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**COMMISSION STAFF WORKING DOCUMENT**

*Accompanying the document*

**Proposal for a  
COUNCIL DECISION**

**on the position to be taken on behalf of the European Union within the Council of  
Members of the International Olive Council (IOC), as regards one method of analysis  
and the IOC Trade Standard for olive oil and olive-pomace oil**

{COM(2024) 416 final}

DRAFT DECISION No DEC III.X/119-VI/2024

CONCERNING THE METHOD FOR THE DETERMINATION OF  
THE CONTENT OF WAXES, FATTY ACID ETHYL ESTERS BY  
CAPILLARY GAS CHROMATOGRAPHY



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## **DRAFT DECISION N° DEC-III.X/119-VI/2024**

### **CONCERNING THE METHOD FOR THE DETERMINATION OF THE CONTENT OF WAXES, FATTY ACID ETHYL ESTERS BY CAPILLARY GAS CHROMATOGRAPHY**

#### **THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,**

**Having regard** to the International Agreement on Olive Oil and Table Olives, 2015, in particular Article 1 “Objectives of the Agreement” concerning standardisation and research, as regards achieving uniformity in national and international legislation, and the harmonisation of physico-chemical and organoleptic analysis, to improve knowledge of the composition and quality characteristics of olive products, and in particular Chapter VI “Standardisation provisions”;

**Having regard** to the recommendation made by the Chemistry and Standardisation Committee at its 14th meeting during the 119th session of the Council of Members;

**Considering** the ring tests carried out by the chemists on waxes and ethyl esters;

**Considering** the unanimous position of the chemistry experts appointed by Members at their meeting on 3 and 4 April 2024, and the recommendation to incorporate the modification into document COI/T.20/Doc. No 28/Rev. 4;

#### **DECIDES**

To adopt revision 4 of the document COI/T.20/Doc. No 28 on the method of analysis for the determination of the content of waxes and fatty acid ethyl esters by capillary gas chromatography.

Both methods A and B can be used for official control. Method A (15 g of silica) is the reference method for counter-assessment.

Document COI/T.20/Doc. No. 28/Rev. 4 June 2024 replaces and repeals document COI/T.20/Doc. No 28/Rev. 3 Corr 1 June 2024.

Madrid (Spain), 25 June 2024

Javad Mir Arabrazi  
Chair of the International Olive Council



## METHOD OF ANALYSIS

### DETERMINATION OF THE CONTENT OF WAXES AND FATTY ACID ETHYL ESTERS BY CAPILLARY GAS CHROMATOGRAPHY

#### METHOD A (15 g of silica)

##### 1. PURPOSE

This method is for the determination of the content of waxes and fatty acid ethyl esters in olive oils. The individual waxes and alkyl esters are separated according to the number of carbon atoms.

##### 2. SCOPE

The method is recommended as a tool for distinguishing between olive oil and olive-pomace oil, and as a quality parameter for extra virgin oils, as it facilitates the identification of false blends of extra virgin olive oils and low-quality oils and determines whether they are virgin, lampante or deodorized low quality oils that may have underwent a deodorization process. This document presents two methods that can be used both for official control. Method A (15 g of silica) is the reference method for counter assessment.

##### ~~2.~~ 3. PRINCIPLE

Addition of suitable internal standards to the oil and fractionation by chromatography on hydrated silica gel column. Recovery of the fraction eluted under the test conditions (with a lower polarity than that of the triacylglycerols) and direct analysis by capillary gas chromatography. Waxes and alkyl esters can be analysed using either the reference method of part A or the alternative method of part B. Both methods share the same principle, but the simplified method uses a reduced quantity of sample, reagents, and solvents. Method A is the reference method for counter assessment In case of disputes the results obtained using the reference method (part A) should prevail.

**PART A — REFERENCE METHOD METHOD A – 15 GRAMS OF SILICA**

**3. A4 -APPARATUS**

**3.1. A4.1.** Test tube, 10 ml.

**3.2. A4.2.** Glass column for liquid chromatography, internal diameter 15 mm, length 40 cm, fitted with a suitable stopcock.

**3.3. A4.3.** Gas chromatograph suitable for use with a capillary column, equipped with a system for direct, on-column injection comprising:

**3.3.1. A4.3.1** Thermostat-controlled oven with temperature programming.

**3.3.2. A4.3.2** Cold injector for direct on-column injection

**3.3.3. A4.3.3** Flame ionisation detector and converter-amplifier.

**3.3.4. A4.3.4** ~~Computerized systems recorder~~ Recorder-integrator (*Note*<sup>1</sup>) ~~for use with the converter-amplifier (3.3.3), with a response time of not more than 1 s and a variable paper speed.~~

**3.3.5. A4.3.5** Capillary column, fused silica (for analysis of the waxes and methyl and ethyl esters), length 8-12 m, internal diameter 0.25-0.32 mm, internally coated with liquid phase (*Note*<sup>2</sup>) to a uniform thickness of 0.10-0.25 µm.

**3.4. A4.4.** Microsyringe, 10 µl, ~~with hardened needle,~~ for ~~direct~~ on-column injection.

**3.5. A4.5.** Electric shaker.

**3.6. A4.6.** Rotary evaporator.

**3.7. A4.7.** Muffle oven.

**3.8. A4.8.** Analytical balance for weighing to an accuracy of ± 0.1 mg.

**3.9. A4.9.** Usual laboratory glassware.

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*Note*<sup>1</sup> ~~Computerised systems may also be used where the gas chromatography data are entered through a PC. also to use with the converter-amplifier (A4.3.3) a recorder with a response time of not more than 1 s and a variable paper speed can be used.~~

*Note*<sup>2</sup> Suitable commercial liquid phases are available for this purpose such as SE52, SE54 (methyl silicon with 5% phenyl), etc. or other phase with similar or lower polarity.

## **4. A5 -REAGENTS**

**4.1 A5.1.**      **Silica gel**, 60-200 µm mesh. Place the silica gel in the muffle oven at 500 °C for at least 4 hours. Allow to cool and then add 2% water in relation to the quantity of silica gel used. Shake well to homogenise slurry and keep in the desiccator for at least 12 hours prior to use. If the silica gel is ultra-pure grade, the muffle oven treatment will may not be necessary. In any case each batch of silica must be checked for suitability.

**4.2 A5.2.**      **n-Hexane**, chromatography (or residue) grade – the purity must be checked as follows: 100 ml of n-hexane are evaporated to dryness, residue is re-dissolved in 100 µl n-heptane, and analysed as solvent blank applying the same gas chromatographic conditions. There must be no peak in the elution alkyl esters area. (Hexane can be replaced by Isooctane) Hexane – Chromosolv Pestanal is available from Honeywell-Riedel-de Haen (code 34484).

This reference is an example of suitable products, which are available commercially. This information is given for the convenience of users of this Standard and does not constitute an endorsement of these products.

WARNING – Fumes may ignite. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid build-up of fumes and remove any possible fire risk, such as heaters or electric apparatus not manufactured from non-inflammable material. Harmful if inhaled because it may cause nerve cell damage. Avoid breathing in the fumes. Use a suitable respiratory apparatus if necessary. Avoid contact with eyes and skin.

**4.3 A5.3.**      **Ethyl ether**, chromatography grade.

WARNING – Highly inflammable and moderately toxic. Irritates the skin. Harmful if inhaled. May cause damage to eyes. Effects may be delayed. It can form explosive peroxides. Fumes may ignite. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid build-up of fumes and remove any possible fire risk, such as heaters or electric apparatus not manufactured from non-inflammable material. Do not evaporate to dryness or near dryness. The addition of water or an appropriate reducing agent can reduce peroxide formation. Do not drink. Avoid breathing in the fumes. Avoid prolonged or repeated contact with skin.

**4.4 A5.4.**      **n-hHeptane**, chromatography grade, or **iso-octane isooctane**

WARNING – *Inflammable*. Harmful if inhaled. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid breathing in the fumes. Avoid prolonged or repeated contact with skin.

**4.5 A5.5.**      **Standard solution of lauryl arachidate** (*Note* <sup>3</sup>) at 0.02% (m/V) in heptane (internal standard for waxes).

**4.6 A5.6.**      **Standard solution of methyl heptadecanoate** at 0.005% (m/V) in heptane (internal standard for methyl and ethyl esters).

**4.7 A5.7.**      **Sudan 1 (1-phenylazo-2-naphthol)** optional (attention: azo-compounds have mutagenic and carcinogenic properties)

**4.8 A5.8.**      **Carrier gas:** hydrogen or helium, pure, gas chromatography grade.

**WARNING**

**Hydrogen.** Highly inflammable, under pressure. Keep away from sources of heat, sparks, naked flames or electric apparatus not manufactured from non-inflammable material. Make sure the bottle valve is shut when not in use. Always use with a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not stand in front of the bottle outlet when opening the valve. Ensure proper ventilation during usage. Do not transfer hydrogen from one bottle to another. Do not mix gas in the bottle. Make sure the bottles cannot be knocked over. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles.

**WARNING**

**Helium.** Compressed gas at high pressure. It reduces the amount of oxygen available for breathing. Keep the bottle shut. Ensure proper ventilation during usage. Do not enter storage areas unless they are properly ventilated. Always use with a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not transfer gas from one bottle to another. Make sure the bottles cannot be knocked over. Do not stand in front of the bottle outlet when opening the valve. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles. Do not inhale. Use solely for technical purposes.

**4.9 A5.9.**      **Auxiliary gases:**

- Hydrogen, pure, gas chromatography grade.
- Air, pure, gas chromatography grade.

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*Note* <sup>3</sup> *Palmityl palmitate, myristyl stearate or arachidyl laureate may also be used.*

## WARNING

*Air.* Compressed gas at high pressure. Use with caution in the presence of combustible substances as the self-ignition temperature of most of the organic compounds in the air is considerably lower under high pressure. Make sure the bottle valve is shut when not in use. Always use a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not stand in front of the bottle outlet when opening the valve. Do not transfer gas from one bottle to another. Do not mix gas in the bottle. Make sure the bottles cannot be knocked over. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles. Air intended for technical purposes must not be used for inhaling or respiratory apparatus.

## **5. A6. PROCEDURE**

### **5.1. A6.1. Preparation of the ~~chromatography~~ chromatographic column**

Suspend 15 g of silica gel (A4.5.1) in n-hexane (A4.5.2) and introduce into the column (A3.4.2). Allow to settle spontaneously. Complete settling with the aid of an electric shaker (A3.4.5) to make the chromatographic bed more homogeneous. Percolate 20 ml of n-hexane to remove any impurities. Weigh exactly, about 500 mg of the sample into the 10-ml test tube (A3.4.1), using the analytical balance (A3.4.8), and add a suitable amount of internal standard (A4.5.5) depending on the assumed wax content, e.g. add 0.10 mg of lauryl arachidate in the case of extra virgin olive oil, virgin olive oil, refined olive oil and olive oil, 0.25-0.50 mg in the case of olive-pomace oil and 0.05 mg of methyl heptadecanoate for extra virgin olive oils (A4.5.6). Transfer the prepared sample to the chromatography column with the aid of two 2-ml portions of n-hexane (A4.5.2). Allow the solvent to flow to 1 mm above the upper level of the absorbent. Percolate a 50 ml n-hexane/ethyl ether (99:1) to further remove hydrocarbons (alkanes and sterenes). Then continue the chromatographic elution of n-hexane/ethyl ether (99:1) (*Note* <sup>4</sup>) and collect 150 ml at a flow of about 15 drops every 10 seconds. (**This fraction contains ethyl esters and waxes**) (*Note* <sup>5</sup>).

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*Note* <sup>4</sup> The n-hexane/ethyl ether (99:1) mixture should be freshly prepared every day, n-hexane can be replaced with the same amount of ~~iso octane~~ isooctane

*Note* <sup>5</sup> ~~100 µl of Sudan I dye at 1% in the elution mixture can be added to the sample solution to check visually that the waxes are eluted properly. The retention time of the dye lies in between that of the waxes and triacylglycerols. Hence, when the dye reaches the bottom of the chromatography column, elution must be suspended because all the waxes have been eluted. Verify the correct elution by checking the presence on the chromatogram at the same time of squalene and epoxy squalene.~~

The elution volumes indicated in the text are those which usually are employed. However, if needed, they can be modified to achieve a satisfactory separation of the fractions. For this purpose, 100 µl of Sudan I dye at 1% in the elution mixture can be added to the sample solution to check visually that the waxes are eluted properly. The retention volume of the dye lies in between that of the waxes and triacylglycerols. Hence, when the dye reaches the bottom of the chromatography column, elution must be suspended because all the waxes have been eluted. Verify the correct elution by checking the presence on the chromatogram at the same time of squalene and epoxy squalene.



Evaporate the resultant fractions in a rotary evaporator (A3.4.6) until the solvent is almost removed. Remove the last 2 ml under a weak current of nitrogen. Collect the fraction containing the ethyl esters and waxes diluted with 1-2 ml of n-heptane or iso-octane.

## **5.2. A6.2. Gas chromatography analysis**

### **5.2.1. A6.2.1. Preliminary procedure**

~~Fit the column to the gas chromatograph (3.3), connecting the inlet port to the on-column system and the outlet port to the detector.~~ Check the gas chromatography apparatus (operation of gas loops, efficiency of detector and recorder system, etc.).

If the column is being used for the first time, it is advisable to condition it. Run a light flow of gas through the column, then switch on the gas chromatography apparatus. Gradually heat to 350 °C (approximately 4 hours).

Maintain this temperature for at least 2 hours, then regulate the apparatus to the operating conditions (regulate gas flow, light flame, connect to electronic recorder (3-A.4.3.4), regulate oven temperature for column, regulate detector, etc.). Record the signal at a sensitivity at least twice as high as required for the analysis. The base line should be linear, with no peaks of any kind, and must not have any drift.

Negative straight-line drift indicates that the column connections are not correct while positive drift indicates that the column has not been properly conditioned.

### **5.2.2. A6.2.2. Choice of operating conditions for waxes and ethyl esters (Note <sup>6</sup>)**

The operating conditions are generally as follows:

- Column temperature:

20°C/min      5°C/min

80°C at first (1') → 140°C → 335°C (20') for ethyl esters and waxes

20°C/min      5°C/min      20°C/min

80°C at first (1') → 240°C → 325°C (6') → 340°C (10') for waxes only

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*Note <sup>6</sup>. Due to the high final temperature, positive drift is allowed but may not exceed more than 10% of the full-scale value.*

- Detector temperature: 350°C.
- Amount injected: 1 µl of n-heptane solution (1-2ml).
- Carrier gas: helium or hydrogen at the optimal linear speed for the gas chosen (see Annex A B).
- Instrument sensitivity: ~~suitable for fulfilling the above conditions (a detection limit of about 1 mg/Kg for each identified peak recommended)~~ suitable for the quantification of the analytes.

These conditions may be modified to suit the characteristics of the column and the gas chromatograph ~~in order~~ to separate all the waxes and fatty acid ethyl esters and to obtain satisfactory peak separation (see Figures 1 ~~and 2~~) ~~and a retention time of  $18 \pm 3$  minutes for the lauryl arachidate internal standard. The most representative peak of the waxes must be over 60% of the full scale value in the case of refined olive oil, olive oil, olive pomace oils, while it should be between 60% and full scale in the case of extra virgin olive oil and virgin olive oil. The methyl heptadecanoate internal standard for the ethyl esters must fit the full-scale value.~~

### **5.3. A6.3. Performance of the analysis**

Take up 1-2 µl of the solution with the aid of the 10 µl micro-syringe, drawing back the plunger until the needle is empty. Introduce the needle into the injection system and inject quickly after 1–2 s. After about 5 s, gently extract the needle.

Perform the recording until the waxes (C40-C46) are completely eluted, ~~depending on the fraction being analysed.~~

The base line must always meet the required conditions.

### **5.4. A6.4. Peak identification**

Identify the peaks from the retention times by comparing them with mixtures of waxes with known retention times, analysed under the same conditions. The alkyl esters are identified from mixtures of methyl and ethyl esters of the main fatty acids in olive oils (palmitic and oleic).

Figure 1. (Annex A) ~~S~~shows a chromatogram of the FAEE and waxes in an extra virgin olive oil using the method A (15 g).

Figure 2. (Annex A) ~~S~~shows a chromatogram of the FAEE and waxes in a lampante olive oil using the method A (15 g).

#### **5.5. A6.5. Quantitative analysis of the waxes**

Determine the area of the peaks corresponding to the lauryl arachidate internal standard and the aliphatic esters from C42 to C46 in the case of extra virgin olive oil and virgin olive oil and from C40 to C46 in the case of other oils, with the aid of the integrator.

Determine the content of each individual wax, in mg/kg of fat, as follows:

$$Waxes, mg/kg = \frac{A_x * m_s * 1000}{A_s * m}$$

where:

- $A_x$  = area corresponding to the peak for the individual ester, in computer counts (~~peak no. 11-13, 14-15, 16-17-18 in fig. 2~~ sum of peaks: C42, C44, C46 for EVOO and VOO; sum of peaks: C40, C42, C44 and C46 for the other oil)
- $A_s$  = area corresponding to the peak for the lauryl arachidate internal standard, in computer counts
- $m_s$  = mass of the lauryl arachidate internal standard added, in milligrams
- $m$  = mass of the sample taken for determination, in grams

#### **5.6. A6.6. Quantitative analysis of the ethyl esters**

Using the integrator, determine the areas of the peaks corresponding to the methyl heptadecanoate internal standard, the ethyl esters of the C16 and C18 fatty acids.

Determine the content of ethyl ester, in mg/kg of fat, as follows:

$$Ester, mg/kg = \frac{A_x * m_s * 1000}{A_s * m}$$

where:

- $A_x$  = area corresponding to all the peak for the individual C16 and C18 ethyl ester, in computer counts (~~sum of EtC16, EtC18:2, EtC18:1, EtC18~~).
- $A_s$  = area corresponding to the peak for the methyl heptadecanoate internal standard, in computer counts
- $m_s$  = mass of the methyl heptadecanoate internal standard added, in milligrams;
- $m$  = mass of the sample taken for determination, in grams.

## **6. A7.      EXPRESSION OF RESULTS**

~~Report the sum of the contents of the different waxes from C40 to C46 in milligrams per kilograms of fat for Ordinary Olive oil (OOO), Lampante olive oil (LOO), Refined olive oil (ROO), Olive oil (ROO+VOOs), Crude olive pomace oil (COPO), Refined olive pomace oil and Olive pomace oil (ROPO+VOOs). Report the sum of the contents of the different waxes from C42 to C46 (Note <sup>7</sup>) in the case of extra virgin and virgin olive oils and from C40 to C46 in the case of other oils (Note 10) in milligrams per kilograms of fat (ppm) in milligrams per kilograms of fat for Extra virgin Olive oil (EVOO) and Virgin Olive oil (VOO). Report the sum of the content of C40 to C46 in the case of the other categories of olive or olive pomace oils.~~

Report the sum of the contents of the ethyl esters from C16 to C18 and the total of the two.

Results should be expressed to the nearest mg/kg.

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*Note <sup>7</sup>. The components for quantification refer to the peaks with even carbon numbers amongst the C40-C46 esters, according to the specimen chromatogram of the waxes in olive oil provided in the attached figure. Peaks identification of the individual waxes, especially if C46 peak is splitted, can be made by comparing the retention time of the wax fraction of an olive-pomace oil.*

## **METHOD B (3 g GRAMS OF SILICA)**

### **1. PURPOSE**

~~This method is for the determination of the content of waxes and fatty acid ethyl esters in olive oils. The individual waxes and alkyl esters are separated according to the number of carbon atoms. The method is recommended as a tool for distinguishing between olive oil and olive pomace oil and as a quality parameter for extra virgin oils, as it facilitates the identification of false blends of extra virgin olive oils and low-quality oils and determines whether they are virgin, lampante or deodorized oils.~~

### **2. PRINCIPLE**

~~Addition of suitable internal standards to the oil and fractionation by chromatography on a hydrated silica gel column. Recovery of the fraction eluted under the test conditions (with a lower polarity than that of the triacylglycerols) and direct analysis by capillary gas chromatography.~~

### **3. B4. APPARATUS**

**3.1. B4.1.** Test tube, 10 ml.

**3.2. B4.2.** Glass column for liquid chromatography, internal diameter 10 mm, length 40 cm, fitted with a suitable stopcock.

**3.3. B4.3.** Gas chromatograph suitable for use with a capillary column, equipped with a system for direct, on-column injection comprising:

**3.3.1. B4.3.1.** Thermostat-controlled oven with temperature programming.

**3.3.2. B4.3.2.** Cold injector for direct on-column injection

**3.3.3. B4.3.3.** Flame ionisation detector and converter-amplifier

**3.3.4. B4.3.4.** ~~Recorder~~ **Computerized systems recorder**-integrator (*Note <sup>1 8</sup>*) for use with the converter-amplifier (3.3.3), with a response time of not more than 1 s and a variable paper speed.

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*Note <sup>1 8</sup>. ~~Computerised systems may also be used where the gas chromatography data are entered through a PC; also to use with the converter-amplifier (A4.3.3) a recorder with a response time of not more than 1 s and a variable paper speed can be used.~~*

**3.3.5. B4.3.5. Capillary column**, fused silica (for analysis of the waxes and methyl and ethyl esters), length 8-12 m, internal diameter 0.25-0.32 mm, internally coated with liquid phase (*Note <sup>2 2</sup>*) to a uniform thickness of 0.10-0.25  $\mu\text{m}$ .

**3.4. B4.4. Microsyringe**, 10  $\mu\text{l}$ , ~~with hardened needle~~, for direct on-column injection.

**3.5. B4.5. Electric shaker**

**3.6. B4.6. Rotary evaporator**

**3.7. B4.7. Muffle oven**

**3.8. B4.8. Analytical balance** for weighing to an accuracy of  $\pm 0.1$  mg.

**3.9. B4.9. Usual laboratory glassware**

#### **4. B5. REAGENTS**

**4.1. B5.1. Silica gel**, 60-200  $\mu\text{m}$  mesh. Place the silica gel in the muffle oven at 500°C for at least 4 hours. Allow to cool and then add 2% water in relation to the quantity of silica gel used. Shake well to homogenise slurry and keep in the desiccator for at least 12 hours prior to use. If the silica gel is ultra-pure grade, the muffle oven treatment ~~will~~ may not be necessary. In any case each batch of silica must be checked for suitability.

**4.2. B5.2. n-Hexane**, chromatography (or residue) grade the purity must be checked as follows 200 100 ml of n-hexane are evaporated to dryness, residue is ~~redissolved~~ re-dissolved in 100  $\mu\text{l}$  n-heptane and analysed as solvent blank applying the same gas chromatographic conditions. There must be no peak in the elution alkyl esters area. (Hexane can be replaced by Isooctane) Hexane – Chromosolv Pestanal is available from Honeyell-Riedel-de Haen (code 34484).

This reference is an example of suitable products which are available commercially. This information is given for the convenience of users of this Standard and does not constitute an endorsement of these products.

WARNING – Fumes may ignite. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid build-up of fumes and remove any possible fire risk, such as heaters or electric apparatus not manufactured from non-inflammable material. Harmful if inhaled because it may cause nerve cell damage. Avoid breathing in the fumes. Use a suitable respiratory apparatus if necessary. Avoid contact with eyes and skin.

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*Note <sup>2 2</sup>. Suitable commercial liquid phases are available for this purpose such as SE52, SE54 (methyl silicon with 5% phenyl), etc. or other phase with similar or lower polarity.*

**4.3. B5.3. Ethyl ether**, chromatography grade.

WARNING – Highly inflammable and moderately toxic. Irritates the skin. Harmful if inhaled. May cause damage to eyes. Effects may be delayed. It can form explosive peroxides. Fumes may ignite. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid build-up of fumes and remove any possible fire risk, such as heaters or electric apparatus not manufactured from non-inflammable material. Do not evaporate to dryness or near dryness. The addition of water or an appropriate reducing agent can reduce peroxide formation. Do not drink. Avoid breathing in the fumes. Avoid prolonged or repeated contact with skin.

**4.4. B5.4. n-~~h~~Heptane**, chromatography grade, or **iso-octane isooctane**.

WARNING – Inflammable. Harmful if inhaled. Keep away from sources of heat, sparks or naked flames. Make sure the bottles are always properly closed. Ensure proper ventilation during usage. Avoid breathing in the fumes. Avoid prolonged or repeated contact with skin.

**4.5. B5.5. Standard solution of lauryl arachidate** (*Note<sup>3 10</sup>*), at ~~0.01%~~ 0.002% (m/V) in heptane (internal standard for waxes).

**4.6. B5.6. Solution of methyl heptadecanoate**, at ~~0.002%~~ 0.0005% (m/V) in heptane (internal standard for methyl and ethyl esters).

**4.7. B5.7. Sudan 1 (1-phenylazo-2-naphthol)** optional (attention: azo-compounds have mutagenic and carcinogenic properties)

**4.8. B5.8. Carrier gas**: hydrogen or helium, pure, gas chromatography grade.

WARNING

Hydrogen. Highly inflammable under pressure. Keep away from sources of heat, sparks, naked flames or electric apparatus not manufactured from non-inflammable material. Make sure the bottle valve is shut when not in use. Always use with a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not stand in front of the bottle outlet when opening the valve. Ensure proper ventilation during usage. Do not transfer hydrogen from one bottle to another. Do not mix gas in the bottle. Make sure the bottles cannot be knocked over. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles.

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*Note <sup>3 10</sup> Palmityl palmitate, myristyl stearate or arachidyl laureate may also be used.*

WARNING - *Helium*. Compressed gas at high pressure. It reduces the amount of oxygen available for breathing. Keep the bottle shut. Ensure proper ventilation during usage. Do not enter storage areas unless they are properly ventilated. Always use with a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not transfer gas from one bottle to another. Make sure the bottles cannot be knocked over. Do not stand in front of the bottle outlet when opening the valve. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles. Do not inhale. Use solely for technical purposes.

**4.9. B5.9. Auxiliary gases:**

- Hydrogen, pure, gas chromatography grade.
- Air, pure, gas chromatography grade.

**WARNING**

*Air*: Compressed gas at high pressure. Use with caution in the presence of combustible substances as the self-ignition temperature of most of the organic compounds in the air is considerably lower under high pressure. Make sure the bottle valve is shut when not in use. Always use a pressure reducer. Release the tension of the reducer spring before opening the bottle valve. Do not stand in front of the bottle outlet when opening the valve. Do not transfer gas from one bottle to another. Do not mix gas in the bottle. Make sure the bottles cannot be knocked over. Keep them away from sunlight and sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles. Air intended for technical purposes must not be used for inhaling or respiratory apparatus.

**5. B6. PROCEDURE**

**5.1. B6.1. Preparation of the ~~chromatography~~ chromatographic column**

Suspend 3 g of silica gel (4 B5.1) in n-hexane (4 B5.2) and introduce into the column (3 B4.2). Allow to settle spontaneously. Complete settling with the aid of an electric shaker (3 B4.5) to make the chromatographic bed more homogeneous. Percolate 10 ml of n-hexane to remove any impurities. Weigh exactly, about 100 mg of the sample into the 10-ml test tube (3 B4.1), using the analytical balance (3 B4.8), and add a suitable amount of internal standard (4 B5.5) depending on the assumed wax content; e.g., add ~~0.01~~ 0.02 mg (1 ml) of lauryl arachidate in the case of ~~extra virgin~~ olive oil, ~~virgin olive oil, refined olive oil and olive oil~~, 0.025 0.05-0.10 mg in the case of olive-pomace oil and ~~0.002~~ 0.005 mg (1 ml) of methyl heptadecanoate ~~for extra virgin olive oil and olive oil (4-6)~~. Transfer the prepared sample to the chromatography column with the aid of two ~~2~~-1-ml portions of n-hexane (4 B5.2).



Allow the solvent to flow to 1 mm above the upper level of the absorbent. Percolate a 12-15 ml n-Hexane(\*) to further remove hydrocarbons (alkanes and sterenes). Then start the chromatographic elution of n-hexane/ethyl ether (99:1) (~~Note<sup>4</sup>~~) and collect 40-45 ml at a flow of about 15 drops every 10 seconds.

**This fraction contains the ethyl esters and waxes (~~Note<sup>5</sup>~~ <sup>8</sup> and ~~Note<sup>9</sup>~~).**

(\*) Not necessary for the determination of waxes only.

Evaporate the resultant fractions in a rotary evaporator (~~3~~ B4.6) until the solvent is almost removed. Remove the last 2 ml under a weak current of nitrogen. Collect the fraction containing the methyl and ethyl esters diluted with 0.5-1 ml of n-heptane or ~~iso-octane~~ isooctane.

## **5.2. B6.2. Gas chromatography analysis**

### **5.2.1. B6.2.1. Preliminary procedure**

Fit the column to the gas chromatograph (~~3~~ B4.3), connecting the inlet port to the on-column system and the outlet port to the detector. Check the gas chromatography apparatus (operation of gas loops, efficiency of detector and recorder system, etc.).

If the column is being used for the first time, it is advisable to condition it. Run a light flow of gas through the column, then switch on the gas chromatography apparatus. Gradually heat to 350 °C (approximately 4 hours).

Maintain this temperature for at least 2 hours, then regulate the apparatus to the operating conditions (regulate gas flow, light flame, connect to electronic recorder (3.3.4), regulate oven temperature for column, regulate detector, etc.). Record the signal at a sensitivity at least twice as high as ~~that~~ required for the analysis. The base line should be linear, with no peaks of any kind, and must not have any drift. Negative straight-line drift indicates that the column connections are not correct while positive drift indicates that the column has not been properly conditioned.

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*Note<sup>4</sup><sup>11</sup>. The n-hexane/ethyl ether (99:1) mixture should be freshly prepared every day, n-hexane can be replaced with the same amount of ~~iso-octane~~ isooctane.*

*~~Note<sup>5</sup>. 100 µl of Sudan I dye at 1% in the elution mixture can be added to the sample solution to check visually that the waxes are eluted properly. The retention time of the dye lies in between that of the waxes and triacylglycerols. Hence, when the dye reaches the bottom of the chromatography column, elution must be suspended because all the waxes have been eluted.~~*

*Note<sup>12</sup>. The elution volumes indicated in the text are those which usually are employed. However, if needed, they can be modified to achieve a satisfactory separation of the fractions. For this purpose, 100 µl of Sudan I dye at 1% in the elution mixture can be added to the sample solution to check visually that the waxes are eluted properly. The retention volume of the dye lies in between that of the waxes and triacylglycerols. Hence, when the dye reaches the bottom of the chromatography column, elution must be suspended because all the waxes have been eluted. Verify the correct elution by checking the presence on the chromatogram at the same time of squalene and epoxy squalene.*

**5.2.2. B6.2.2. Choice of operating conditions for waxes and ethyl esters (Note <sup>6 13</sup>)**

The operating conditions are generally as follows:

- Column temperature:

20°C/min      5°C/min  
80°C at first (1') → 140 °C → 335°C (20') for ethyl esters and waxes

20°C/min      5°C/min  
80°C at first (1') → 200°C → 335°C (20') for waxes only

- Detector temperature: 350°C.
- Amount injected: 1 µl of n-heptane solution (0.5-1ml).
- Carrier gas: helium or hydrogen at the optimal linear speed for the gas chosen (see Annex A B).
- Instrument sensitivity: ~~suitable for fulfilling the above conditions (a detection limit of about 1 mg/Kg for each identified peak recommended)~~ suitable for the quantification of the analytes.

These conditions may be modified to suit the characteristics of the column and the gas chromatograph in order to separate all the waxes and fatty acid ethyl esters and to obtain satisfactory peak separation (see Figures 1-2) ~~and a retention time of  $18 \pm 3$  minutes for the lauryl arachidate internal standard.~~

~~The most representative peak of the waxes must be over 60% of the full scale value in the case of refined olive oil, olive oil, olive pomace oils, while it should be between 60% and full scale in the case of extra virgin olive oil and virgin olive oil. The methyl heptadecanoate internal standard for the ethyl esters must fit the full scale value.~~

- ~~— Carrier gas: helium or hydrogen at the optimal linear speed for the gas chosen (see Annex A).~~
- ~~Instrument sensitivity: suitable for fulfilling the above conditions.~~

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Note <sup>6 13</sup> Due to the high final temperature, positive drift is allowed but may not exceed more than 10% of the full-scale value.

### **5.3. B6.3. Performance of the analysis**

Take up 1-2 µl of the solution with the aid of the 10 µl micro-syringe, drawing back the plunger until the needle is empty. Introduce the needle into the injection system and inject quickly after 1–2 s. After about 5 s, gently extract the needle

Perform the recording until the waxes (C40-C46) are completely eluted, ~~depending on the fraction being analysed.~~

The base line must always meet the required conditions.

### **5.4. B6.4. Peak identification**

Identify the peaks from the retention times by comparing them with mixtures of waxes with known retention times, analysed under the same conditions. The alkyl esters are identified from mixtures of methyl and ethyl esters of the chief main fatty acids in olive oils (palmitic and oleic).

Annex A reports some examples of chromatograms of ethyl esters and waxes suitable to identify related peaks.

Figure 3. Shows a chromatogram of the FAEE and waxes in an extra virgin olive oil using the method B (3 g).

Figure 4. Shows the chromatograms of FAEE and waxes in a virgin olive oil using the method B (3 g).

### **5.5. B6.5. Quantitative analysis of the waxes**

Determine the area of the peaks corresponding to the lauryl arachidate internal standard and the aliphatic esters from C42 to C46 in the case of extra virgin olive oil and virgin olive oils and from C40 to C46 ~~for in the case of~~ other oils, with the aid of the integrator.

Determine the content of each individual wax, in mg/kg of fat, as follows:

$$Waxes, mg/kg = \frac{A_x * m_s * 1000}{A_s * m}$$

where:

$A_x$  = area corresponding to the peak for the individual ester, in computer counts (~~peak n° 11–13, 14–15, 16–17–18 in fig.2~~ sum of peaks: C42, C44, C46 for EVOO and VOO; sum of peaks: C40, C42, C44 and C46 for the other oil)

$A_s$  = area corresponding to the peak for the lauryl arachidate internal standard, in computer counts

$m_s$  = mass of the lauryl arachidate internal standard added, in milligrams

$m$  = mass of the sample taken for determination, in grams.

#### **5.6. B6.6. Quantitative analysis of the ethyl esters**

~~With the aid of~~ Using the integrator, determine the areas of the peaks corresponding to the methyl heptadecanoate internal standard, the ethyl esters of the C16 and C18 fatty acids.

Determine the content of ethyl ester, in mg/kg of fat, as follows:

$$Ester, mg/kg = \frac{A_x * m_s * 1000}{A_s * m}$$

where:

$A_x$  = area corresponding to the peak for all the individual C16 and C18 ethyl ester, in computer counts (~~sum of EtC16, EtC18:2, EtC18:1, EtC18~~).

$A_s$  = area corresponding to the peak for the methyl heptadecanoate internal standard, in computer counts.

$m_s$  = mass of the methyl heptadecanoate internal standard added, in milligrams.

$m$  = mass of the sample taken for determination, in grams.

#### **6. B7. EXPRESSION OF RESULTS**

Report the sum of the contents of the different waxes from C42 to C46 in the case of extra virgin olive oil and virgin olive oil and from C40 to C46 in the case of other categories of olive or olive pomace oils (Note 7) in milligrams per kilograms of fat (Note 14).

Report the sum of the contents of the ethyl esters from C16 to C18 and the total of the two.

Results should be expressed to the nearest mg/kg.

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*Note <sup>7,14</sup> The components for quantification refer to the peaks with even carbon numbers amongst the C40-C46 esters, according to the specimen chromatogram of the waxes in olive oil provided in the attached figure. Peaks identification of the individual waxes, especially if C46 peak is splitted, can be made by comparing the retention time of the wax fraction of an olive-pomace oil.*

## ANNEX A

### Examples of chromatograms:

The following chromatograms are reported as an aid to identify peaks as well as to give information about the separation to be obtained.

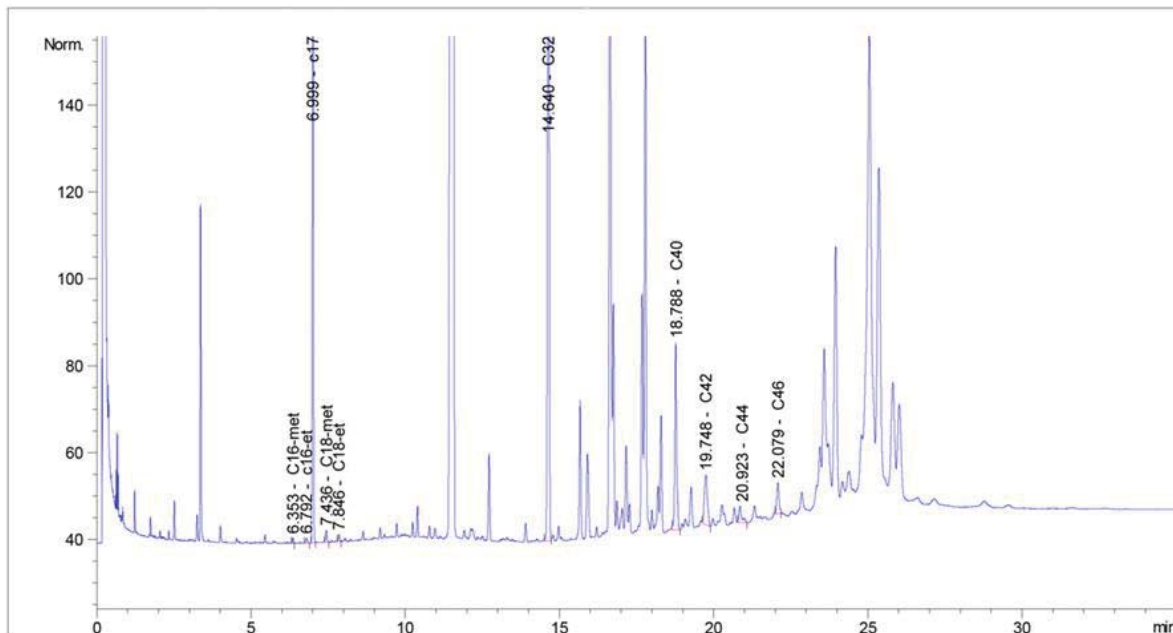


Figure 1: Chromatogram of FAEE and waxes of EVOO using method A (15 g).

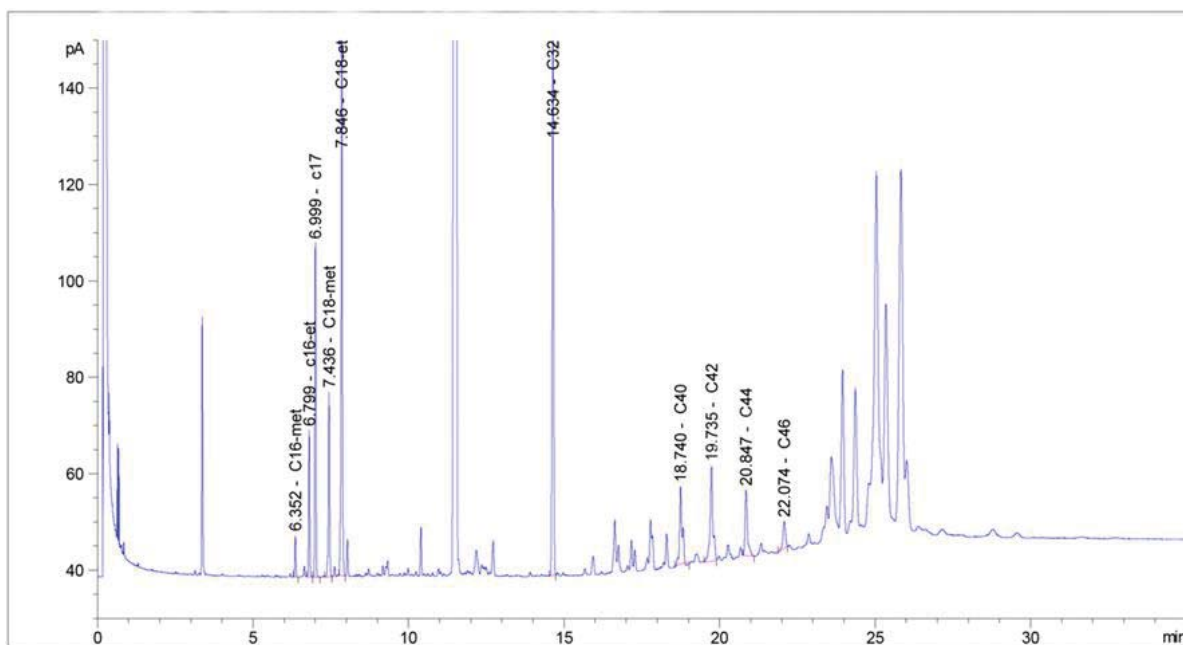


Figure 2: Chromatogram of FAEE and waxes of Lampante olive oil using method A (15 g).

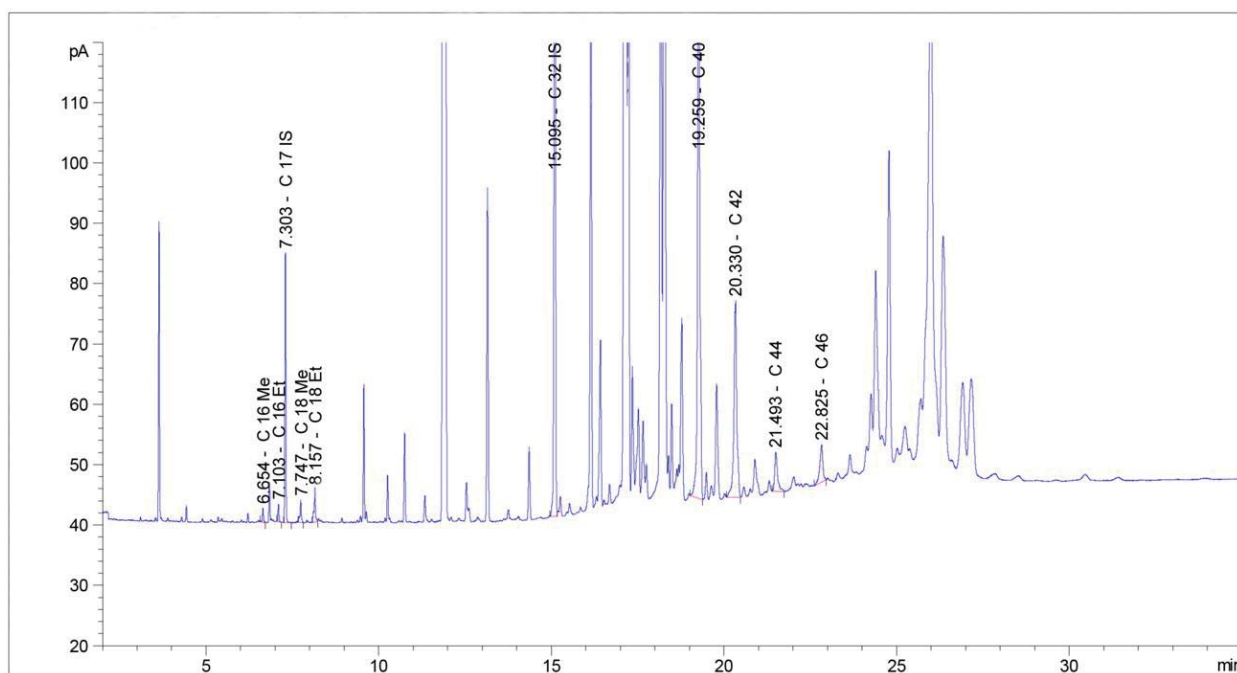


Figure 3: Chromatogram of FAEE and waxes of EVOO using method B (3 g).

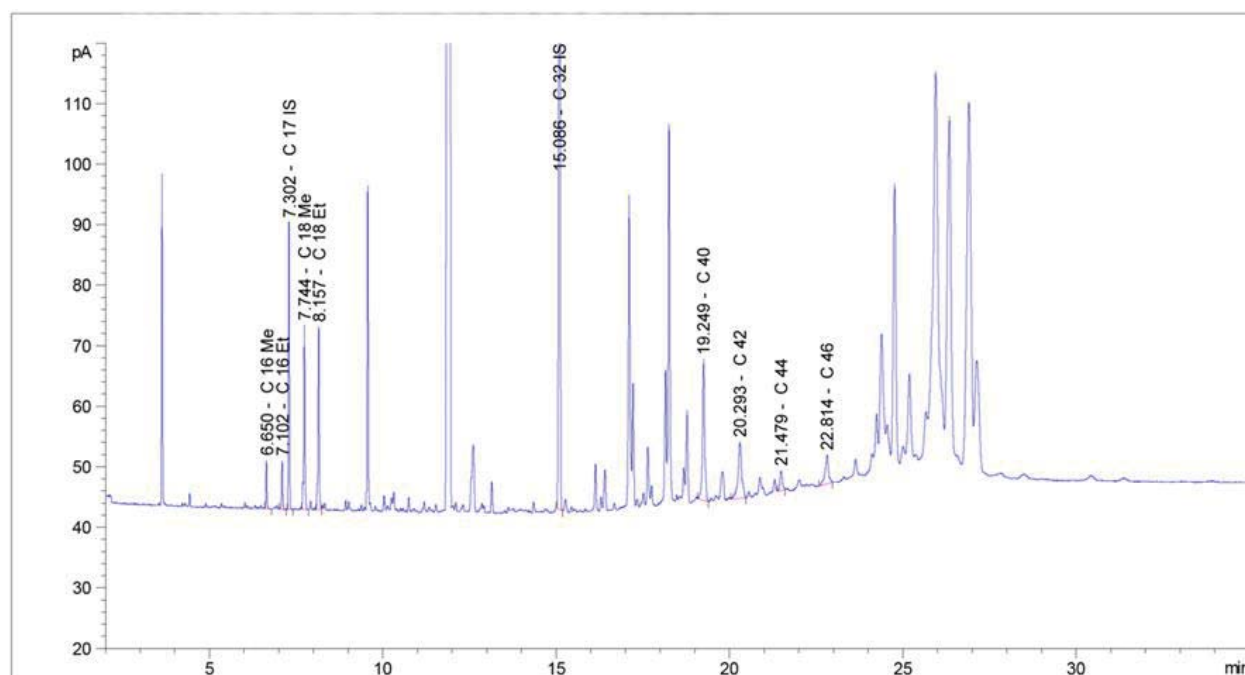


Figure 4: Chromatogram of FAEE and waxes of VOO using method B (3 g).

## **ANNEX B**

### **Determination of linear gas speed**

Inject 1:3 µl of methane (or propane) into the gas chromatograph after adjusting it to the normal operating conditions. Measure the time the gas takes to run through the column from the moment it is injected until the peak emerges ( $t_M$ ).

The linear speed in cm/s is given by  $L/t_M$  where  $L$  is the length of the column, in cm, and  $t_M$  is the time measured in s.

## **ANNEX C**

### **Precision values of the ethyl esters and wax method**

#### **Analysis of the collaborative test results**

The results of the collaborative test organised by the IOC Executive Secretariat were statistically processed according to the rules laid down in the international standards ISO 5725.

#### **~~Accuracy (trueness and precision) of measurement methods and results.~~**

The test was performed on five samples.

M1: EVOO to determine FAEE and waxes.

M2: EVOO to determine FAEE and waxes.

M3: VOO to determine FAEE and waxes.

M4: LOO to determine FAEE and waxes.

M5: OPO to determine waxes.

Outliers were examined by applying Cochran's and Grubbs's test to the laboratory results for each determination (replicates a and b).

The precision values of the method are given in the table overleaf.

The table lists:

<b>n</b>	number of participating laboratories
<b>outliers</b>	number of laboratories with outlying values
<b>mean</b>	mean of the accepted results
<b>r</b>	value below which the absolute difference between two single independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time may be expected to lie with a probability of 95%
<b>S<sub>r</sub></b>	repeatability standard deviation.

- RSD<sub>r</sub> (%)**      repeatability coefficient of variation ( $S_r \times 100 / \text{mean}$ )
- R**                      value below which the absolute difference between two single test results obtained with the same method on identical test material in different laboratories with different operators using different equipment may be expected to lie with a probability of 95%.
- S<sub>r</sub>**                      reproducibility standard deviation
- RSD<sub>R</sub> (%)**      reproducibility coefficient of variation ( $S_r \times 100 / \text{mean}$ )

Ethyl esters (mg/kg) – Method A 15 g of silica				
Sample	M1	M2	M3	M4
Mean	10.00	8.21	36.97	50.83
n	16	16	16	16
outliers	3	2	0	0
S <sub>r</sub>	0.574	0.330	1.316	1.934
RSD <sub>r</sub> (%)	5.74	4.02	3.56	3.80
r	1.61	0.92	3.68	5.41
S <sub>R</sub>	0.759	0.915	3.720	6.736
RSD <sub>R</sub> (%)	7.59	11.15	10.06	13.25
R	2.12	2.56	10.42	18.86

<del>Waxes (mg/kg) Method A 15 g of silica</del>					
Sample	<del>M1</del>	<del>M2</del>	<del>M3</del>	<del>M4</del>	<del>M5</del>
Mean	93.00	45.17	38.54	323.17	2350.72
n	16	16	16	16	16
outliers	4	1	2	1	0
S <sub>r</sub>	0.610	1.406	1.315	4.346	35.728
RSD <sub>r</sub> (%)	0.66	3.11	3.41	1.34	1.52
r	1.71	3.94	3.68	12.17	100.04
S <sub>R</sub>	8.053	4.879	5.590	23.649	247.180
RSD <sub>R</sub> (%)	8.66	10.80	14.51	7.32	10.52
R	22.55	13.66	15.65	66.22	692.10



Ethyl esters (mg/kg) -Method B (3 g of silica)				
Sample	M1	M2	M3	M4
Mean	10.00	8.15	39.22	52.46
<i>n</i>	15	15	15	15
Outliers	4	4	0	0
S <sub>r</sub>	0.570	0.225	1.234	1.903
RSD <sub>r</sub> (%)	5.90	2.76	3.15	3.63
<i>r</i>	1.65	0.63	3.46	5.33
S <sub>R</sub>	1.681	0.991	4.450	4.691
RSD <sub>R</sub> (%)	14.00	12.16	11.34	8.94
R	3.92	2.77	12.46	13.13

For waxes determination, for both methods A and B:

M1, M2 and M3 (sum of waxes from C42 to C46); M4 and M5 (sum of waxes from C40 to C46)

Waxes (mg/kg) Method A 15 g of silica					
Sample	M1 <sub>*</sub>	M2 <sub>*</sub>	M3 <sub>*</sub>	M4 <sub>**</sub>	M5 <sub>**</sub>
Mean	93.00	45.17	38.54	323.17	2350.72
<i>n</i>	16	16	16	16	16
outliers	4	1	2	1	0
S <sub>r</sub>	0.610	1.406	1.315	4.346	35.728
RSD <sub>r</sub> (%)	0.66	3.11	3.41	1.34	1.52
<i>r</i>	1.71	3.94	3.68	12.17	100.04
S <sub>R</sub>	8.053	4.879	5.590	23.649	247.180
RSD <sub>R</sub> (%)	8.66	10.80	14.51	7.32	10.52
R	22.55	13.66	15.65	66.22	692.10

\* Sum C42-C46; \*\* Sum C40-C46

<b>Waxes (mg/kg) Method B (3 g of silica)</b>					
<b>Sample</b>	<b>M1*</b>	<b>M2*</b>	<b>M3*</b>	<b>M4**</b>	<b>M5**</b>
<b>Mean</b>	90.45	45.53	38.81	325.47	2354.21
<b>n</b>	15	15	15	15	15
<b>Outliers</b>	2	1	2	0	0
<b>S<sub>r</sub></b>	1.770	2.306	1.745	7.758	50.548
<b>RSD<sub>r</sub> (%)</b>	1.96	5.06	4.50	2.38	2.15
<b>r</b>	4.96	6.46	4.89	21.72	141.54
<b>S<sub>R</sub></b>	15.287	7.066	8.236	23.614	213.990
<b>RSD<sub>R</sub> (%)</b>	16.90	15.52	21.22	7.26	9.09
<b>R</b>	42.80	19.79	23.06	66.12	599.17

\* Sum C42-C46; \*\* Sum C40-C46

## **ANNEX D**

### **References**

ISO 5725-1:1994 Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions

ISO 5725-2:~~1994~~ 2019 Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of the repeatability and reproducibility of a standard measurement method

ISO 5725-5:1998 Accuracy (trueness and precision) of measurement methods and results – Part 5: Alternative methods for the determination of the precision of a standard measurement method

ISO 5725-6:1994 Accuracy (trueness and precision) of measurement methods and results – Part 6: Use in practice of accuracy values

DECISION N°. DEC-III. X/119-VI/2024

REVISING THE TRADING STANDARD FOR OLIVE OIL AND OLIVE-POMACE OIL



INTERNATIONAL  
OLIVE  
COUNCIL

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OLEICOLA  
INTERNACIONAL

CONSEIL  
OLEICOLE  
INTERNATIONAL

CONSIGLIO  
OLEICOLO  
INTERNAZIONALE

المجلس  
الدولي  
للزيتون

## **DRAFT DECISION N°. DEC-III. X/119-VI/2024**

### **REVISING THE TRADING STANDARD FOR OLIVE OIL AND OLIVE-POMACE OIL**

#### **THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,**

**Having regard to** the International Agreement on Olive Oil and Table Olives 2015, and in particular its article 1 'Objectives of the Agreement' on standardisation and research, concerning the standardisation of national and international legislation relating to the physico-chemical and organoleptic characteristics of olive oils, olive-pomace oils and table olives in order to avoid any obstacle to trade, and its Chapter VI 'Provisions concerning standardisation';

**Having regard to** the recommendation made by the Committee on Chemistry and Standardisation at its 14th meeting during the 119th session of the Council of Members;

**Considering** the work carried out by the chemists on the application of methods and studies on olive oils with non-standard parameters;

**Considering** the unanimous position of the expert chemists appointed by Members, formulated at the expert chemists' meeting of 3 and 4 April 2024, on:

- The inclusion of the revision 11 of the method for the organoleptic assessment of virgin olive oil (T.20/Doc. No. 15/Rev. 11).
- The inclusion of revision 3 Corr 1 of the method of analysis for the determination of the content of waxes and fatty acid ethyl esters by capillary gas chromatography (COI/T.20/Doc. No 28/Rev. 3 Corr. 1).

#### **DECIDES**

1. To adopt the Trade Standard for Olive Oils and Olive Pomace Oils COI/T.15/NC No. 3/Rev. 20, attached to this decision, which replaces and repeals the Trade Standard for Olive Oils and Olive Pomace Oils COI/T.15/NC No. 3/Rev. 19 of November 2022;
2. Members shall, subject to their respective legislation, take all relevant measures to implement the adopted Standard and shall communicate these measures to the Executive Secretariat once they are implemented;
3. Non-Member States interested in international trade in olive oils and olive-pomace oils are invited to take the adopted Standard into consideration and to adapt their regulations to the provisions of this Standard.

Madrid (Spain), 25 June 2024

Javad Mir Arabrazi  
Chair of the International Olive Council



**TRADE STANDARD APPLYING TO OLIVE OILS**  
**AND OLIVE POMACE OILS**

**1. SCOPE**

This standard applies to olive oils and olive pomace oils that are the object of international trade or of concessional or food aid transactions.

**2. DESIGNATIONS AND DEFINITIONS**

**2.1. Olive oils**

**2.1.1.** Virgin olive oils are oils which are obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. Virgin olive oils shall be classified and designated as follows:

**2.1.1.1.** Virgin olive oils fit for consumption as they are:

(i) Extra virgin olive oil: virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.80 grams per 100 grams and the other physico-chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard.

(ii) Virgin olive oil: virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 2.0 grams per 100 grams and the other physico–chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard.

(iii) Ordinary virgin olive oil: virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 3.3 grams per 100 grams and the other physico–chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard.<sup>1/</sup>

**2.1.1.2. Virgin olive oils that must undergo processing prior to consumption:**

Lampante virgin olive oil: virgin olive oil which has a free acidity expressed as oleic acid, of more than 3.3 grams per 100 grams and/or the physico–chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard. It is intended for refining or for technical use.

**2.1.2. Refined olive oil**: olive oil obtained from virgin olive oils by refining methods which do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.30 grams per 100 grams and its other physico–chemical and organoleptic characteristics correspond to those fixed for this category in this standard.<sup>2/</sup>

**2.1.3. Olive oil composed of refined olive oil and virgin olive oils**: oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption as they are. It has a free acidity, expressed as oleic acid, of not more than 1.00 gram per 100 grams and its other physico–chemical and organoleptic characteristics correspond to those fixed for this category in this standard.

**2.2. Olive pomace oil**<sup>3/</sup> is the oil obtained by treating olive pomace with solvents or other physical treatments, to the exclusion of oils obtained by re-esterification processes and of any mixture with oils of other kinds. It is marketed in accordance with the following designations and definitions:

**2.2.1. Crude olive pomace oil**: olive pomace oil, the physico–chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard. It is intended for refining for use for human consumption, or it is intended for technical use.

<sup>1/</sup> This product may only be sold direct to the consumer if permitted in the country of retail sale. If not permitted, the designation of this product shall comply with the legal provisions of the country concerned.

<sup>2/</sup> This product may only be sold direct to the consumer if permitted in the country of retail sale.

<sup>3/</sup> Olive pomace oil cannot be sold with the designation or definition “olive oil”.

- 2.2.2. Refined olive pomace oil: oil obtained from crude olive pomace oil by refining methods which do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.30 grams per 100 grams and its other physico-chemical and organoleptic characteristics correspond to those fixed for this category in this standard.<sup>1/</sup>
- 2.2.3. Olive pomace oil composed of refined olive pomace oil and virgin olive oils: oil consisting of a blend of refined olive pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity of not more than 1.00 gram per 100 grams and its other physico-chemical and organoleptic characteristics correspond to those fixed for this category in this standard.<sup>2/</sup> In no case shall this blend be called "olive oil".

### 3. PURITY CRITERIA

The identity characteristics comprising the purity criteria shall be applicable to olive oils and olive pomace oils.

The limits established for each criterion include the precision values of the attendant recommended method.

#### 3.1. Fatty acid composition as determined by gas chromatography (% m/m methyl esters):

- Myristic acid	< 0.03
- Palmitic acid	7.00 - 20.00
- Palmitoleic acid	0.30 - 3.50
- Heptadecanoic acid	< 0.40
- Heptadecenoic acid	< 0.60
- Stearic acid	0.50 - 5.00
- Oleic acid	55.00 - 85.00
- Linoleic acid	2.50 - 21.00
- Linolenic acid	< 1.00 <sup>3/</sup>
- Arachidic acid	< 0.60
- Gadoleic acid (eicosenoic)	< 0.50
- Behenic acid	≤ 0.20*
- Lignoceric acid	< 0.20

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<sup>1/</sup> This product may only be sold direct to the consumer if permitted in the country of retail sale.

<sup>2/</sup> The country of retail sale may require a more specific designation.

<sup>3/</sup> When an edible virgin olive oil exhibits  $1.00 < \text{linolenic acid} \% \leq 1.40$ , then this oil is authentic, provided that App.  $\beta$ -sito/Campe content  $\geq 24$  and all other purity criteria lie within the official limit

\* Limit raised to  $\leq 0.30$  for olive pomace oils.

### 3.2. Trans fatty acid content (% trans fatty acids)

	C18:1 T %	C18:2 T + C18:3 T %
- Edible virgin olive oils	$\leq 0.05$	$\leq 0.05$
- Lampante virgin olive oil	$< 0.10$	$< 0.10$
- Refined olive oil	$< 0.20$	$< 0.30$
- Olive oil (ROO+VOOs) <sup>1</sup>	$< 0.20$	$< 0.30$
- Crude olive pomace oil	$< 0.20$	$< 0.10$
- Refined olive pomace oil	$< 0.40$	$< 0.35$
- Olive pomace oil (ROPO+VOOs) <sup>2</sup>	$< 0.40$	$\leq 0.35$

### 3.3. Sterol and triterpene dialcohol composition

#### 3.3.1. Desmethylsterol composition (% total sterols)

- Cholesterol	$\leq 0.5$
- Brassicasterol	$\leq 0.1^*$
- Campesterol	$\leq 4.0^{**}$
- Stigmasterol	$< \text{campesterol in edible oils}$
- Delta 7 stigmastenol	$\leq 0.5^{***}$
- Apparent beta sitosterol: beta-sitosterol + delta-5-avenasterol + delta-5-23-stigmastadienol + clerosterol + sitostanol + delta 5-24-stigmastadienol	$\geq 93.0$

<sup>1</sup> Blend of refined olive oil and virgin olive oils

<sup>2</sup> Blend of refined olive pomace oil and virgin olive oils

\* Limit raised to  $\leq 0.2$  for olive pomace oils.

\*\* An extra virgin or virgin olive oil that exhibits  $4.0 < \text{campesterol \%} \leq 4.5$  is authentic provided that stigmasterol  $\leq 1.4\%$ ,  $\Delta 7$ -stigmastenol  $\leq 0.3\%$  and all other parameters lie within the limits fixed in this standard.

\*\*\* An olive oil or olive-pomace oil that exhibits  $0.5 < \Delta 7$ -stigmastenol  $\% \leq 0.8$  is authentic provided that:

- app.  $\beta$ -sitosterol/campesterol  $\geq 28$ ,  $\Delta \text{ECN}42 \leq \begin{cases} 0.10 \\ 0.15 \end{cases}$  (for extra virgin or virgin olive oil)
- app.  $\beta$ -sitosterol/campesterol  $\geq 28$ ,  $\Delta \text{ECN}42 \leq \begin{cases} 0.10 \\ 0.15 \end{cases}$ , stigmastadiene  $\leq 0.30$  (for lampante virgin olive oil)
- app.  $\beta$ -sitosterol/campesterol  $\geq 28$ ,  $\Delta \text{ECN}42 \leq \begin{cases} 0.10 \\ 0.15 \end{cases}$  (for refined olive oil or olive oil (ROO+VOOs))
- stigmasterol  $\leq 1.4\%$ ,  $\Delta \text{ECN}42 \leq \begin{cases} 0.40 \\ 0.40 \end{cases}$  (crude olive-pomace oil, refined olive-pomace oil or olive pomace oil (ROPO+VOOs))

In all the above cases, all other parameters lie within the limits fixed in this standard.



### 3.3.2. Total sterol content (mg/kg)

- Virgin olive oils	}	$\geq 1000$
- Refined olive oil		
- Olive oil (ROO+VOOs)		
- Crude olive pomace oil		$\geq 2500$
- Refined olive pomace oil		$\geq 1800$
- Olive pomace oil (ROPO+VOOs)		$> 1600$

### 3.3.3. Erythrodiol and uvaol content (% total sterols)

- Edible virgin olive oils	$\leq 4.5$
- Lampante virgin olive oil	$\leq 4.5^{1/}$
- Refined olive oil	$\leq 4.5^{2/}$
- Olive oil (ROO+VOOs)	$\leq 4.5$
- Crude olive pomace oil	$> 4.5^{3/}$
- Refined olive pomace oil	$> 4.5$
- Olive pomace oil (ROPO+VOOs)	$> 4.5$

### 3.4. Wax content (mg/kg)

C42 + C44 + C46 (mg/kg)

- Extra virgin olive oil and virgin olive oil	$\leq 150$
---	------------

C40 + C42 + C44 + C46 (mg/kg)

- Ordinary virgin olive oil	$\leq 250$
- Lampante virgin olive oil	$\leq 300^{1/}$
- Refined olive oil	$< 350$
- Olive oil (ROO+VOOs)	$< 350$
- Crude olive pomace oil	$> 350^{2/}$
- Refined olive pomace oil	$> 350$
- Olive pomace oil (ROPO+VOOs)	$> 350$

<sup>1/</sup> When the oil has a wax content between 300 mg/kg and 350 mg/kg it is considered a lampante virgin olive oil if the total aliphatic alcohol content is  $\leq 350$  mg/kg or the erythrodiol + uvaol content is  $\leq 3.5\%$ .

<sup>2/</sup> When the oil has an erythrodiol + uvaol content of between 4.5 and 6 %, the erythrodiol content must be  $< 75$  mg/kg.

<sup>3/</sup> When the oil has a wax content between 300 mg/kg and 350 mg/kg it is considered a crude olive pomace oil if the total aliphatic alcohol content is  $> 350$  mg/kg and the erythrodiol + uvaol content is  $> 3.5\%$ .

**3.5. Maximum difference between the actual and theoretical ECN 42 triacylglycerol content (%)**

- Edible virgin olive oils	≤  0.20
- Lampante virgin olive oil	≤  0.30
- Refined olive oil	≤  0.30
- Olive oil (ROO+VOOs)	≤  0.30
- Crude olive pomace oil	≤  0.60
- Refined olive pomace oil	≤  0.50
- Olive pomace oil (ROPO+VOOs)	≤  0.50

**3.6. Stigmastadiene content (mg/kg)**

- Extra virgin olive oil and virgin olive oil	≤ 0.05
- Ordinary virgin olive oil	≤ 0.10
- Lampante virgin olive oil	≤ 0.50

**3.7. Content of 2-glyceryl monopalmitate (%)**

- Edible virgin olive oils and olive oil (ROO+VOOs):

C16:0 < 14.00%; 2P < 0.9%

C16:0 > 14.00%, 2P < 1.0%

- Non-edible virgin olive oils and refined olive oils:

C16:0 < 14.00%; 2P < 0.9%

C16:0 > 14.00%, 2P < 1.1%

- Olive pomace oil (ROPO+VOOs) < 1.2%
- Crude and refined olive pomace oils < 1.4%

**3.8. Unsaponifiable matter (g/kg)**

- Olive oils < 15
- Olive pomace oils < 30



#### 4. QUALITY CRITERIA

The limits established for each criterion and designation include the precision values of the attendant recommended method

	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil*	Refined olive oil	Olive Oil (ROO+VO Os)	Crude olive pomace oil	Refined olive pomace oil	Olive pomace oil (ROPO+VO Os)
4.1 <u>Organoleptic characteristics</u>									
- odour and taste					acceptable	good		acceptable	good
- median of defect	Me = 0.0	0.0 < Me < 3.5	3.5 < Me < 6.0**	Me > 6.0					
- median of the fruity attribute	Me > 0.0	Me > 0.0							
- colour					Light yellow	Light yellow to green		Light yellow to brownish yellow	Light yellow to green
- aspect at 20°C for 24 hours					limpid	limpid		limpid	limpid
4.2. <u>Free acidity</u> % m/m expressed in oleic acid	≤ 0.80	≤ 2.0	≤ 3.3	> 3.3	≤ 0.30	≤ 1.00	no limit	≤ 0.30	≤ 1.00
4.3. <u>Peroxide value</u> in milleq. peroxide oxygen per kg/oil	≤ 20.0	≤ 20.0	≤ 20.0	no limit	≤ 5.0	≤ 15.0	no limit	≤ 5.0	≤ 15.0

\* ..... It is not obligatory for the criteria in 4.1, 4.2 and 4.3 to be concurrent; one is sufficient.

\*\* ... Or when the median of the defect is less than or equal to 3.5 and the median of the fruity attribute is equal to 0.0.

EN

EN

**4. QUALITY CRITERIA (contd.)**

	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive Oil (ROO+VOOs)	Crude olive pomace oil	Refined olive pomace oil	Olive pomace oil (ROPO+VOOs)
4.4. <u>Absorbency in ultra-violet</u> (K <sup>1%</sup> <sub>1cm</sub> ) - 270 nm (cyclohexane) / 268 nm (iso-octane) - ΔK - 232 nm*	≤ 0.22	≤ 0.25	≤ 0.30		≤ 1.25	≤ 1.15		≤ 2.00	≤ 1.70
	≤ 0.01	≤ 0.01	≤ 0.01		≤ 0.16	≤ 0.15		≤ 0.20	≤ 0.18
	≤ 2.50**	≤ 2.60**							
4.5. <u>Moisture and volatile matter</u> (% m/m)	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.3	≤ 0.1	≤ 0.1	≤ 1.5	≤ 0.1	≤ 0.1
4.6. <u>Insoluble impurities in light petroleum</u> (% m/m)	≤ 0.10	≤ 0.10	≤ 0.10	≤ 0.20	≤ 0.05	≤ 0.05		≤ 0.05	≤ 0.05
4.7. <u>Flash point</u>							≥ 120°C		
4.8. <u>Trace metals</u> (mg/kg)									
Iron	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0		≤ 3.0	≤ 3.0
Copper	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1		≤ 0.1	≤ 0.1
4.9. Fatty acid ethyl esters (FAEEs)	≤ 35 mg/kg								
4.10. <u>Phenols content</u>	See section 11.21								

\* ..... This determination is solely for application by commercial partners on an optional basis.

\*\* ... Commercial partners in the country of retail sale may require compliance with these limits when the oil is made available to the end consumer.

**EN****EN**

## 5. FOOD ADDITIVES

### 5.1. Virgin olive oils and crude olive pomace oil:

none permitted

### 5.2. Refined olive oil, olive oil (ROO+VOOs), refined olive pomace oil and olive pomace oil (ROPO+VOOs): alpha-tocopherol permitted to restore natural tocopherol lost in the refining process.

Maximum level: According to the Good Manufacturing Practices (GMP)

## 6. CONTAMINANTS

### 6.1. Heavy metals

The products covered by this standard shall comply with the maximum levels of the General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995).

### 6.2. Pesticide residues

The products covered by this standard shall comply with those maximum residue limits established by the Codex Alimentarius Commission for these commodities.

### 6.3. Halogenated solvents

- |  |           |
|--|-----------|
| - Maximum content of each halogenated solvent            | 0.1 mg/kg |
| - Maximum content of the sum of all halogenated solvents | 0.2 mg/kg |

## **7. HYGIENE**

- 7.1. It is recommended that the products intended for human consumption covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the General Principles of Food Hygiene (CAC/RP 1-1969), and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.
- 7.2. The products intended for human consumption should comply with any microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria (CAC/GL 21-1997).

## **8. PACKING**

Olive oils and olive pomace oils intended for international trade shall be packed in containers complying with the General Principles of Food Hygiene recommended by the Codex Alimentarius Commission (CAC/RCP 1 - 1969), and other relevant texts such as Codes of Hygienic Practice and Codes of Practice.

The containers used may be:

- 8.1. tanks, containers, vats, which permit the transportation in bulk of olive oils and olive pomace oils;
- 8.2. metal drums, in good condition, hermetically-sealed, which should be internally covered with a suitable varnish;
- 8.3. metal tins and cans, lithographed, new, hermetically-sealed, which should be internally covered with a suitable varnish;
- 8.4. demi-johns, glass bottles or bottles made of suitable macromolecular material.

## **9. CONTAINER FILLING TOLERANCE**

The volume occupied by the contents shall under no circumstances be less than 90% of the capacity of the container, except in the case of tin containers with a capacity of, or less than, 1 L in which the volume occupied shall under no circumstances be less than 80% of the capacity of the container; this capacity is equal to the volume of distilled water at 20°C which the container can hold when full.

## **10. LABELLING**

In addition to the appropriate sections of the Codex General Standard for the Labelling of Pre-packaged Foods (CODEX STAN 1-1985) and the guidelines applying to food not intended for direct sale to consumers, the specific provisions providing the following information shall be applied:

### **10.1. On containers intended for direct sale to consumers**

#### **10.1.1. Name of the product**

The labelling on each container shall indicate the specific designation of the product contained, complying in every way with the relevant provisions of this standard.

##### **10.1.1.1. Designations of olive oils:**

- Extra virgin olive oil
- Virgin olive oil
- Ordinary virgin olive oil<sup>1/</sup>
- Refined olive oil<sup>1/</sup>
- Olive oil (ROO+VOOs)<sup>2/</sup>

##### **10.1.1.2. Designations of olive pomace oils:**

- Refined olive pomace oil<sup>1/</sup>
- Olive pomace oil (ROPO+VOOs)<sup>2/</sup>

#### **10.1.2. Net contents**

The net contents shall be declared by volume in the metric system ("Système International" units).

#### **10.1.3. Name and address**

The name and address of the manufacturer, packer, distributor, importer, exporter or seller shall be declared.

<sup>1/</sup> This product may only be sold direct to the consumer if permitted in the country of retail sale.

<sup>2/</sup> The country of retail sale may require a more specific designation.



**10.1.4. Country of origin**

The name of the country of origin shall be declared. When the product undergoes substantial processing in a second country, the country in which such processing is carried out shall be considered as the country of origin for labelling purposes.

**10.1.5. Geographical indications and designations of origin**

**10.1.5.1. Geographical indications**

The labels of virgin olive oils may state their geographical indication (country, region or locality) when they have been empowered to do so by their country of origin and when such virgin olive oils have been produced, packed and originate exclusively in the country, region or locality mentioned.

**10.1.5.2. Designations of origin**

The labels of extra virgin olive oils may indicate their designation of origin (country, region or locality) when they have been awarded such a designation, in accordance with the terms provided under the regulations of their country of origin and when such extra virgin olive oil has been produced, packed and originates exclusively in the country, region or locality mentioned.

**10.1.6. Lot identification**

Each container shall be embossed or otherwise permanently marked in code or in clear to identify the producing factory and the lot.

**10.1.7. Date marking and storage conditions**

**10.1.7.1. Date of minimum durability**

In the case of pre-packaged products intended for the end consumer, the date of minimum durability (preceded by the words "best before end") shall be declared by the month and year in uncoded numerical sequence. The month may be indicated by letters in those countries where such use will not confuse the consumer; if the shelf life of the product is valid to December, the expression "end (stated year)" may be used as an alternative.

**10.1.7.2. Storage instructions**

Any special conditions for storage shall be declared on the label if the validity of the date of minimum durability depends thereon.

**10.2.** On forwarding packs of oils intended for human consumption

In addition to the details noted under section 10.1., the following inscription shall appear:  
- number and type of containers held in pack.

**10.3.** On containers allowing the transportation in bulk of olive oils and olive pomace oils

The labelling on each container shall include:

**10.3.1.** Name of the product

The name shall indicate the specific designation of the product contained, complying in every way with the provisions of this standard.

**10.3.2.** Net contents

The net contents shall be declared by weight or volume in the metric system ("Système International" units).

**10.3.3.** Name and address

The name and address of the manufacturer, distributor or exporter shall be declared.

**10.3.4.** Country of origin

The name of the exporting country shall be declared.

**11.** **METHODS OF ANALYSIS AND SAMPLING**

The methods of analysis and sampling given below are international referee methods. The latest version of these methods should be used.

**11.1.** Sampling

According to ISO 5555, "Animal and vegetable fats and oils - Sampling".

**11.2.** Preparation of the test sample

According to ISO 661, "Animal and vegetable fats and oils - Preparation of the test sample".

**11.3.**     Determination of the fatty acid composition and *trans* fatty acid content

According to COI/T.20/Doc. No 33/Rev.2, “Determination of the fatty acid composition in olive and olive-pomace oils by gas chromatography”

**11.4.**     Determination of the sterol composition and content and alcoholic compounds

According to COI/T.20/Doc. No 26/Rev. 5, “Determination of the composition and content of sterols, triterpenic dialcohols and aliphatic alcohols by capillary column gas chromatography”.

**11.5.**     Determination of the difference between the actual and theoretical ECN 42 triacylglycerol content

According to COI/T.20/Doc. No 20/Rev. 4, "Determination of the difference between actual and theoretical content of triacylglycerols with ECN 42", or AOCS 5b-89.

**11.6.**     Determination of the stigmastadiene content

According to COI/T.20/Doc. No 11/Rev.4, "Determination of stigmastadienes in vegetable oils", or COI/T.20/Doc. no. 16/Rev.2, "Determination of sterenes in refined vegetable oils", or ISO 15788-1 or AOCS Cd 26-96.

**11.7.**     Determination of the content of 2-glyceryl monopalmitate

According to COI/T.20/Doc. No 23/Rev.1, "Determination of the percentage of 2-glyceryl monopalmitate" or to ISO 12872.

**11.8.**     Determination of the unsaponifiable matter

According to ISO 3596, “Determination of the unsaponifiable matter – Method using diethyl ether extraction”, or AOCS Ca 6b-53 or ISO 18609.

The results should be expressed in g/unsaponifiable matter per kg/oil.

**11.9.**     Determination of the organoleptic characteristics

According to COI/T.20/Doc. No 15/Rev. ~~40~~ 11, "Organoleptic assessment of virgin olive oil".

**11.10.**    Determination of the free acidity

According to COI/T.20/Doc. No 34/Rev.1, “Determination of free fatty acids, cold method”.

**11.11. Determination of the peroxide value**

According to COI/T.20/Doc. No 35/Rev.1, "Determination of the peroxide value", ISO 3960, or AOCS Cd 8b-90.

**11.12. Determination of the absorbency in ultra-violet**

According to COI/T.20/Doc. No 19/Rev.5, "Spectrophotometric investigation in the ultraviolet", or ISO 3656 or AOCS Ch 5-91.

**11.13. Determination of the moisture and volatile matter**

According to ISO 662, "Determination of the moisture and volatile matter".

**11.14. Determination of the insoluble impurities in light petroleum**

According to ISO 663, "Determination of the insoluble impurities".

**11.15. Determination of the flash point**

According to the FOSFA International method.

**11.16. Detection of trace metals**

According to ISO 8294, "Determination of copper, iron and nickel by direct graphite furnace atomic absorption spectrometry".

**11.17. Determination of the alpha-tocopherol**

According to ISO 9936, "Determination of tocopherols and tocotrienols contents – Method using high-performance liquid chromatography".

**11.18. Determination of traces of heavy metals**

- Determination of lead: according to ISO 12193 or AOCS Ca 18c-91 or AOAC 994.02.
- Determination of arsenic: according to AOAC 952.13 or AOAC 942.17 or AOAC 985.16.

**11.19. Detection of traces of halogenated solvents**

According to COI/T.20/Doc. No 8, "Determination of tetrachloroethylene in olive oils by gas-liquid chromatography".

**11.20.** Determination of the content of waxes and alkyl esters

[According to COI/T.20/Doc. No 28/Rev.3 Corr 1, “Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography“.

**11.21.** Determination of biophenols

According to COI/T.20/Doc. No 29/Rev.2, “Document to declare the use of IOC methods for phenol determination”

**11.22.** Determination of the coherence of TAG composition with the fatty acid composition: Screening method (not legal in nature and cannot downgrade an oil)

According to COI/T.20/Doc. No 25/Rev.2, “Method for the evaluation of the coherence of TAG composition with the fatty acid composition”.

**11.23.** Determination of the methanol and ethanol content in virgin olive oils

According to COI/T.20/Doc. No 36, “Method of determination of ethanol and methanol content on virgin olive oils”.