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COVER NOTE

From:	Secretary-General of the European Commission, signed by Ms Martine DEPREZ, Director
date of receipt:	28 September 2022
To:	General Secretariat of the Council
No. Cion doc.:	SWD(2022) 309 final
Subject:	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 the trade standard applying to olive oils and olive-pomace oils

Delegations will find attached document SWD(2022) 309 final.

Encl.: SWD(2022) 309 final

EUROPEAN
COMMISSION

Brussels, 28.9.2022
SWD(2022) 309 final

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 the trade standard applying to olive oils and olive-pomace oils

{COM(2022) 487 final}

Draft Decision NO DEC-III.X/116-VI/2022 revising the trade standard applying to olive oils and olive-pomace oils

DRAFT DECISION Nº DEC-III.X/116-VI /2022

REVISING THE TRADE STANDARD APPLYING TO OLIVE OILS AND OLIVE POMACE OILS

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 11th meeting during the 116th 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 10 and 11 March 2022, on:

- The removal of Annex 1 and the simplification of the delta 7 stigmastenol decision trees.

- The inclusion of revision 3 of the method on the determination of waxes and ethyl esters.

Considering the work carried out by the expert chemists as part of the collaborative trials on the validation of the method to determine waxes and ethyl esters by the revision COI/T.20/Doc. No. 28/Rev. 3 'Determination of the content of waxes and fatty acid ethyl esters by capillary column gas chromatography';

DECIDES

1. To adopt the Trade Standard for Olive Oils and Olive Pomace Oils COI/T.15/NC No. 3/Rev. 19, 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. 18 of June 2022;

2. Members shall, in accordance with their respective legislation, take all appropriate measures to implement the adopted Standard and shall communicate these measures to the Executive Secretariat as soon as they are taken;

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), November 2022

Mr Kaled Musa Al Henefat

Chair of the International Olive Council

COI/T.15/NC No 3/Rev. 19
November 2022

ENGLISH
Original: FRENCH

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.[1/]

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.[2/]

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[3/] 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.

1/ 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.

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

3/ 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.[1/]

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.[2/] 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[3/]
- Arachidic acid	< 0.60
- Gadoleic acid (eicosenoic)	< 0.50
- Behenic acid	≤ 0.20*
- Lignoceric acid	< 0.20

_____

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

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

3/ When an edible virgin olive oil exhibits 1.00<linolenic acid%≤1.40, then this oil is authentic, provided that App. β-sito/Campe content ≥ 24 and all other purity criteria lie within the official limit

* Limit raised to ≤ 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	≤ 0.05	≤ 0.05
- Lampante virgin olive oil	< 0.10	< 0.10
- Refined olive oil	< 0.20	< 0.30
- Olive oil (ROO+VOOs)1	< 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)2	< 0.40	≤ 0.35

3.3. Sterol and triterpene dialcohol composition

3.3.1. Desmethylsterol composition (% total sterols)

- Cholesterol	≤ 0.5
- Brassicasterol	≤ 0.1*
- Campesterol	≤ 4.0**
- Stigmasterol	< campesterol in edible oils
- Delta 7 stigmastenol	≤ 0.5***
- Apparent beta sitosterol: beta-sitosterol + delta-5-avenasterol + delta-5-23-stigmastadienol +clerosterol + sitostanol + delta 5-24-stigmastadienol	≥ 93.0

[1] Blend of refined olive oil and virgin olive oils
[2] Blend of refined olive pomace oil and virgin olive oils

* Limit raised to ≤ 0.2 for olive pomace oils.

** An extra virgin or virgin olive oil that exhibits 4.0< campesterol % ≤4.5 is authentic provided that stigmasterol ≤1.4%, Δ7-stigmastenol ≤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< Δ7-stigmastenol % ≤0.8 is authentic provided that:

a) app. β-sitosterol/campesterol≥28, ΔECN42 ≤ | 0.10 | (for extra virgin or virgin olive oil)
b) app. β-sitosterol/campesterol≥28, ΔECN42 ≤ | 0.15 |, stigmastadiene≤0.30 (for lampante virgin olive oil)
c) app. β-sitosterol/campesterol≥28, ΔECN42≤ | 0.15 | (for refined olive oil or olive oil (ROO+VOOs))
d) stigmasterol ≤1.4%, ΔECN42 ≤ | 0.40 | (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 - Refined olive oil - Olive oil (ROO+VOOs)	≥ 1000
- Crude olive pomace oil	≥ 2500
- Refined olive pomace oil	≥ 1800
- Olive pomace oil (ROPO+VOOs)	> 1600

3.3.3. Erythrodiol and uvaol content (% total sterols)

- Edible virgin olive oils	≤ 4.5
- Lampante virgin olive oil	≤ 4.5[1/]
- Refined olive oil	≤ 4.5[2/]
- Olive oil (ROO+VOOs)	≤ 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	≤ 150
C40 + C42 + C44 + C46 (mg/kg)	
- Ordinary virgin olive oil	≤ 250
- Lampante virgin olive oil	≤ 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

1/ 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 ≤ 350 mg/kg or the erythrodiol + uvaol content is ≤ 3.5%.

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

3/ 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+VOOs)	Crude olive pomace oil	Refined olive pomace oil	Olive pomace oil (ROPO+VOOs)
4.1 Organoleptic characteristics									
- 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 20ºC for 24 hours					limpid	limpid		limpid	limpid
4.2. Free acidity % 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. Peroxide value 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.

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. Absorbency in ultra-violet ($K^{1\%}_{1cm}$)									
- 270 nm (cyclohexane) / 268 nm (iso-octane)	≤ 0.22	≤ 0.25	≤ 0.30		≤ 1.25	≤ 1.15		≤ 2.00	≤ 1.70
- ΔK	≤ 0.01	≤ 0.01	≤ 0.01		≤ 0.16	≤ 0.15		≤ 0.20	≤ 0.18
- 232 nm*	≤ 2.50**	≤ 2.60**							
4.5. Moisture and volatile matter (% m/m)	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.3	≤ 0.1	≤ 0.1	≤ 1.5	≤ 0.1	≤ 0.1
4.6. Insoluble impurities in light petroleum (% m/m)	≤ 0.10	≤ 0.10	≤ 0.10	≤ 0.20	≤ 0.05	≤ 0.05		≤ 0.05	≤ 0.05
4.7. Flash point							≥ 120ºC		
4.8. Trace metals (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. Phenols content	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.

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 20ºC 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[1/]
- Refined olive oil[1/]
- Olive oil (ROO+VOOs)[2/]

10.1.1.2. Designations of olive pomace oils:

- Refined olive pomace oil[1/]
- Olive pomace oil (ROPO+VOOs)[2/]

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.

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

2/ 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.10, "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, "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".

DECISION Nº DEC-III-X/116-VI/202 concerning the method for the determination of the content of waxes and fatty acid ethyl esters by capillary gas chromatography

DECISION Nº DEC-III-X/116-VI/2022

CONCERNING THE METHOD FOR THE DETERMINATION OF THE CONTENT OF WAXES AND 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 the recommendation made by the Chemistry and Standardisation Committee at its 11th meeting during the 116th session of the Council of Members;

Considering the chemistry experts studied the application of method COI/T.20/Doc. No 28 and its fusion with method COI/T.20/Doc. No 31;

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 10 and 11 March 2022;

DECIDES

To adopt revision 3 of the document COI/T.20/Doc. No 28/Rev. 3 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.

This revised method COI/T.20/Doc. No 28/Rev. 3 shall replace the methods COI/T.20/Doc. No 28/Rev. 2 and COI/T.20/Doc. No 31 and will be listed in the IOC trade standard.

Madrid (Spain), XX November 2022

Mr Kaled Musa Al Henefat
Chair of the International Olive Council

COI/T.20/Do. No 28/Rev. 3
November 2022

ENGLISH

Original: ENGLISH

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. 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.

This document presents two methods that can be used for official control. Method A (15 g of silica) is the reference method for counter-assessment.

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. APPARATUS

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

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

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

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

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

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

3.3.4. **Recorder-integrator** (*Note 1*) 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. **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 2*) to a uniform thickness of 0.10-0.25 μm.

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

3.5. **Electric shaker.**

3.6. **Rotary evaporator.**

3.7. **Muffle oven.**

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

3.9. Usual laboratory glassware.

4. REAGENTS

4.1. **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 not be necessary.

4.2. **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 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. **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

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Note 1: Computerised systems may also be used where the gas chromatography data are entered through a PC.
Note 2: 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

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. **n-heptane,** chromatography grade, or **iso-octane.**

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. **Standard solution of lauryl arachidate** (*Note 3*) at 0.02% (m/V) in heptane (internal standard for waxes).

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

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

4.8. **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.

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.

Note 3: Palmityl palmitate, myristyl stearate or arachidyl laureate may also be used.

4.9. 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. PROCEDURE

5.1. Preparation of the chromatography column

Suspend 15 g of silica gel (4.1) in n-hexane (4.2) and introduce into the column (3.2). Allow to settle spontaneously. Complete settling with the aid of an electric shaker (3.5) to make the chromatographic bed more homogeneous. Percolate 20 ml of n-hexane to remove any impurities. Weigh exactly 500 mg of the sample into the 10-ml test tube (3.1), using the analytical balance (3.8), and add a suitable amount of internal standard (4.5) 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 (4.6). Transfer the prepared sample to the chromatography column with the aid of two 2-ml portions of n-hexane (4.2). 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) of n-hexane/ethyl ether (99:1) and collect 150 ml at a flow of about 15 drops every 10 seconds. (**This fraction contains ethyl esters and waxes).**

Evaporate the resultant fractions in a rotary evaporator (3.6) 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. Gas chromatography analysis

5.2.1. 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 (3.3.4), 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.

Note 4: 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.

Note 5: 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.

5.2.2. Choice of operating conditions for waxes and ethyl esters (*Note 6*)

The operating conditions are generally as follows:

- Column temperature:

$$80°C \text{ at first } (1') \xrightarrow{20°C/min} 140°C \xrightarrow{5°C/min} 335°C\ (20') \text{ for ethyl esters and waxes}$$

$$80°C \text{ at first } (1') \xrightarrow{20°C/min} 240°C \xrightarrow{5°C/min} 325°C\ (6') \xrightarrow{20°C/min} 340ºC\ (10') \text{ for waxes only}$$

- 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).
- Instrument sensitivity: suitable for fulfilling the above conditions.

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 Figures1-2) and a retention time of 18 ± 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.

5.3. 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. 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. Shows a chromatogram of the FAEE and waxes in an extra virgin olive oil using the method A (15 gr).

Figure 2. Shows a chromatogram of the FAEE and waxes in a lampante olive oil using the method A (15 gr).

5.5. 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 form 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)

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

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

5.6. 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:

Ax= area corresponding to the peak for the individual C16 and C18 ethyl ester, in computer counts

As= area corresponding to the peak for the methyl heptadecanoate internal standard, in computer counts

ms= mass of the methyl heptadecanoate internal standard added, in milligrams;

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

6. EXPRESSION OF RESULTS

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

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 one decimal place.

Note 7: 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.

METHOD B (3 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. 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. APPARATUS

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

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

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

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

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

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

3.3.4. **Recorder-integrator** (*Note 1*) for use with the converter-amplifier (3.3.3), with a response time of not more than 1 s and a variable paper speed.

3.4.5. **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 2*) to a uniform thickness of 0.10-0.25 μm.

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

3.5. **Electric shaker.**

3.6. **Rotary evaporator.**

3.7. **Muffle oven.**

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

3.9. Usual laboratory glassware.

REAGENTS

4.1. **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 not be necessary.

4.2. **n-Hexane,** chromatography or residue grade the purity must be checked as follows:

200 ml of n-hexane are evaporated to dryness, residue is redissolved in 100 µl n-heptane and analysed applying the same gas chromatographic conditions. There must be no peak in the elution alkyl esters area. (Hexane can be replaced by Isoctane) 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.

4.3. **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. **n-heptane,** chromatography grade, or **iso-octane.**

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. **Standard solution of lauryl arachidate** (*Note 3*), at 0.01% (m/V) in heptane (internal standard for waxes).

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

4.7. **Sudan 1 (1-phenylazo-2-naphthol)**

4.8. **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.

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 purpose.

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Note 1: Computerised systems may also be used where the gas chromatography data are entered through a PC.

Note2: 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

Note 3: Palmityl palmitate, myristyl stearate or arachidyl laureate may also be used.

4.9. 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. PROCEDURE

5.1. Preparation of the chromatography column

Suspend 3 g of silica gel (4.1) in n-hexane (4.2) and introduce into the column (3.2). Allow to settle spontaneously. Complete settling with the aid of an electric shaker (3.5) to make the chromatographic bed more homogeneous. Percolate 10 ml of n-hexane to remove any impurities. Weigh exactly 100 mg of the sample into the 10-ml test tube (3.1), using the analytical balance (3.8), and add a suitable amount of internal standard (4.5) depending on the assumed wax content, e.g. add 0.01 mg of lauryl arachidate in the case of extra virgin olive oil, virgin olive oil, refined olive oil and olive oil, 0.025-0.10 mg in the case of olive-pomace oil and 0.002 mg 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-ml portions of n-hexane (4.2).
Allow the solvent to flow to 1 mm above the upper level of the absorbent. Percolate 12-15 ml n-Hexane (*) to further remove hydrocarbons (alkanes and sterenes) of n-hexane/ethyl ether (99:1) (*Note 4*) and collect 40-45 ml at a flow of about 15 drops every 10 seconds. (**This fraction contains the ethyl esters and waxes).**
Evaporate the resultant fractions in a rotary evaporator (3.7) 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.

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

5.2. Gas chromatography analysis

5.2.1. 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 (3.3.4), 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.

Note 4: 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

Note 5: 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.

5.2.2. Choice of operating conditions for waxes and ethyl esters (*Note 6*)

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).
- Instrument sensitivity: suitable for fulfilling the above conditions.

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 Figures1-2) and a retention time of 18 ± 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.

5.3. 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 are completely eluted, depending on the fraction being analysed.
The base line must always meet the required conditions

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

5.4. 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 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 gr).

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

5.5. 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 and virgin olive oils and from C40 to C46 for 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)

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. Quantitative analysis of the ethyl esters

With the aid of 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 the individual C16 and C18 ethyl ester, in computer counts

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. 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 oils (*Note 7*) in milligrams per kilograms of fat.

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 one decimal place.

Results should be expressed to two decimal places, up to 1 mg/kg above this with one decimal place.

Note 7: 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.

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.

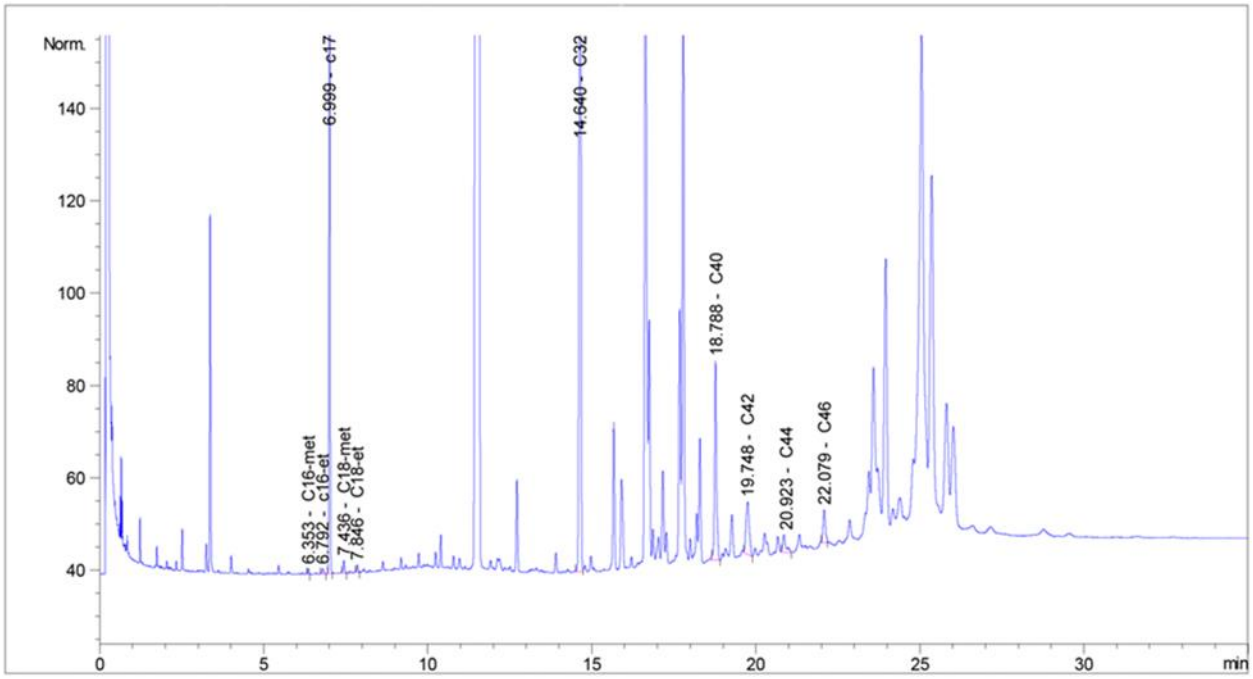

Figure 1: Chromatogram of FAEE and waxes of EVOO using method A (15 gr).

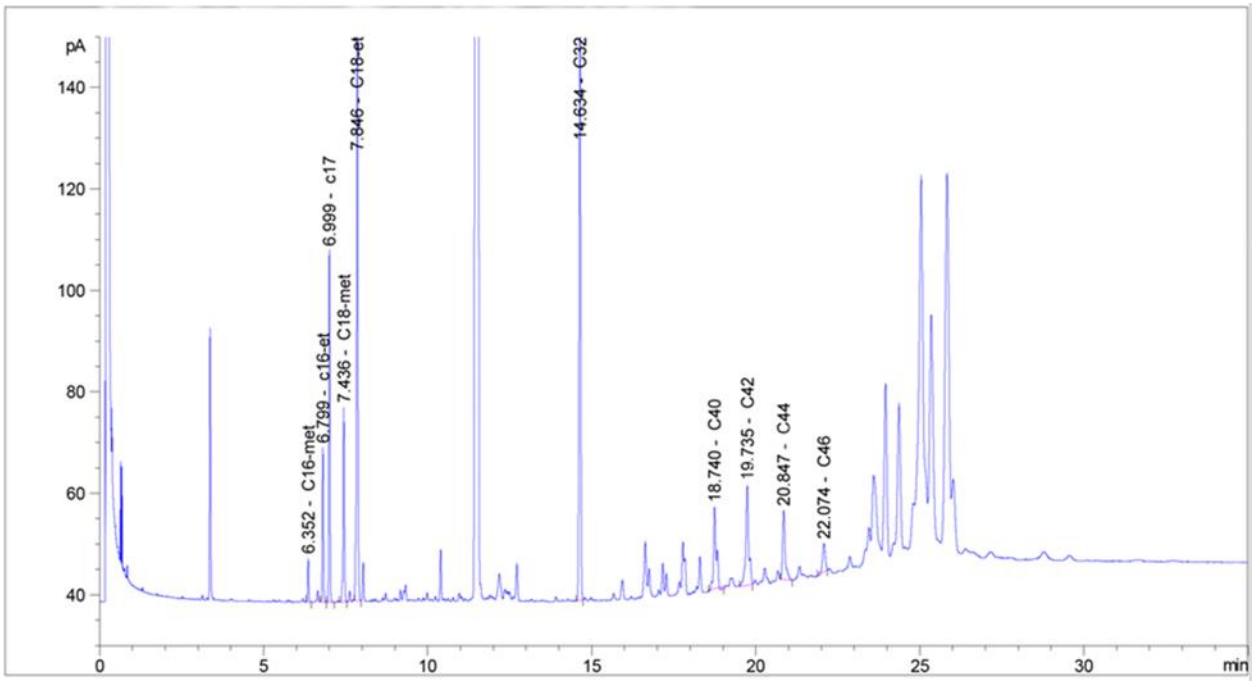

Figure 2: Chromatogram of FAEE and waxes of Lampante olive oil using method A (15 gr).

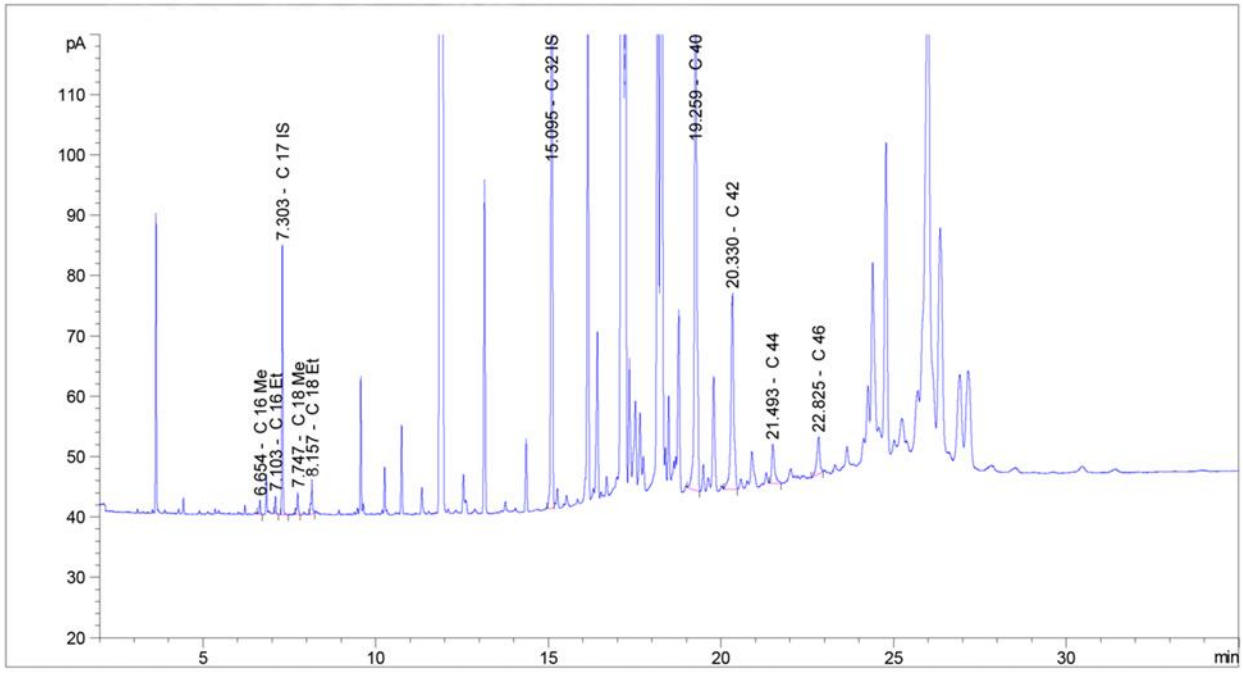

Figure 3: Chromatogram of FAEE and waxes of EVOO using method B (3 gr).

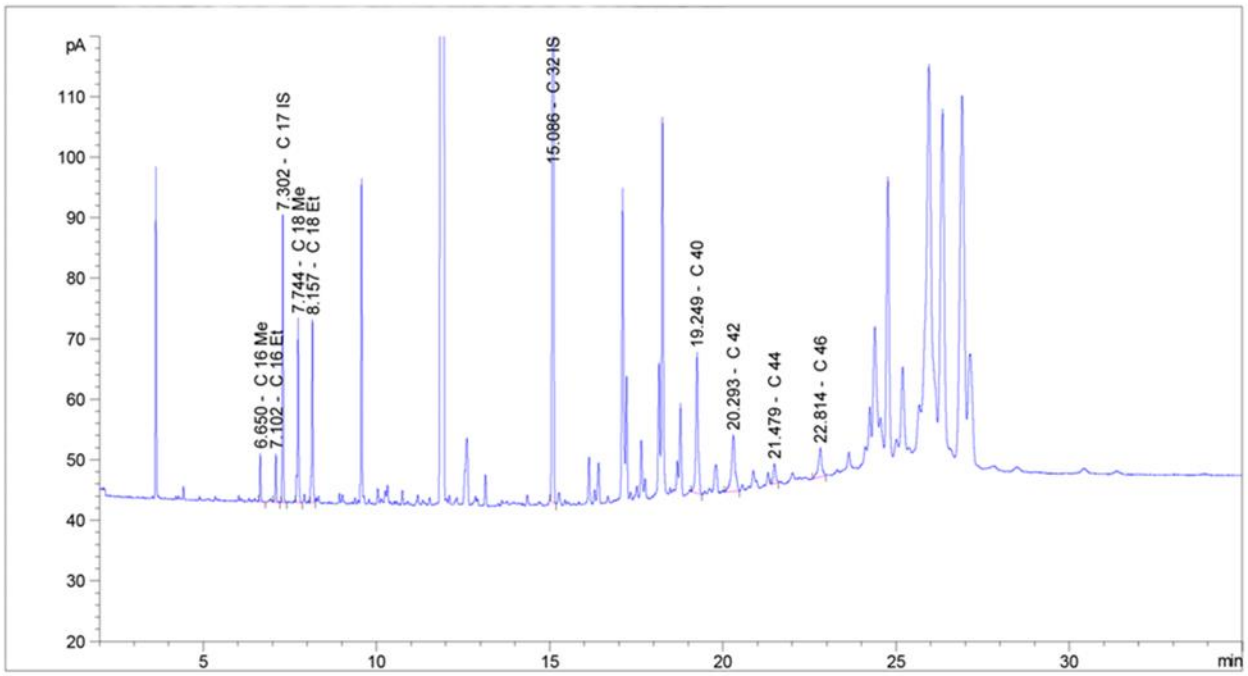

Figure 4: Chromatogram of FAEE and waxes of VOO using method B (3 gr).

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 (tM).

The linear speed in cm/s is given by L/tM where L is the length of the column, in cm, and tM 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. 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:

n	number of participating laboratories
outliers	number of laboratories with outlying values
mean	mean of the accepted results
r	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%
S_r	repeatability standard deviation
RSD_r (%)	repeatability coefficient of variation (S_r x 100 / 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_r	reproducibility standard deviation
RSD_r (%)	reproducibility coefficient of variation (S_r x 100 / mean)

Ethyl esters (mg/kg) – Method A 15 g of silica				
Sample	**M1**	**M2**	**M3**	**M4**
Mean	10	8.21	36.97	50.83
n	16	16	16	16
outliers	3	2	0	0
Sr	0.574	0.330	1.316	1.934
RSDr (%)	5.74	4.02	3.56	3.80
r	1.61	0.92	3.68	5.41
S_R	0.759	0.915	3.720	6.736
RSD_R (%)	7.59	11.15	10.06	13.25
R	2.12	2.56	10.42	18.86

Waxes (mg/kg) Method A 15 g of silica					
Sample	**M1**	**M2**	**M3**	**M4**	**M5**
Mean	93.00	45.17	38.54	323.17	2350.72
n	16	16	16	16	16
outliers	4	1	2	1	0
Sr	0.610	1.406	1.315	4.346	35.728
RSDr (%)	0.66	3.11	3.41	1.34	1.52
r	1.71	3.94	3.68	12.17	100.04
S_R	8.053	4.879	5.590	23.649	247.180
RSD_R (%)	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
n	15	15	15	15
Outliers	4	4	0	0
S_r	0.570	0.225	1.234	1.903
RSDr (%)	5.90	2.76	3.15	3.63
r	1.65	0.63	3.46	5.33
S_R	1.681	0.991	4.450	4.691
RSD_R (%)	14.00	12.16	11.34	8.94
R	3.92	2.77	12.46	13.13

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

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 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