together with their odour activity values, limits of detection and quantification, the mapping of the brain response and the synergy and masking effects among compounds. The aim of this work is to underpin the results of sensory panel tests, which are often a bottleneck in the official quality assessment of OO.

Edwin Frankel from the University of California, UC Davis Olive Center made a **review of the published literature on the adulteration of EVOO** with deodorised OO (including soft deodorisation), highlighting potential markers and the methods applied for their determination. Soft deodorisation is a process to remove at low temperatures undesirable volatiles derived from lipid oxidation that characterise low quality OO having sensory defects. It can reasonably be achieved by two main methods, either

separately or combined: i) physical stripping and ii) membrane filtration. An initial separation of deodorised distillates into two fractions of differing polarity may be of great utility for the accurate quantitation of the groups of compounds of interest, whatever the type of refining process or the composition of the distillate. The nonpolar fraction contains hydrocarbons, alkyl esters (AEs) and triglycerides. The polar fraction includes partial glycerides, fatty acids (FAs) and sterols as the main groups.

Tullia Gallina Toschi from the University of Bologna (IT) suggested alternative methods to the lengthy and laborious determination of fatty acid alkyl esters (FAAEs) by liquid chromatography – gas chromatography (LC-GC) having potential to detect adulteration of edible VOO with soft deodorised OO. These alternative methods include **Fourier Transformed-IR (FT-IR) and Time Domain Reflectometry (TDR)** both coupled to chemometry. Soft physical stripping deodorisation may produce other effects such as: i) the appearance of anomalous sensory attributes (e.g. "cardboard" like flavour); ii) changes in the ratio of volatile compounds (e.g. ethanol / *E*-2-hexenal ratio); iii) the lowering of water content in oils; and iv) changes in the DAGs content and the proportional amount of FFAs.

Nelson Marmiroli from the University of Parma (IT) illustrated the range of molecular marker platforms that can be used for the d**etermination of the plant genotype in VOO**. As the composition of VOO is the result of a complex interaction among olive variety, environmental conditions, fruit ripening, and oil extraction technology, application of DNA-based markers was recently promoted since they are independent from environmental conditions and less influenced by processing conditions. DNA extraction from VOO of different degrees of processing was possible and the obtained DNA could be amplifiable. However, DNA is continuously degraded during OO storage. Current DNA-based methods offering perspectives for the future are: i) Simple Sequence Repeats (SSRs) applying capillary electrophoresis (CE) or High Resolution Melting analysis (HRM) with real-time polymerase chain reaction (rt-PCR); ii) Single Nucleotide Polymorphisms (SNPs) analysed by rt-PCR or HRM-rtPCR or an array. The simultaneous detection of multiple SNPs from a single DNA sample is of particular interest.

Rodney Mailer from the Australian Oils Research showed how the emergence of new countries producing OO outside the primary gene pool of *Olea europaea* is requiring an open discussion on the **influence of natural variation** on the provisions stated in IOC and Codex Alimentarius standards and on the relevance of new parameters (e.g. PPPs and DAGs) to estimate edible VOO quality.

Lanfranco Conte presented a **critical review of FAs, PPPs, DAGs, sterols and AEs used to detect adulteration of OOs.** Vegetable oils are checked for FA composition, as some of them are typical of selected botanical groups (e.g. oleic acid naturally most abundant in OO). FA composition is, however, influenced by the cultivation area. Furthermore, it has been dramatically modified by genetic improvement, so that nowadays, safflower, sunflower, and rapeseed oil may have a very high content of oleic acid. Certain sterols can be used as markers of the botanical origin of oils (e.g. high concentration of Δ7-stigmastenol is characteristic for *Compositae* oil such as sunflower and safflower oil). However, desterolised and refined sunflower oil has a sterol profile similar to OO and their detection remains challenging in adulterated edible VOO. Traditionally, the characterisation of oils has been based on FA composition. Nonetheless, this information is not enough because different triacylglycerol (TAG) blends in the right proportion could lead to similar FA profiles. For this reason, recent studies tend to directly use the TAGs as compositional markers in order to characterise oils. Mr Conte finalised his presentation on the challenges to detect adulteration with soft deodorised OOs by pointing to limitations of DAGs and PPPs in the detection of such adulteration as both are influenced by storage time and storage conditions. On the contrary, FAAEs which are not influenced by storage seem to be more relevant even more so when only fatty acid ethyl esters (FAEEs) are used instead of the official sum of fatty acid alkyl esters (FAAEs) as introduced in the most recent amendment of the IOC trade standard. The lower will be the limit of FAAEs, the higher will be its efficiency in detecting soft deodorised OOs.

Franz Ulberth summarised the first day of presentations by highlighting the following main issues:

- Reducing the number of trade standards by their harmonisation should serve better the consumers.
- Sensory testing is an important tool for the verification of OO quality grade. The steady increase of OO consumption requires an increased number of well-trained sensory panels, which are costly to maintain. Consequently screening methods making use of advanced methods to analyse VOO volatiles are needed to lessen the work of sensory panels.
- It is, however, difficult to relate the volatile compounds to sensory parameters and to set cut-off values for flavour defects as a combination of defects can change these values.
- Identification of research needs for robust methods to replace or complement official methods. Within that framework, PPPs, DAGs and FAAEs were evaluated for their relevance to quality and/or purity of EVOO.
- Using genomics information to discriminate vegetable oils and quantify the relative addition of seed oils to VOO.

Session 3 - World Café

The second day was based on an interactive approach where participants were invited to discuss analytical strategies to detect soft deodorisation and adulteration of VOO. In the morning, the World Café created lively dialogues around the following questions:

- What are the relevant analytes?
- Which are the analytical techniques used?
- Which are the major limitations?
- What are the alternatives/improvements wished for?

Before starting the day, Panayotis Barzoukas (DG AGRI) recalled the objectives of the workshop before highlighting the anticipated outcome of the workshop: (a) to evaluate the capabilities and limitations of currently used methods to monitor VOO authenticity, and (b) receiving input for setting up a research program about this topic. For reaching these objectives it is appropriate to **join efforts at international level** and evaluate the advantages/disadvantages of the official methods, of alternative methods that, ideally, should be faster, cheaper, and more robust and be accepted worldwide.

For the **detection of soft deodorised OO** blended into edible VOO, participants clearly mentioned that the major difficulty to tackle this issue concerns the poor knowledge regarding the

deodorisation processes used by industries and to get access to representative samples of soft deodorised OO. Such information is a prerequisite that will help to identify potential markers, to develop appropriate analytical methods, and to estimate limits of detection and quantitation. In addition to what was mentioned during the presentations of the first day, additional analytes that could be used for the detection of soft deodorisation of OO are: carotenes, polar compounds, steryl glucosides, steradienes, and in a broader sense chemical profiling (including volatile profiles) and sensory parameters. For that purpose, common analytical methods in use are sensory panel testing, chromatography (liquid or gas) and spectrometry (fluorometry, NIR spectroscopy, nuclear magnetic resonance - NMR, mass spectrometry).

In addition to technical limitations with specific analytical methods - such as a lengthy procedure for the determination of DAGs, the cost of solvents, or the influence of storage condition on the level of PPPs - the main limitation is the fact that certain methods used for the detection of soft deodorised oil are not fully validated. Drawbacks with the specificity of methods (e.g. interferences of methanol liberated by the action of pectinase during the malaxation of olives), the sensitivity (e.g. stigmastadiene could not be detected in oils deodorised at low temperatures, or low levels of degradation products in edible VOO blended with low levels of soft deodorised oil), and the calibration (e.g. NIR) of methods were mentioned. The number of available Certified Reference Materials (CRMs) (e.g. for training sensory panels) and the need to perform systematically ring tests to assess the performance of the most relevant methods were also highlighted.

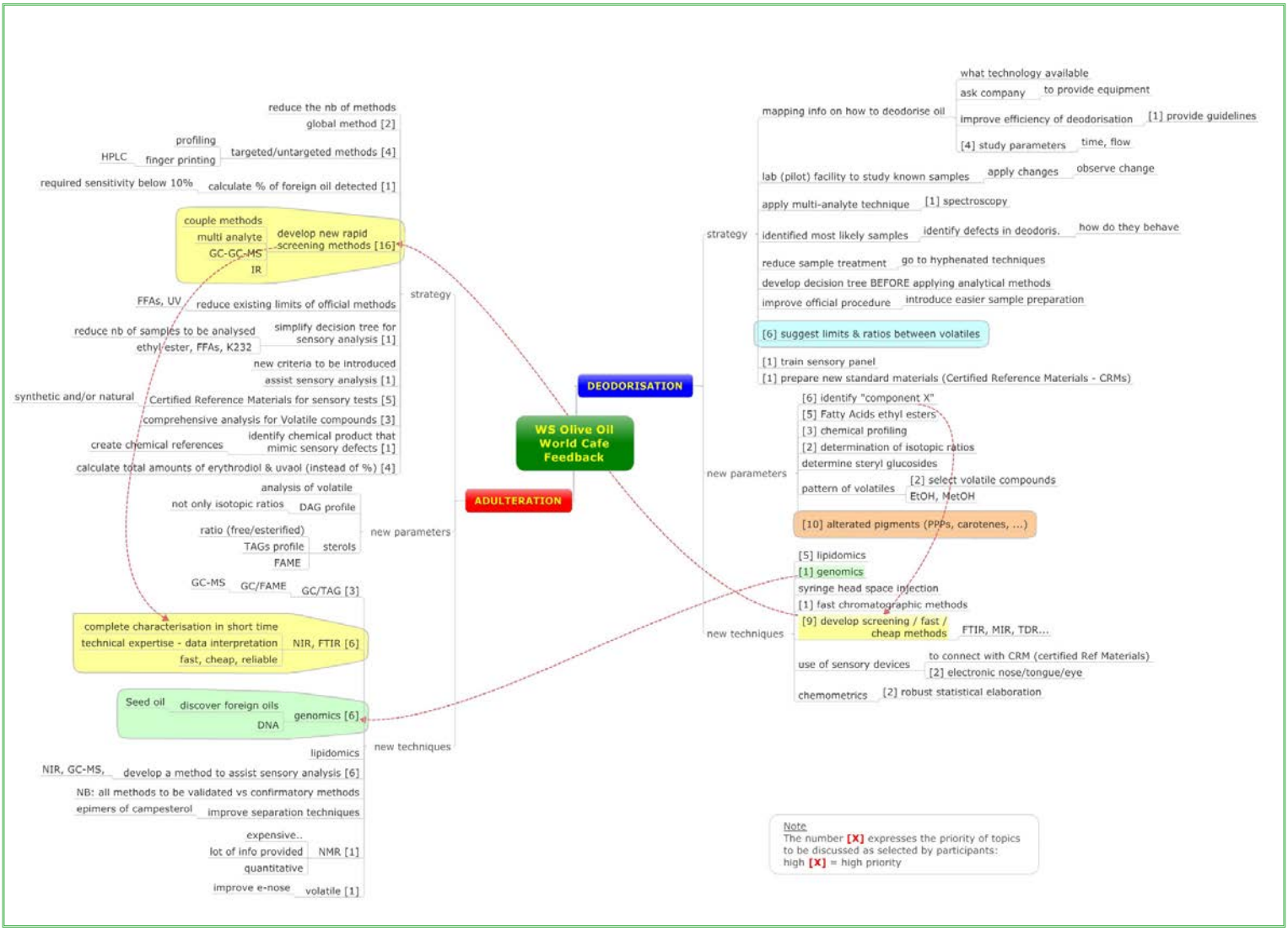
As a result, participants suggested evaluating the following strategy for detecting adulteration with soft deodorised oils:

- to collect reliable information on the soft deodorisation process(es),
- to consider creating a facility to produce soft deodorised OO under conditions that are close to industrial refining, and
- to study in a systematic way the changes in oil composition due to soft deodorisation.

Efforts should also be invested in the improvement of official procedures, to define limits and ratios among volatiles influenced by soft deodorisation, to train more sensory panels and to make more CRMs available. Participants expressed an urgent need for fast screening methods (e.g. FTIR, MIR, TDR, etc.) coupled to chemometrics as well as the amelioration of sensory devices and fast chromatographic methods. The development of shotgun lipidomics should help to deepen the understanding of the alterations of the lipidome during the processing of OO. Technological processes will also have an influence on OO pigments (e.g. PPPs, carotenes, etc.), which represent another set of potential markers to be investigated in the future.

For the detection of other **adulterant oils blended into edible VOO** two main groups of oil used for co-mingling can be distinguished: i) vegetable/edible oil and ii) low quality OO (e.g. lampante oil or OPO). In addition to the markers discussed during the presentations made during the first day, many others are also available and deserve future investigations like: alcohols (methanol and ethanol), solvent residues, steradienes, terpenes, tocopherols, waxes, volatile profiling and sensory parameters. Recently, DNA is becoming more attractive for VOO authentication as huge progress was made for its extraction from oil. On the other hand, most of the limitations are similar to those already described in case of adulteration with soft deodorised oils. The techniques used for adulterating edible VOO are not known in detail and in case quantification of the added amount of foreign oil would be required, no validated methods are available. Alternative methods like GC-MS or on-line LC-GC and PCR could be used to complement or improve the official methods and commonly applied techniques (e.g. solid phase extraction (SPE), solid phase micro extraction (SPME), LC, GC, HS-GC, etc.). Participants expressed once more the need to develop a broader array of CRMs in lipid matrices for validation of analytical methods and sensory panellist training and highlighted the need to perform ring tests in order to assess the performance of selected methods.

In order to improve the detection of adulterants in edible VOO, participants recommended mainly strategic actions and development of new techniques. There was a great interest in the application of fast screening methods for targeted and untargeted analyses using for instance comprehensive GC coupled to MS or other hyphenated methods, and rapid screening methods such as FTIR spectroscopy. Another advice was to complement sensory tests with a comprehensive analysis of volatile compounds and the identification of chemical products that are responsible for certain sensory defects. Considering the official methods, existing limits for FFAs and UV should be reduced and total amounts of erythrodiol and uvaol should be calculated instead of a percentage of total sterols. DNA-based methods, and to some extent also lipidomics, were proposed as an alternative for the determination of the genuineness of edible VOO. Obviously, newly developed methods should be properly validated according to internationally accepted principles.



Session 4 - Open Space

After having highlighted the state-of-the-art of OO authentication as well as alternatives to solve current limitations, participants were invited to start the Open Space session. The goal of an Open Space is to create time and space for people to engage deeply and creatively around issues of concern to them. Therefore the participants set themselves the agenda for the afternoon by selecting the following four issues for further discussions:

1. rapid screening methods;
2. alterated pigments;
3. threshold limits and ratio among volatiles;
4. genomics.

They were asked to participate in conversations related to each issue and answer the following questions:

- Which are the strong points?,
- What are the difficulties foreseen?,
- What can be done to solve them?,
- How can the institutions contribute?, and
- The timing required.

About the **development of screening / fast / cheap methods**, which are relevant for both adulterations of edible VOO with soft deodorised OO or other oils, the experts summed up as strong points that well established IR spectrometric methods are already used for screening the purity of edible VOO. However, they rely on the availability of spectral libraries of authentic oils which are in many cased proprietary knowledge only available from the instrument vendor. To solve the current difficulties it was suggested to implement the technology in more laboratories, to extend compositional data banks and spectral libraries by including more cultivars and geographical locations, to develop standards / reference materials, and to organise ring tests. For the last two actions, IOC and JRC could contribute significantly. Approximately two years will be necessary to achieve those objectives.

The second main issue requiring future action concerned the **research on alterated pigments**, mainly chlorophyll and carotenoids. Despite the fact that those pigments are sensitive to light exposure, temperature or oil ageing, various strong points should be taken into account: existence of a standardised method for the determination of the degradation products of chlorophylls a and a' (ISO 29841:2009), possibility to apply rapid methods to assess the total amounts of chlorophyll and carotene as already applied in industry, to measure not only the

total amounts of different pigments but also their ratios (e.g. in case of carotenoids, the trans/cis and trans/total ratio), where chlorophyll a and β-carotene could be used as standards. By harmonising the current procedures, standardising the way how calibration curves are set up, and improving accuracy most of the difficulties could be solved. It is also essential to collect data on the occurrence of pigments in a representative number of olive cultivars. Participants highlighted the needs of reference materials for chlorophyll a and β-carotene and of support from IOC and JRC to coordinate ring tests for method validations. The objectives could be reached within a nearly three years period of research.

The third research axis to be investigated in relation to adulteration of EVOO with soft deodorised OO encompasses the **definition of thresholds and ratios among volatiles**. Knowing that sensory panellists are able to detect blends with deodorised oils, there is a possibility to correlate their volatile profiles to various deodorisation practices applied to OO with different flavour defects. Another difficulty concerns the selection of the most appropriate analytical methods among SPME, dynamic HS (DHS) or rapid methods.

The main recommended actions to solve this issue were:

- to set up a pilot industrial plant to provide a panel of soft deodorised oils,
- to determine which volatile compounds change during deodorisation,
- to estimate the performance parameters of the most efficient method to detect changes in the volatile profile,
- to evaluate the odour thresholds of aroma impact compounds in oil,
- to understand how different flavour defects influence the volatile profile of soft deodorised oils,
- to initiate work with more stable oils by selecting few cultivars containing high oleic acid concentrations.

The EU is expected to help in the setting up of the pilot plant that will provide reference samples to the research community, to support the compiling of available information on current deodorisation processes and for creating a platform to share data. The suggested objectives could be finalised within a period of approximately four years.

The last issue discussed during the Open Space was mainly dedicated to the detection of VOO adulteration with vegetable oils. Indeed **DNA-based methods** are independent of external influencing factors like pedo-climatic conditions, are characterised by high sensitivity and selectivity, can be applied to any categories of oils even refined ones, and multiplexing to detect more than one adulterant is possible. Nonetheless, these methods are not applicable to detect admixture of low quality OOs or soft deodorised OOs. It was also noted that DNA extraction protocols need harmonisation, DNA is degenerated during OO storage, some DNA markers (e.g. SNPs) require the genetic characterisation of the plant, heterozygosity due to cross pollinations could interfere in interpreting DNA profiles, and there is a need for CRMs for authentic varieties and species. Consequently, a common strategy for DNA extraction should be provided. The complete sequence of the olive genome that will be shortly available will provide genomic information for developing more efficient DNA-markers and the use of DNA from organelles (i.e. chloroplasts and mitochondria) with uniparental inheritance should help to reduce the risk of mis-interpretation of DNA profiles due to cross pollination. A common database recording DNA extraction protocols as well as DNA markers and DNA methods should be made available. Support from JRC and IOC were requested for elaborating Standard Operating Procedures (SOPs) for DNA extraction and purification, for developing CRMs and for organising ring tests to compare extraction methods. The experts foresee that the objectives could be finalised in a short period of time.

The workshop finished with a closing session where participants were invited to share additional comments and insights arising from the Open Space process. Mr J.M. Silva Rodriguez acknowledged IOC for hosting the workshop and providing all the necessary facilities. He also thanked the experts for their active participation in envisioning the future and suggesting the strategic direction-setting of a research agenda and he highlighted the success of the workshop. The full list of identified areas for innovation as well as the input on how to accelerate innovation will feed the call for a research project on OO authentication that should be launched by the end of this year / beginning of next year. The call will be opened to EU Member States and any international cooperation in science and technology.

List of abbreviations

AE: alkyl ester
AGRI: Agriculture and Rural Development
CE: capillary electrophoresis
CRM: Certified Reference Material
CSIC: Spanish National Research Council
DAG: diacylglycerol
DG: Directorate General
DGF: German Society for Fat Science
DHS: dynamic head-space
e-eye: electronic-eye
e-nose: electronic nose
e-tongue: electronic-tongue
EC: European Commission
EVOO: Extra Virgin Olive Oil
FA: fatty acid
FAAE: fatty acid alkyl ester
FAEE: Fatty acid ethyl ester
FFA: free fatty acid
FTIR: Fourier Transforme Infra-Red
GC: gas chromatography
GC-MS: gas chromatography-mass spectrometry
HO: hazelnut oil
HRM: high resolution melting
HS: head-space
HS-GC: head-space gas chromatography
IOC: International Olive Council
IR: infra-red
IRMM: Institute for Reference Materials and Measurements
JRC: Joint Research Centre
LC: liquid chromatography
LC-GC: liquid chromatography-gas chromatography
MIR: medium infra-red
NIR: near infra-red
NMR: nuclear magnetic resonance
OO: olive oil
OPO: Olive-pomace oil
PCR: polymerase chain reaction
PPP: pyropheophytin a
rt-PCR: real-time polymerase chain reaction
SNP: single nucleotide polymorphism
SOP: Standard operating procedure
SPE: solid phase extraction
SPME: solid phase micro extraction
SSR: simple sequence repeat
TAG: triacylglycerol
TDR: Time Domain Reflectometry
UV: ultra-violet
VOO: Virgin Olive Oil

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In order to be updated on the development of this call as well as on the documents provided during the workshop please consult the following links:

http://ec.europa.eu/agriculture/events/olive-oil-workshop-2013_en.htm

http://ec.europa.eu/agriculture/events/2013/olive-oil-workshop/proceedings_en.pdf