OLEUM: Advanced solutions for assuring authenticity and quality of olive oil at global scale

DELIVERABLE 4.1

Title: Protocol for the standardization of a procedure in terms of homogeneity and representativeness of the samples to be analysed in T4.2 and T4.3

Date: 28 November 2016

LEAD BENEFICIARY:

Fera Science Ltd (Fera)

OTHER BENEFICIARIES

UNIBO

CSIC

ITERG

EUROFINS

UB

CONICET

SMART ASSAYS

UNIUD

NESTEC

Project funded by the European Commission within the Horizon 2020 Programme (2014- 2020)		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (Including the Commission Services)	
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Table of contents:

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1. Executive summary

2. Introduction - Rationale for the Protocol

3. Protocol for sample preparation and homogeneity assessment

4. References

5. Annex – Partner organisations

1. Executive Summary

Different partners will be carrying out analysis of various blends of extra virgin olive oils (EVOOs) with -deodorised olive oils (sd-OOs) and blends of EVOOs or olive oils (OOs) with other vegetable oils. Interlaboratory comparisons will establish the extent to which different laboratories can detect the same adulterations by applying the same methods.

A successful interlaboratory comparison depends on the production of the test materials.

Test material production and characterisation (homogeneity testing) will rely on the established experience of Fera's proficiency testing group, who routinely carry out interlaboratory tests for olive oil quality parameters.

This document is a protocol for the production and homogeneity assessment of olive oil test materials blended at different proportions both by Fera and by the partner organisations.

2. Introduction - Rationale for the Protocol

The Fera Proficiency Testing Group (FAPAS) undertakes a number of proficiency tests (PTs) each year using oil as the food matrix. These include olive oil for quality parameters, as well as other edible oils for contaminants analysis (pesticide residues and heavy metals). The procedure for their preparation varies depending on the PT and whether additional components need to blended in. Oils for contaminants analysis are spiked with small volumes of standards and mixed for 12 hours. Olive oils for quality parameters are not spiked (although BHT is added as an anti-oxidant) and therefore mixed only for one hour.

Having prepared the bulk material, this is then aliquoted into small volumes (typically 50 – 100 ml) and stored. Prior to the distribution of samples to participants in the PT, a homogeneity test is undertaken [1, 2] to establish that any between-sample homogeneity will not affect the outcome of the PT. This test for sufficient homogeneity does not test the within-sample homogeneity (but does test the analytical variance of the method). Importantly, the homogeneity test is carried under repeatability conditions, i.e. the analysis is completely in a single analytical batch with

randomisation of sample selection and analytical sequence. Normally, 10 samples are selected and analysed in duplicate.

This procedure for the preparation of PT materials will not be entirely suitable for the OLEUM project T4.2 and T4.3. The reasons are that some of the samples will be diluted by individual laboratories, the methods of analysis are being developed, the analytes of interest include also volatile compounds, a complete homogeneity test on each blend will be expensive and time consuming. Therefore, a more pragmatic approach needs to be adopted which will maintain sufficient statistical control.

An oil is a liquid and is therefore inherently homogeneous, but some sediments and particles in suspension may also occur. The blends being prepared will be at percentage levels (not trace levels) and the blends are produced with all oils of similar viscosity. It can therefore be assumed that controlled mixing of the blends will result in a sufficiently homogeneous material. An assessment of homogeneity can be made from the distribution of data received from each collaborator. There are precedents for this assumption [3, 4]. In the case of [3], it was demonstrated that a representative analysis was sufficient to establish homogeneity in aqueous liquid test materials (fruit juices and soft drinks). In the case of [4] the samples are drinking water which are taken to be standard solutions. Participants also receive spiking standards which they are instructed to mix (typically 100 µl) into one litre of water before carrying out their analysis. Although this is aqueous matrix, not lipidic, the analysis is at trace levels and this protocol has been adopted and applied successfully for many years.

3. Protocol for sample preparation and homogeneity assessment

3.1 Samples preparation and dispatch

Fera will receive *i)* sd-OOs and the respective defective OOs from ITERG and *ii)* EVOOs from UNIBO (T.4.2); moreover, Fera will receive *i)* vegetable oils from NESTEC and ITERG and *ii)* OO and EVOO from UNIBO (T.4.3). Before sending these samples to the partners, Fera will protect them from light and heat (a temperature of around 10-12°C is recommended, in accordance with ISO 5555:2001). Sample dispatch to the partner organisations will be managed by Fera PT group. This is routine work for the group and presents low risk. All the dispatched samples will be adequately coded, clearly indicating in the label the kind of sample and also the Task for which they will be used for. All the samples will be aliquoted by Fera and send to the involved partners into tins or amber bottles with limited headspace (almost completely filled). The materials of all the containers have to satisfy food contact requirement and guarantee no release of volatile compounds to avoid contaminations, since some samples may be also sensory evaluated (e.g. olfactory evaluation). Samples to be dispatched will include the blended sd-OOs with EVOOs, legal/illegal blends of OOs and EVOOs with other vegetable oils, as well as the raw materials (EVOOs, OOs, sd-OOS and related lampante defective OOs, vegetable oils) to ensure consistent and centralised distribution. Fera will dispatch all the samples to the OLEUM partners (see Annex for partner organisation receipt). Once delivered in each partner laboratory, all the samples shall be protected from light and heat (a temperature of around 10-12°C is recommended) and stored prior to analysis in appropriate containers that have to be almost completely filled: the air space (headspace) shall be minimal, since air has a detrimental action on most fats (gentle nitrogen sparging should be applied, if possible).

3.1.1 Sample dispatch in T.4.2

In M6, each partner involved in T.4.2 will receive from Fera one sample of each type of raw material (ten sd-OOs and respective defective OOs, two EVOOs) for preparing the needed blends in its own laboratory.

In M11, each partner involved in T.4.2 will receive from Fera one sample of each type of raw material (ten sd-OOs and respective defective OOs, two EVOOs) and also a set of blends prepared by FERA in percentages to be defined according to the results obtained on the samples of the first dispatch.

In M23, each partner involved in T.4.2 will receive from Fera one sample of each type of raw material (ten sd-OOs and respective defective OOs, two EVOOs, which will be the same oils sampled in M10) and also the same set of blends sent by Fera in M11, after storage from M11 to M23.

3.1.2 Sample dispatch in T.4.3

In M5, each partner involved in T.4.3 will receive from Fera one sample of each type of raw material (one EVOO, one OO and six vegetable oils, namely both virgin and refined hazelnut and avocado oils, one refined palmolein oil and one desterolized high oleic sunflower oil) for preparing the needed illegal blends in its own lab.

In M11, each partner involved in T.4.3 will receive from Fera one sample of each type of raw material (one EVOO, one OO and six vegetable oils, namely both virgin and refined hazelnut and avocado oils, one refined palmolein oil and one desterolized high oleic sunflower oil) and also a set of illegal blends prepared by Fera in percentages to be defined according to the results obtained on the samples of the first dispatch.

In M5 and M11, each partner involved in T.4.3 will receive from Fera one sample of each type of raw material (one EVOO, one OO and two vegetable oils, namely one sunflower and one high oleic sunflower oil) and legal blends prepared by Fera in previously established percentages.

3.2 Preparation of blends

Deviations from regular FAPAS OO preparations are that no BHT will be used and mixing time for preparing blends will be minimal (30 minutes). It is very important that, before preparing any blends, the operator will keep for at least six hours at room temperature the recipients in which the oils are stored and shake them vigorously before withdrawing the aliquots to prepare the blends.

Small volume blends, prepared within partner laboratories, can be achieved via magnetic stirrer in suitably sized glassware, preferably enclosed. If possible, first of all each partner has to add the internal standard to the blend in order to test homogeneity (see instructions in paragraph 3.3). The mixing needs to be done slowly over a period of 30 minutes at cool ambient temperatures and with a minimal air gap above the oil to reduce the effect of oxidation. The glassware needs to be tightly covered (e.g. with inert lab film); the use of amber glass bottle with screw cap is recommended to avoid loss of volatiles. Each partner will have the possibility to prepare blends at different percentages to test its own analytical method (the preparation of blends at least 3 different percentages is recommended); each blend has to be prepared in a total volume of 50 g, by using glassware with a capacity of 50 mL to avoid headspace. For blends prepared by each partner, the use of amber glassware can be avoided, provided that analysis is undertaken immediately following preparation. However, the use of amber glassware is highly recommended, if possible.

Larger volume blends undertaken at Fera will use an enclosed mixing vessel operated at low speed and cool ambient temperatures. The bulk material will be aliquoted into amber bottles (100 ml, plastic with characteristics reported in paragraph 3.1 with leak-proof lids. Nitrogen will be

applied to sparge the headspace. The lids will be attached immediately and secured tightly. Laboratories preparing their own preparations will not be expected to have mixing equipment on the scale that Fera has access to. Given that the difference in preparations will be one of volume only, smaller scale mixing can be assumed to be equivalent to larger scale mixing. The accuracy of differently scaled preparations will be reflected in the appropriateness of measuring glassware, pipettes and balances used by the different participant laboratories.

The exact percentages for the blends of EVOOs with sd-OOs and of the illegal blends of EVOO/OO with vegetable oils will be defined later, but are expected to be at levels consistent with economically-viable adulteration (i.e. percentage levels). In addition, blends with vegetable oils will be prepared by Fera at the legal levels (information to be supplied to Fera before M4).

3.3 Homogeneity assessment

The homogeneity data assessment includes assessment of formal homogeneity data on the blends prepared by Fera, as well as data generated by the partner labs for blends prepared internally.

The homogeneity assessment will be achieved by the use of an internal standard, tridecanoic acid methyl ester (ideal purity ≥ 99.5 %, preferably liquid at room temperature) added to each blend at a defined concentration (see below), before the mixing step. After mixing, each blend will be analysed for the internal standard by Fera (for the blends prepared by Fera) and, if possible, by each partner (for the blends prepared internally by each partner).

- *For blended oils prepared by the partner in their own laboratory*, the preparation effectively will be a single sample (not further aliquoted out). Therefore, within-sample homogeneity can be assumed and between-sample homogeneity is not applicable. The only relevant measure of precision remaining is that of analytical variance. The accuracy of the partner-prepared blends will also be taken into account in the comparison of data generated by each partner. No between-sample homogeneity assessment will be undertaken for these internally-blended preparations. To determine the analytical variance of these blended oils, if possible each partner can evaluate the concentration of the internal standard (tridecanoic acid methyl ester, added at 1 g/50 g of blends) for five replicate GC-FID analyses performed on the same blend (samples withdrawn at different depths of the recipient in which the blend is contained). In particular, 1.0 g of internal standard will be added by the partner to 50 g of the blend: in a first step, this aliquot of standard will be completely dissolved by the partner in a small aliquot of oil and then transferred in the recipient used for preparing the blend. After that, the blend will be prepared according to the chosen percentages, adequately mixed (see paragraph 3.1), and the fatty acid composition of the blends will be determined by GC-FID, according to the EEC Reg. 2568/1991 (and following amendments). The concentrations of the fatty acids (calculated respect to the internal standard) and the area of the peak related to the internal standard, assuming Krisp=1 for all the fatty acids, will be send by the partner to Fera to carry out the statistical assessment of variance and relative accuracy of the blends, also compared to the other partners. N.B. This is a voluntary and not compulsory test that each partner can perform - or not – before starting to analyse the blends by using its own analytical method.

- *For blended oils prepared by Fera*, representative homogeneity testing (i.e. between-sample) will be carried out by adding the internal standard to the blends (similarly as described above), according to an established GC-FID method. Since the object of the homogeneity testing is to establish between-sample heterogeneity, undertaking this exercise on the internal standard will be a sufficient measure. This analysis will be undertaken at Fera which is in routine use, although not within scope of Fera's ISO 17025 accreditation. The data analysis of the homogeneity data will be carried out at Fera before the shipment of the blended oils to the partners, using the established statistical process [1, 2].

3.4 Key risks

Stability – mitigation by use of appropriate sample containers and flushing with nitrogen. Courier shipment to partner laboratories with tracking and short delivery times (typically within 3 days within the EU). Fera has experience of handling and shipping these types of test materials over many years.

In addition, Fera would capture data on sample receipt by partners and the dates of analysis, to ensure timeliness of the experimental work.

Homogeneity – use of established homogeneity procedures and representative analyte.

4. References

[1] FAPAS Protocol for Proficiency Testing Schemes, Part 1 – Common Principles

[2] Thompson, Ellison, Wood, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, 2006

[3] FAPAS internal report, Tom Langford, Replacing Full Homogeneity Testing with a Representative Analysis in Liquid Test Materials, 2016

[4] FAPAS Water and Environmental Scheme, Protocol and Sample preparation instructions, www.fapas.com

5. Annex – Partner Organisations receipt

For T4.2, the partners receiving from Fera the sd-OOs, the related defective OOs, the EVOOs and the blends are: CSIC, ITERG, EUROFINS, UB, UNIBO.

For T4.3, the partners receiving from Fera the EVOOs, the olive oils, the vegetable oils, and the blends are: CONICET, CSIC, ITERG, EUROFINS, SMART ASSAYS, UB, UNIUD.