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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 in the Council of Members of the International Olive Council as regards the trade standard applying to olive oils and olive pomace oils and two methods of analysis

{COM(2026) 562 final}

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DRAFT DECISION No DEC III.X/123-VI/2026

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

DECISION DEC-III.X/123-VI/2026

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 Article I "Objectives of the Agreement" 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 barriers to trade, and Chapter VI "Standardisation provisions";

Having regard to the recommendation made by the Committee on Chemistry and Standardisation at its 18th meeting, within the framework of the 123rd session of the Council of Members;

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

Considering the unanimous position agreed by the chemists at their meeting on 24 and 25 March 2026 regarding:

- The replacement of the flash point with residual hexane content, with a limit of 1,000 mg/kg.
- The replacement of the FOSFA International method with the ISO 9832 method.
- The inclusion of the method for the determination of triacylglycerol composition and the composition and content of diacylglycerols in vegetable oils by capillary gas chromatography (COI/T.20/Doc. No. 32/Rev. 1).
- The inclusion of the method for the determination of the fatty acid composition in olive oils and olive pomace oils by gas chromatography (COI/T.20/Doc. No. 33/Rev.2).
- The inclusion of the revised method for the determination of acidity in olive oils and olive pomace oils (COI/T.20/Doc. No. 34/Rev. 2).

Decides

1. To adopt the Trade Standard for Olive Oils and Olive-Pomace Oils COI/T.15/NC No. 3/Rev. 23, attached to this decision, which replaces and repeals the previous version (COI/T.15/NC No. 3/Rev. 22) of June 2026.

2. Members shall, in accordance with their respective legislations, take all appropriate measures to apply the adopted standard and shall communicate these measures to the Executive Secretariat as soon as they have been implemented.

3. Non-member countries engaged in international trade in olive oils and olive pomace oils are invited to take the adopted standard into consideration and to align their regulations with its provisions.

Lisbon, 30 June 2026

Tawfik EL ACHCHABI

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COI/T.15/NC No 3/Rev. ~~21~~ **22**
June 2026
English/Original: French

International Olive Council

Trade Standard Applying to Olive Oils and Olive Pomace Oils

COI/T.15/NC. No. 3/Rev. 2~~2~~3
June 2026
English / Original: French

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Trade Standard Applying to Olive Oils and Olive Pomace Oils

01. 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.

02. Designations and Definitions

2.1. Olive Oils

2.1.1. Virgin olive oils

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:

1. Extra virgin olive oil: virgin olive oil which has an ~~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.

2. Virgin olive oil: virgin olive oil which has an ~~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.

3. Ordinary virgin olive oil: virgin olive oil which has an ~~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 an ~~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.

[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.

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2. 1. 2. Refined olive oil

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 an ~~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

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 an ~~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

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. 1. Crude olive pomace oil

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.

2.2.2. Refined olive pomace oil

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 an ~~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.[4]

2.2.3. Olive pomace oil composed of refined oil and virgin olive oils

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 an ~~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.[5] In no case shall this blend be called "olive oil".

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”.
4 This product may only be sold direct to the consumer if permitted in the country of retail sale.
5 The country of retail sale may require a more specific designation.

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03. 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[6]
• Arachidic acid	≤ 0.60
• Gadoleic acid (eicosenoic)	≤ 0.50
• Behenic acid	≤ 0.20*
• Lignoceric acid	≤ 0.20

[6] 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

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

	%	
	C18:1 T	C18:2 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)[7]	< 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)[8]	< 0.40	≤ 0.35

3.3. Sterol and tripertene 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

7 Blend of refined olive oil and virgin olive oils

8 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|, in case of crude olive-pomace oil or refined olive-pomace oil or olive pomace oil (ROPO+VOOs).

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

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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[9]
• Refined olive oil	< 4.5[10]
• Olive oil (ROO + VOOs)	< 4.5
• Crude olive pomace oil	> 4.5[11]
• 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[9]
• Refined olive oil	<350
• Olive oil (ROO+VOOs)	< 350
• Crude olive pomace oil	> 350[11]
• Refined olive pomace oil	> 350
• Olive pomace oil (ROPO+VOOs)	> 350

[9] 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%.

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

[11] 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%.

* Pending further scientific studies and by way of exception, for extra virgin olive oils produced exclusively from olives of the Koroneiki, Nocellara del Belice or Coratina varieties—where the definition of additional parameters to establish a decision tree for verifying the authenticity of single-varietal oils is considered a priority—the minimum limit for total sterol content is set at ≥ 800 mg/kg.

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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 $\underline{\leq}$ 14.00%; 2P $\underline{\leq}$ 0.9%
  C16:0 > 14.00%, 2P $\underline{\leq}$ 1.0%

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

  C16:0 $\underline{\leq}$ 14.00%; 2P $\underline{\leq}$ 0.9%
  C16:0 > 14.00%, 2P $\underline{\leq}$ 1.1%

- Olive pomace oil (ROPO+VOOs) $\underline{\leq}$ 1.2%
- Crude and refined olive pomace oils $\underline{\leq}$ 1.4%

3.8. Unsaponifiable matter (g/kg)

- Olive oils < 15
- Olive pomace oils < 3

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04. 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)	Me = 0.0	0.0 < Me < 3.5	3.5 <Me < 6.0**	Me > 6.0					
	Median of the fruity attribute (Me)	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 ~~Free a~~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 milliequivalents of active oxygen per kg		≤ 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.

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		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 ($K1^{\%}$) 1cm	270 nm (cyclohexane) / 268nm (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 (°C)~~ Residual hexane content (mg/kg)								~~> 120°C~~ ≤ 1°000		
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, mg/kg))		≤ 35								
4.10. Phenols content		See section 11.20								

* 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.

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05. 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).

06. 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.

07. 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).

08. 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;

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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.

09. 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[12]
- Refined olive oil[12]
- Olive oil (ROO+VOOs)[13]

[12] This product may only be sold direct to the consumer if permitted in the country of retail sale.
[13] The country of retail sale may require a more specific designation.

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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.

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, thecountry in whichsuchprocessingiscarriedoutshall be considered as the countryoforiginfor 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.

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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:

10.3.1.1. 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.

10.3.1.2. Net contents

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

10.3.1.3. Name and address

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

10.3.1.4. Country of origin

The name of the exporting country shall be declared.

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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.

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.~~1~~2, "Determination of fatty acid ~~methyl esters~~ composition in olive oils 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.

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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.11, "Organoleptic assessment of virgin olive oil".

11.10 Determination of the ~~free~~ acidity

According to COI/T.20/Doc. No. 34/Rev.~~1~~2, “Method for the determination of acidity in olive oils and olive pomace oils ~~Determination of free fatty acids, cold method~~”.

11.11 Determination of the peroxide value

According to COI/T.20/Doc. No. 38 and 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~~ residual hexane content

According to ISO 9832:2002, “Technical residual hexane content in animal and vegetable oils” ~~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”.

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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 Determination of the content of waxes and alkyl esters

According to COI/T.20/Doc. No. 28/Rev.4, "Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography".

11.20 Determination of phenolic compounds

According to COI/T.20/Doc. No. 29/Rev.2, "Document to declare the use of IOC methods for phenolic compounds determination"

11.21 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".

11.22 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".

11.23 Determination of the triacylglycerols and di-acylglycerols

According to COI/T.20/Doc. No. 32/Rev.1, "Method of determination of composition of triacylglycerols and composition and content of di-acylglycerols by capillary gas chromatography, in vegetable oils".

11.24. Determination of volatile compounds

According to COI/T.20/Doc. No. 37, "Method of determination of volatile compounds in virgin olive oils by SPME-GC-FID/MS".

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DRAFT DECISION DEC-III.X/123-VI/2026

Adopting the method of analysis for the determination of acidity in olive oils and olive pomace oils

DECISION NO DEC-III.X/123-VI/2026

Adopting the method of analysis for the determination of acidity in 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" relating to standardisation and research aimed at harmonising national and international legislation, as well as physicochemical and organoleptic analysis in order to improve knowledge of the composition and quality characteristics of olive products;

Having regard to the recommendation made by the Committee on Chemistry and Standardisation at its 18th meeting, within the framework of the 123rd session of the Council of Members;

Considering the unanimous position agreed by the chemist experts at their meeting of 24 and 25 March 2026, with a view to the duly justified revision of the said method;

Decides

To revise document COI/T.20/Doc. No. 34/Rev. 1 “Determination of free fatty acids, cold method”. Document COI/T.20/Doc. No. 34/Rev. 2, “Method for the determination of acidity in olive oils and olive pomace oils”, replaces and repeals method COI/T.20/Doc.No. 34/Rev. 1.

Lisbon, 30 June 2026

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METHOD OF ANALYSIS

Determination of ~~FREE FATTY ACIDS~~ acidity, ~~COLD METHOD~~ in olive oils and olive pomace oils ~~TITRIMETRIC OR POTENTIOMETRIC DETERMINATION~~

1. Scope and Field of Application

This document describes a method for the determination of free fatty acids in olive oils and olive pomace oils, using either titrimetric endpoint detection with a suitable indicator or potentiometric endpoint detection. The free fatty acid content is expressed as acidity, calculated as a percentage of oleic acid.

2. Principle

A sample is dissolved in a mixture of solvents and the free fatty acids present are titrated using a potassium hydroxide or sodium hydroxide solution.

3. Reagents

All the reagents should be of recogni~~z~~sed analytical quality and the water used either distilled or of equivalent purity.

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3.1. Diethyl ether; and 95 % ethanol (v/v) :, mixture of equal parts by volume. Neutrali~~z~~se precisely at the moment of use with the potassium hydroxide solution (3.2), with the addition of 0.3 m~~l~~L of the phenolphthalein indicator solution (3.3) per 100 mlL of mixture.

Ethanol may be replaced by propanol-2 (99 %) and diethyl ether may be replaced by ter-butylmethylether or petroleum ether[1].

~~*Note 1: Diethyl ether is highly inflammable and may form explosive peroxides. Special care should be taken in its use.*~~

~~*Note 2: If it is not possible to use diethyl ether, a mixture of solvents containing ethanol and toluene may be used. If necessary, ethanol may be replaced by propanol-2.*~~

3.2. Potassium hydroxide or sodium hydroxide, titrated ethanolic or aqueous solution, c(KOH) about 0.1 mol/~~l~~L or, if necessary, c(KOH) about 0.5 mol/~~l~~L. Commercial solutions are available. The exact concentration of the potassium hydroxide solution (or sodium hydroxide solution) must be known and checked prior to use. Use a solution prepared at least five days before use and decanted into a brown glass bottle with a rubber stopper. The solution should be colourless or straw coloured.

If phase separation is observed when using an aqueous solution of potassium hydroxide (or sodium hydroxide), replace the aqueous solution by an ethanolic solution[2].

3.3. Phenolphthalein, 10 g/~~l~~L solution in 95 to 96 % ethanol (v/v), alkali blue 6B or thymolphthalein, 20 g/L~~l~~ solution in 95 to 96 % ethanol (v/v). In the case of strongly coloured oils, alkali blue or thymolphthalein shall be used.

4. Apparatus

Usual laboratory equipment, including:

4.1 Analytical balance, capable of ~~being read~~ reading to the nearest 0.~~,~~001 g~~;~~

[1] Diethyl ether is highly ~~in~~flammable and may form explosive peroxides. Special care ~~should~~ shall be taken in its use. Petroleum ether is ~~In~~flammable and potentially toxic. Phenolphthalein is classified as CMR substance, whereas thymolphthalein and alkali blue are not. Phenolphthalein is a known carcinogenic compound and ~~should~~ shall only be used when no suitable alternative is available

[2] A stable colourless solution of potassium hydroxide (or sodium hydroxide) may be prepared as follows:. B~~b~~ring to the boil 1 000 m~~l~~L of ethanol or water with 8 g of potassium hydroxide (or sodium hydroxide) and 0.5 g of aluminium shavings, and continue boiling under reflux for one hour. Distil immediately. Dissolve in the distillate the required quantity of potassium hydroxide (or sodium hydroxide). Leave for several days and decant the clear supernatant liquid from the precipitate of potassium carbonate (or sodium carbonate). The solution may also be prepared without distillation as follows: ~~to 1 000 ml of ethanol (or water)~~ add 4 mL~~l~~ of aluminium butylate to 1000 mL of ethanol (or water) and leave the mixture for several days. Decant the supernatant liquid and dissolve the required quantity of potassium hydroxide (or sodium hydroxide). The solution is then ready for use.

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4.2 250 m~~l~~L conical flask;

4.3 ~~10 ml b~~ Class A ~~B~~burette ~~class A~~ (10 mL~~l~~ or 5 mL~~l~~), graduated in 0,.05 ~~ml~~ mL or 0,02 m~~l~~L, or an equivalent automatic burette.

4.4 Automatic titrator with processor, dosing device, stirrer and electrodes (4.5), or a potentiometer.

~~If other apparatus is used, the procedure shall be optimized for the relevant apparatus. The apparatus shall be able to perform a dynamic titration (fast at the beginning, slow near the endpoint). This is necessary to minimize the titration time whilst achieving a slow titration near the endpoint.~~ If a different apparatus is used, the procedure shall be optimised accordingly. The apparatus must be capable of performing a dynamic titration—fast at the start and slower near the endpoint.

This is necessary to minimise titration time while ensuring accuracy near the endpoint.

4.5 Combined pH electrode for non-aqueous acid/base titrations.

4.6 Beaker of 250 mL~~, of~~ capacity ~~250 ml~~, and ~~of~~ a tall form.

5. PROCEDURE

~~5.1 Preparation of the test sample~~

When the sample is cloudy, it ~~should~~ shall be filtered.

~~5.2 Test portion~~

Take a sample depending on the presumed acidity in accordance with the following table:

Expected acidity (oleic acid g/100 g	Mass of sample (g)	Weighing accuracy (g)
0 to 2	10	0.02
> 2 to 7.5	2.5	0.01
> 7.5	0.5	0.001

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5.1. ~~5.3.~~ Titrimetric determination using an indicator

Weigh the sample in the conical flask (4.2).

Dissolve the sample ~~(5.2)~~ in 50 mL to 100 m~~l~~L of the previously neutrali~~z~~sed mixture of diethyl ether and ethanol (3.1).

Titrate while stirring with the 0.1 mol/L~~l~~ solution of potassium hydroxide (3.2)[3] ~~(see Note 4)~~ until the indicator changes (the colour of the coloured indicator persists for at least 10 seconds).[4]

~~Note 4: If the quantity of 0.1 mol/l potassium hydroxide solution required exceeds 10 ml, use the 0.5 mol/l solution or change the sample mass according to the expected free acidity and the proposed table.~~

~~Note 5: If the solution becomes cloudy during titration, add enough of the solvents (3.1) to give a clear solution.~~

Carry out a second determination only if the first result is higher than the specified limit for the oil category ~~of the oil~~.

5.2. Potentiometric determination

Weigh the sample in~~to~~ a 250 mL~~-~~beaker (4.6).

Add 50 mL to 100 mL of the neutralised solvent mixture (3.1) and dissolve the sample, if necessary, with gentle warming.

Introduce the combined electrode (4.5) into the solvent mixture and connect it to the automatic titration apparatus (4.4).

[3] If the quantity of 0.1 mol/L~~l~~ potassium hydroxide solution required exceeds 10 mL~~l~~, use the 0.5 mol/L~~l~~ solution or change the sample mass according to the expected free acidity and the proposed table.
[4] If the solution becomes cloudy during titration, add ~~enough of the solvents~~ sufficient solvent (3.1) to ~~give~~ obtain a clear solution.

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6. Expression of Results

Acidity, as percentage of oleic acid by weight is equal to:

$$V \times c \times \frac{M}{1\,000} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m}$$

where:

- V: = the volume of titrated potassium hydroxide solution used, in milliliters;

- c =: the exact concentration in moles per litre of the titrated solution of potassium hydroxide used;

- M = : 282 g/mol, the molar mass in grams per mole of oleic acid;

- m =: the mass of the sample, in grams.

Oleic acidity is reported as follows:

- ~~(a)~~ to two decimal places for values from 0 up to and including 1;

- ~~(b)~~ to one decimal place for values from 1 up to and including 100

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Precision Values of the Method
Analysis of the collaborative test results

More than ~~20~~ twenty laboratories took part in the collaborative ~~test~~ study ~~arranged~~ conducted by the International Olive Council (IOC) in 2014 and 2015.

The test was organized by the Deutsches Institut für Normung (DIN) in 2004/2005. The results obtained were subjected to statistical analysis in accordance with ISO 5725 1 and ISO 5725 2 to give the precision data shown in the tables 1 and 2.

The ~~test was performed~~ study was carried out on five ~~5~~ samples in 2014 and ~~3~~ three samples in 2015 using titrimetric determination:

- PA 1-2014: crude olive pomace oil
- PA 2-2014: refined olive pomace oil
- PA 3-2014: refined olive oil
- PA 4-2014: extra virgin olive oil (Mario Solinas prizeQualtiy Award) – 2011 crop year
- PA 5-2014: extra virgin olive oil (Mario Solinas prizeQuality Award) – 2014 crop year
- PA 6-2015: 70 % lampante olive oil + 30 % grape seed oil
- PA 7-2015: late harvest extra virgin olive oil
- PA 8-2015: 90 % lampante olive oil + 10 % palm olein

The results of the collaborative tests ~~have been~~ were statistically processed a~~ccording~~ in accordance t~~o the rules laid down in~~ with the international standard~~s~~ ISO 5725-2. Outliers were examined by applying Cochran's and Grubbs' tests to the laboratory results for each determination (replicates a and b) and each sample.

The table lists:

- n: number of participating laboratories
- outliers: number of laboratories with outlying values
- mean: mean of the accepted results (g/100g)

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- S_r: repeatability standard deviation (g/100g)
- R~~D~~SD$_r$ (%) relative standard deviation for repeatability (S_r x 100/mean)
- 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 intervals may be expected to lie with a probability of 95% (S_r ~~multiplied by~~ x 2.8) (g/100g)
- S_R: reproducibility standard deviation (g/100g)
- R~~D~~SD$_R$ (%): relative standard deviation for reproducibility (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~~ by different operators using different equipment may be expected to lie with a probability of 95% (S_R ~~multiplied by~~ x 2.8) (g/100g)

	A	B	C	D	E	F	G	H
n	22	22	22	22	22	20	20	20
outliers	1	1	2	2	0	2	3	2
mean	6,3	0,11	0,07	0,13	0,15	1,4	0,50	0,69
r	0,144	0,019	0,018	0,011	0,021	0,015	0,018	0,022
S_r	0,052	0,007	0,006	0,004	0,007	0,005	0,006	0,008
RSD_r(%)	0,8	6,1	9,3	3,2	4,8	0,4	1,3	1,1
R	0,535	0,074	0,043	0,053	0,100	0,121	0,074	0,085
S_R	0,191	0,027	0,015	0,019	0,036	0,043	0,026	0,030
RSD_R(%)	3,0	24,2	22,7	14,7	23,3	3,1	5,3	4,4

As part of the determination of the precision values of the ISO 660 standard, an international collaborative ~~test~~ study was organi~~z~~sed by ~~the ISO~~ ~~(~~the International Organi~~z~~sation for Standardi~~z~~sation~~)~~ (ISO), involving 41 laboratories from different ~~international~~ countries using the methods described in clauses 5.1 and 5.2. The results obtained ~~on~~ for olive oils were subjected to statistical analysi~~s~~es in accordance with ISO 5725:1986 and ISO 5725 (all parts), in order to ~~give~~ establish the precision data shown in the ~~followed~~ table below:

Sample	Extra virgin olive oil	Lampante olive oil		Olive oil
Number of participating laboratories, N	39	28	28	37

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Number of laboratories retained after eliminating outliers, n	37	26	24	37
Number of individual test results of all laboratories on each sample, n_z	74	52	48	74
Mean value $\overline{W}_{FFA}$, in % mass fraction	0.,343	3.,80	19.,55	0.,604
Repeatability standard deviation, s_r, in %	0.,007	0.,03	0.,09	0.,012
Repeatability coefficient of variation, $C_{V,r}$, in %	1.,9	0.,8	0.,5	1.,9
Repeatability limit, r (s_r × 2,8), in %	0.,018	0.,07	0.,26	0.,033
Reproducibility standard deviation, ~~s~~S_R, in %	0.,019	0.,12	0.,60	0.,035
Reproducibility coefficient of variation, $C_{V,R}$, in %	5.,5	3.,2	3.,1	5.,8
Reproducibility limit, *R* (s_R × 2,8), in %	0.,053	0.,33	1.,67	0.,098

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
- ISO 660:2020 Animal and vegetable fats and oils — Determination of acid value and acidity

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DRAFT DECISION DEC-III-X/12-V3I/2026

Concerning the method of analysis for the determination of the fatty acid composition in olive oils and olive pomace oils by gas chromatography

Decision DEC-III-X/123-VI/2026

Concerning the method of analysis for the determination of the fatty acid composition in olive oils and olive pomace oils by gas chromatography

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”, relating to standardisation and research aimed at harmonising national and international legislation, as well as physico-chemical and organoleptic analysis in order to improve knowledge of the composition and quality characteristics of olive products;

Having regard to the recommendation made by the Committee on Chemistry and Standardisation at its 18th meeting, within the framework of the 123rd session of the Council of Members;

Considering the unanimous position agreed by the chemist experts at their meeting of 24 and 25 March 2026, with a view to the duly justified revision of the said method;

Decides

To revise document COI/T.20/Doc. No. 33/Rev. 1 “Determination of the fatty acid composition in olive oils and olive pomace oils by gas chromatography”.

Document COI/T.20/Doc. No. 33/Rev. 2 replaces and repeals its previous version.

Lisbon, 30 June 2026

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INTERNATIONAL OLIVE COUNCIL

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Method of analysis

Determination of the fatty acid composition in olive and olive pomace oils by gas chromatography

1. Scope

This ~~standard~~ document gives guidance on the gas chromatographic determination of ~~free and~~ bound fatty acids in vegetable fats and oils ~~following their conversion into fatty acid methyl esters (FAME)~~.

The bound fatty acids of the triacylglycerols (TAGs)~~, and, depending on the esterification method, the free fatty acids (FFA),~~ are converted into fatty acid methyl esters (FAME), which are determined by capillary gas chromatography.

This method permits determination of FAME from C12 to C24, including saturated, cis- and trans-monounsaturated and cis- and trans-polyunsaturated fatty acid methyl esters. It also allows the quantification of fatty acid ethyl esters (FAEE) present in an oil.

2. Principle

Gas chromatography (GC) is used for the quantitative analysis of FAME. The FAME are prepared according to Part 1 of this method. They are then injected into and vaporised within the injector. The separation of FAME is performed on analytical columns of specific polarity and length. A Flame Ionisation Detector (FID) is used for the detection of the FAME. The conditions of analysis are given in Part 2 of this method.

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Hydrogen or helium may be used as the carrier gas (mobile phase) in the gas chromatography of FAME with FID. Hydrogen speeds up separation and gives sharper peaks. The stationary phase is a microscopic layer of a thin liquid film on an inert solid surface made of fused silica. As they pass through the capillary column the volatilised compounds being analysed interact with the stationary phase coating the inner surface of the column. Due to this different interaction of different compounds, they elute at a different time, which is called the retention time of the compound for a given set of analysis parameters. The comparison of the retention times is used for the identification of the different compounds.

Part 1. Preparation of the fatty acid methyl esters from olive oil and olive-pomace oil.

1. Scope

This method specifies the preparation of the methyl esters of fatty acids. It includes methods for preparing fatty acid methyl esters from olive and olive-pomace oils.

2. Field of application

Category of oil	Method
Virgin olive oil with acidity ≤ 2.0 %	1. Fatty acids 2. *trans*-Fatty acids 3. ΔECN42 and Global Method (after purification with silica-gel SPE)
Refined olive oil	
Olive oil composed of refined olive oil and virgin olive oils	
Refined olive pomace oil	
Olive pomace oil	
Virgin olive oil with acidity > 2.0 % Crude olive pomace oil	1. Fatty acids (after purification with silica-gel SPE) 2. *trans*-Fatty acids (after purification with silica-gel SPE) 3. ΔECN42 and Global Method (after purification with silica-gel SPE)

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3. Methodology

3.1. Trans-esterification with methanolic solution of potassium hydroxide at room temperature.

3.1.1. Principle

Methyl esters are formed by trans-esterification with methanolic potassium hydroxide as an intermediate stage before saponification takes place.

3.1.2. Reagents

3.1.2.1. Methanol containing not more than 0.5 % (m/m) water.

3.1.2.2. Hexane, chromatographic quality. (Hexane may be replaced by iso-octane (2,2,4-trimethyl pentane in chromatography grade, according with ISO NF EN ISO 12966-2 Method: Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids. However, this is preferred due to the toxicity of hexane.)

3.1.2.3. Heptane, chromatographic quality. Heptane may be replaced by iso-octane (2,2,4-trimethyl pentane in chromatography grade.

3.1.2.4. Diethyl ether, stabilised for analysis.

3.1.2.5. Acetone, chromatographic quality.

3.1.2.6. Elution solvent for purifying the oil by column/SPE chromatography, mixture hexane/diethyl ether 87/13 (v/v).

3.1.2.7. Potassium hydroxide, approximately 2M methanolic solution: dissolve 11.2 g of potassium hydroxide in 100 mL of methanol.

3.1.2.8. Silica gel cartridges, 1 g (6 mL), for solid phase extraction.

3.1.3. Apparatus

3.1.3.1. Screw-top test tubes (5 mL volume) with cap fitted with a PTFE joint.

3.1.3.2. Graduated or automatic pipettes, 2 mL and 0.2 mL.

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3.1.4. Purification of oil samples

When necessary, the samples will be purified by passing the oil through a silica gel solid- phase extraction cartridge. A silica gel cartridge (3.1.2.8) is placed in a vacuum elution apparatus and washed with 6 mL of hexane (3.1.2.2); washing is performed without vacuum. Then a solution of the oil (0.12 g approximately) in 0.5 mL of hexane (3.1.2.2) is loaded onto the column. The solution is pulled down and then eluted with 10 mL of hexane/diethyl ether (87:13 V/V) (3.1.2.6). The combined eluates are homogenised and divided in two similar volumes. An aliquot is evaporated to dryness in a rotary evaporator under reduced pressure at room temperature. The residue is dissolved in 1 mL of heptane for fatty acid methyl ester preparation and analysis by GC (3.1.6). The second aliquot is evaporated and the residue is dissolved in 1 mL of acetone for triglyceride analysis by HPLC, if necessary.

3.1.5. Procedure

In a 5 mL screw-top test tube (3.1.3.1) weigh approximately 0.1 g of the oil sample. Add 2 mL of heptane (3.1.2.2), and shake. Add 0.2 mL of the methanolic potassium hydroxide solution (3.1.2.7), put on the cap fitted with a PTFE joint, tighten the cap, and shake vigorously for 30 seconds. Leave to stratify until the upper solution becomes clear. Decant the upper layer containing the methyl esters. The heptane solution is ready for injection into the gas chromatograph. It is advisable to keep the solution in the refrigerator until gas chromatographic analysis. Storage of the solution for more than 12 hours is not recommended.

Part 2. Analysis of fatty acid methyl esters by gas chromatography

1. Scope

This method gives general guidance for the application of capillary column gas chromatography to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters obtained in accordance with the method specified in Part 1 of this method.

The method is not applicable to polymerised fatty acids.

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2. Reagents

2.1. Carrier gas

Inert gas (helium or hydrogen), thoroughly dried and with an oxygen content of less than 10 mg/kg.[1]

~~Note 3: Hydrogen can double the speed of analysis but is hazardous. Safety devices are available.~~

2.2. Auxiliary gases

2.2.1. Hydrogen (purity ≥ 99.9 %), free from organic impurities.

2.2.2. Air or oxygen, free from organic impurities.

2.2.3. Nitrogen (purity > 99%).

2.3. Reference standard

Mixture of methyl esters of pure fatty acids, or the methyl esters of a fat of known composition, preferably similar to that of the fatty matter to be analysed. Cis and trans isomers of octadecenoic, octadecadienoic and octadecatrienoic methyl esters are useful for the identification of trans isomers of unsaturated acids.

Care should be taken to prevent the oxidation of polyunsaturated fatty acids.

3. Apparatus

The instructions given are for the usual equipment used for gas chromatography, employing capillary columns and a flame-ionisation detector.

3.1. Gas chromatograph

The gas chromatograph shall comprise the following elements.

3.1.1. Injection system

Use an injection system with capillary columns, in which case the injection system should be specially designed for use with such columns. It may be of the split type or the splitless on-column injector type.

3.1.2. Oven

The oven shall be capable of heating the capillary column to a temperature of at least 260 °C and of maintaining the desired temperature to within 0.1°C. The last requirement is particularly important when a fused silica tube is used.

The use of temperature-programmed heating is recommended in all cases, and in particular for fatty acids with less than 16 carbon atoms.

[1] *Hydrogen can reduce analysis time by up to 50% but is hazardous; suitable safety devices shall therefore be used.*

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3.1.3. Capillary column

3.1.3.1. Tube, made of a material inert to the substances to be analysed (usually glass or fused silica). The internal diameter shall be between 0.20 to 0.32 mm. The internal surface shall undergo an appropriate treatment (e.g. surface preparation, inactivation) before receiving the stationary phase coating.

A length of 60 m is sufficient for fatty acid and cis and trans isomers of fatty acids.

3.1.3.2. Stationary phase, polar polysiloxane (cyanopropylsilicone) bonded (cross-linked) columns are suitable.[2]

~~*Note 4: There is a risk that polar polysiloxanes may give rise to difficulties in the identification and separation of linolenic acid and C20 acids.*~~

The coatings shall be thin, i.e, 0.1 to 0.2 µm.

3.1.3.3. Assembly and conditioning of the column

Observe the normal precautions for assembling capillary columns, i.e. arrangement of the column in the oven (support), choice and assembly of joints (leak tightness), positioning of the ends of the column in the injector and the detector (reduction of dead-spaces). Place the column under a flow of carrier gas (e.g. 0.3 bar (30 kPa) for a column of length 25 m and internal diameter 0.3 mm).

Condition the column by temperature programming of the oven at 3 °C/min from ambient temperature to a temperature 10 °C below the decomposition limit of the stationary phase. Maintain the oven at this temperature for one hour until stabilisation of the baseline. Return it to 180 °C to work under isothermal conditions.[3]

~~Note 5: Suitably pre-conditioned columns are available commercially~~

3.1.4. Flame ionisation detector and converter-amplifier

3.2. Syringe

The syringe shall have a maximum capacity of 10 µl, graduated in 0.1 µl divisions.

3.3. Data acquisition system

Data acquisition system connected online with the detectors and employed with a software program suitable for peak integration and normalisation.

[2] *Polar polysiloxanes may cause difficulties in the identification and separation of linolenic acid and C20 acids.*

[3] *Suitable pre-conditioned columns are available commercially available.*

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4. Procedure

The operations described in 4.1 to 4.3 are for the use of a flame-ionisation detector.

4.1. Test conditions

4.1.1. Selection of optimum operating conditions for capillary columns

Owing to the efficiency and permeability of capillary columns, the separation of the constituents and the duration of the analysis are largely dependent on the flow-rate of the carrier gas in the column. It will therefore be necessary to optimise the operating conditions by adjusting this parameter (or simply column head loss) depending on whether the aim is to improve separation or speed up analysis.

The following conditions have proved to be suitable for the separation of FAMEs ~~(C4 to C26)~~. Examples of chromatograms are shown in Annex B:

Injector temperature: 250 °C

Detector temperature: 250 °C

Oven temperature: 165 °C (8 min) to 210 °C at 2 °C/min
Carrier gas hydrogen: column head pressure, 179 kPa
Total flow: 154.0 mL/min;

Split ratio: 1:100

Injection volume: 1 µl

4.1.2. Determination of the resolution (see A~~ppendix~~ Annex A)

Calculate the resolution, R, of two neighbouring peaks I and II, using the formula:

$$R = 2\,\frac{d_{r(II)} - d_{r(I)}}{\omega_{(I)} + \omega_{(II)}} \quad \text{or} \quad R = 2\,\frac{t_{r(II)} - t_{r(I)}}{\omega_{(I)} + \omega_{(II)}}$$

(USP) (United States Pharmacopeia)

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Or $$R = 1.18 \frac{t_{r(II)} - t_{r(II)}}{\omega_{0.5(I)} + \omega_{0.5(II)}}$$ (EP, BP, JP, DAB), (JP (Japanese Pharmacopeia), EP (Pharmacopée Européenne), (BP (British Pharmacopeia))

where:

$d_{r(I)}$ is the retention distance of peak I;

$d_{r(II)}$ is the retention distance of peak II;

$t_{r(I)}$ is the retention time of peak I ;

$t_{r(II)}$ is the retention time of peak II ;

$\omega_{(I)}$ is the width of the base of peak I;

$\omega_{(II)}$ is the width of the base of peak II;

$\omega 0.5$ is the peak width of the specified compound, at mid-height of the peak;

If $\omega_{(I)} \approx \omega_{(II)}$, calculate R using the following formulas:

$$R = \frac{d_{r(II)} - d_{r(I)}}{\omega} = \frac{d_{r(II)} - d_{r(I)}}{4\sigma}$$

where:

σ is the standard deviation (see ~~Appendix~~ Annex A, Figure 1).

If the distance dr between the two peaks $d_{r(II)} - dr_{(I)}$ is equal to 4σ, the resolution factor R = 1.

If two peaks are not separated completely, the tangents to the inflection points of the two peaks

intersect at point C. In order to completely separate the two peaks, the distance between the two peaks must be equal to:

$d_{r(II)} - dr_{(I)} = 6\ \sigma$ from where R = 1.5 (see ~~Appendix~~ Annex A, Figure 3).

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5. Expression of Results

5.1. Qualitative analysis

Identify the methyl ester peaks of the sample from the chromatograms in Annex ~~I~~ B, if necessary by interpolation, or as described in 6.~~4~~.1.

5.2. Quantitative analysis

5.2.1. Determination of the composition

Calculate the mass fraction (wi) of the individual fatty acid methyl esters, and, where present, the ethyl esters, expressed as a percentage by mass of the total methyl and ethyl esters, as follows:

5.2.2. Method of calculation

5.2.2.1. General case

Calculate the content of a given component i, expressed as a percentage by mass of methyl esters, or of methyl and ethyl esters, ~~by determining~~ from the percentage ~~represented by the~~ area of the corresponding peak relative to the sum of the areas of all the peaks, using the following formula:

$$W_i = \frac{A_i}{\sum A} \quad X100$$

where:

Ai is the area under the peak of the individual fatty acid methyl ester i;

ΣA is the sum of the areas under all the peaks of all the individual fatty acid methyl esters or of methyl and ethyl esters. The results are expressed to two decimal places.[4]

~~Note 6: For fats and oils, the mass fraction of the fatty acid methyl esters is equal to the mass fraction of the triacylglycerols in grams per 100 g. For cases in which this assumption is not allowed, see 5.2.2.2.~~

[4] *For fats and oils, the mass fraction of the fatty acid methyl esters is equal to the mass fraction of the triacylglycerols in grams per 100 g.*

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6. Peaks (of methyl and ethyl esters) taking into account for the calculation of each fatty acid

Figure 1 of ANEX B gives the typical gas chromatographic profile of an olive-pomace oil containing methyl and ethyl esters of fatty acids, and trans-isomers of methyl esters.

6.1. For the calculation of the fatty acid composition and Δ ECN42, all the following fatty acids will be taken into account:

Myristic (C14:0)

Palmitic (C16:0). Sum of the areas of the peaks corresponding to the methyl and ethyl esters.

Palmitoleic (C16:1). Sum of the areas of the peaks corresponding to the Δ 9 and Δ 7 isomers of the methyl ester.

Margaric (C17:0).

Margaroleic (C17:1).

Stearic (C18:0).

Oleic (C18:1). Sum of the areas of the peaks corresponding to the Δ 9 and Δ 7 isomers of the

methyl ester, ethyl ester, and trans-isomers of the methyl ester.

Linoleic (C18:2). Sum of the areas of the peaks corresponding to the methyl and ethyl esters, and the trans-isomers of the methyl ester.

Arachidic (C20:0).

Linolenic (C18:3). Sum of the areas of the methyl ester and the trans-isomers of the methyl ester. Eicosenoic (C20:1).

Behenic (C22:0). Lignoceric (C24:0).

Squalene will not be taken into account for the calculation of the total area.

~~Trans-isomers:~~

~~For the calculation of the percentage of trans-C18:1 the peak corresponding to the methyl esters of this fatty acid will be used. For the sum [trans-C18:2 + trans-C18:3], all the peaks corresponding to the trans-isomers of these two fatty acids will be summed. For the calculation of the total area, all the peaks mentioned in 6.1. will be taken into account.Note7: Not all peaks of ethyl esters of fatty acids and trans-isomers of methyl esters may be present in the chromatograms of other oils.~~

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6.2. For the calculation of the percentage of trans fatty acids, the following approach shall be applied[5]:

The percentage of trans-C18:1 shall be calculated ~~using~~ from the peak corresponding to the methyl esters of this fatty acid, where present in the chromatogram.

The percentage of trans-C18:2 shall be obtained by summing all peaks present or appearing in the chromatogram corresponding to the trans isomers of octadecadienoic acid, including:

- trans-trans octadecadienoic isomers,
- cis-trans and trans-cis octadecadienoic isomers.

The percentage of trans-C18:3 shall be obtained by summing all peaks present or appearing in the chromatogram corresponding to the trans isomers of octadecatrienoic acid, including:

- trans-cis-trans,
- cis-cis-trans,
- cis-trans-cis,
- trans-cis-cis isomers.

The total trans fatty acids (%) shall be recorded as the sum of:

- trans-octadecenoic acid (C18:1),
- trans-octadecadienoic acid (C18:2),
- trans-octadecatrienoic acid (C18:3).

Determination of trans fatty acids

For the calculation of the percentage of trans fatty acids, the following approach shall be applied:

The percentage of trans-C18:1 shall be calculated using the peak corresponding to the methyl esters of this fatty acid, where present in the chromatogram.

The percentage of trans-C18:2 shall be obtained as the sum of all peaks present or appearing in the chromatogram corresponding to the trans isomers of octadecadienoic acid, including:

- trans-trans octadecadienoic isomers,
- cis-trans and trans-cis octadecadienoic isomers.

[5] *Not all peaks of ethyl esters of fatty acids and trans-isomers of methyl esters may be present in the chromatograms of other oils.*

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The percentage of trans-C18:3 shall be obtained by summing all peaks present or appearing in the chromatogram corresponding to the trans isomers of octadecatrienoic acid, including:

- trans-cis-trans,
- cis-cis-trans,
- cis-trans-cis,
- trans-cis-cis isomers.

The results ~~are~~ shall be expressed f~~or~~ as:

trans-octadecenoic acid (C18:1),

sum of :the sum of trans-octadecadienoic acid (C18:2) and trans-octadecatrienoic acid (C18:3).

~~For the calculation of the total area, all peaks listed in section 6.1 shall be taken into account.~~ For the calculation of the total area, all peaks listed in section 6.1, including lauric acid (C12:0), shall be taken into account~~. including lauric acid (C12:0)~~.

8.~~7.~~ Test Report

The test report shall specify the methods used for the preparation of the methyl esters and for the gas chromatographic analysis. It shall also mention all operating details not specified in this Standard Method, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all the information necessary for complete identification of the sample.

~~9~~. 8. PRECISION

~~9~~. 8.1 Results of interlaboratory test

Details of an interlaboratory test on the precision of the method are summarised in Annex C. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

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~~9~~. 8.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in ~~not~~ no more than 5% of cases be greater than r given in tables 1 to 15.

~~9~~. 8.3 Reproductibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than R given in tables 1 to 15.

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Annex A

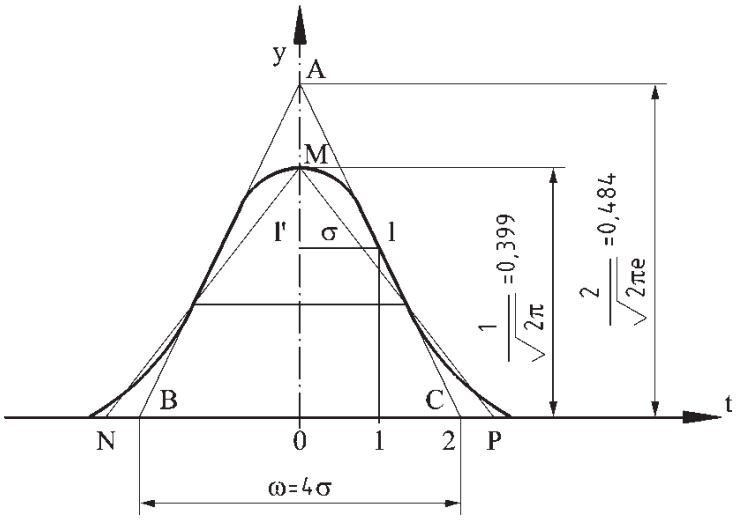


Figure 1: with ω0.5 width at half height of the triangle (ABC) and b width at half height of the triangle (NPM).

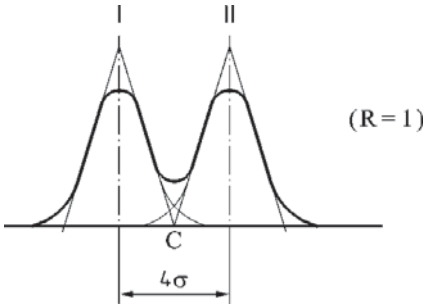


Figure 2

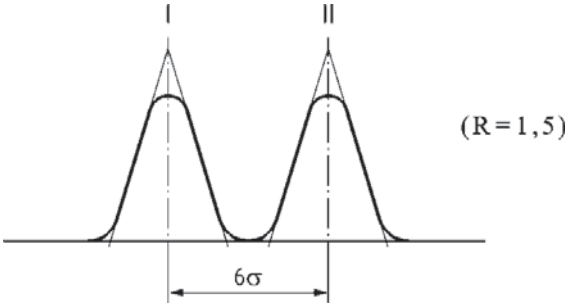
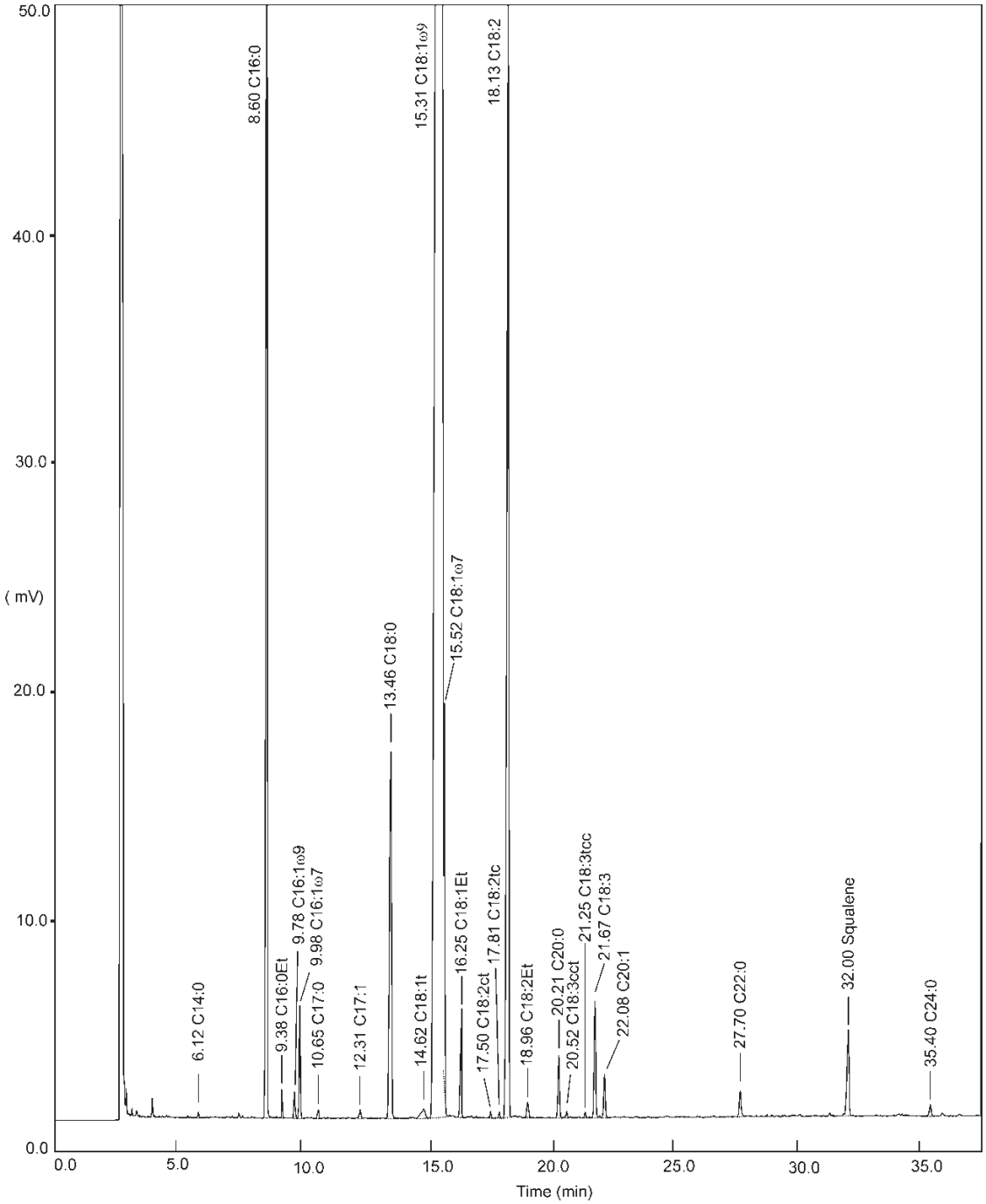


Figure 3

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Annex B



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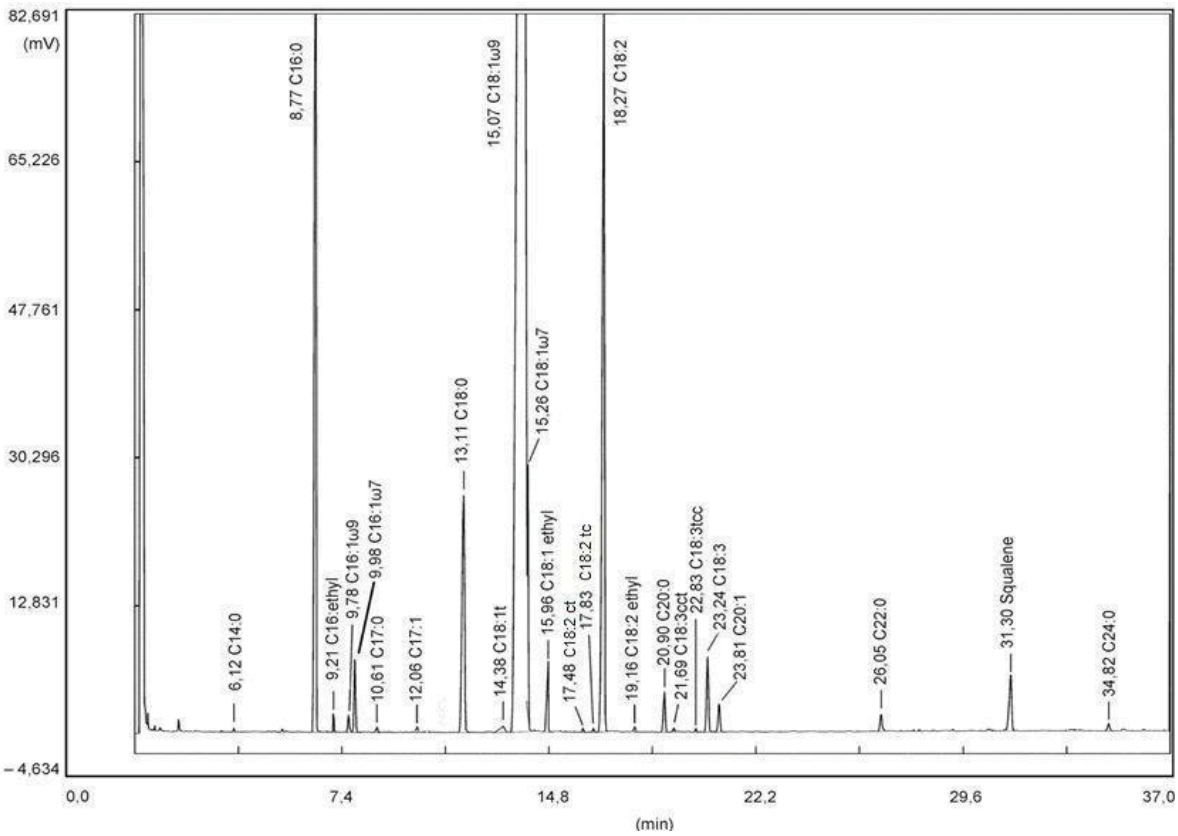


Figure 1: Gas chromatographic profile obtained by the cold methylation method from olive-pomace oil. The chromatographic peaks correspond to the methyl and ethyl esters except where otherwise indicated.

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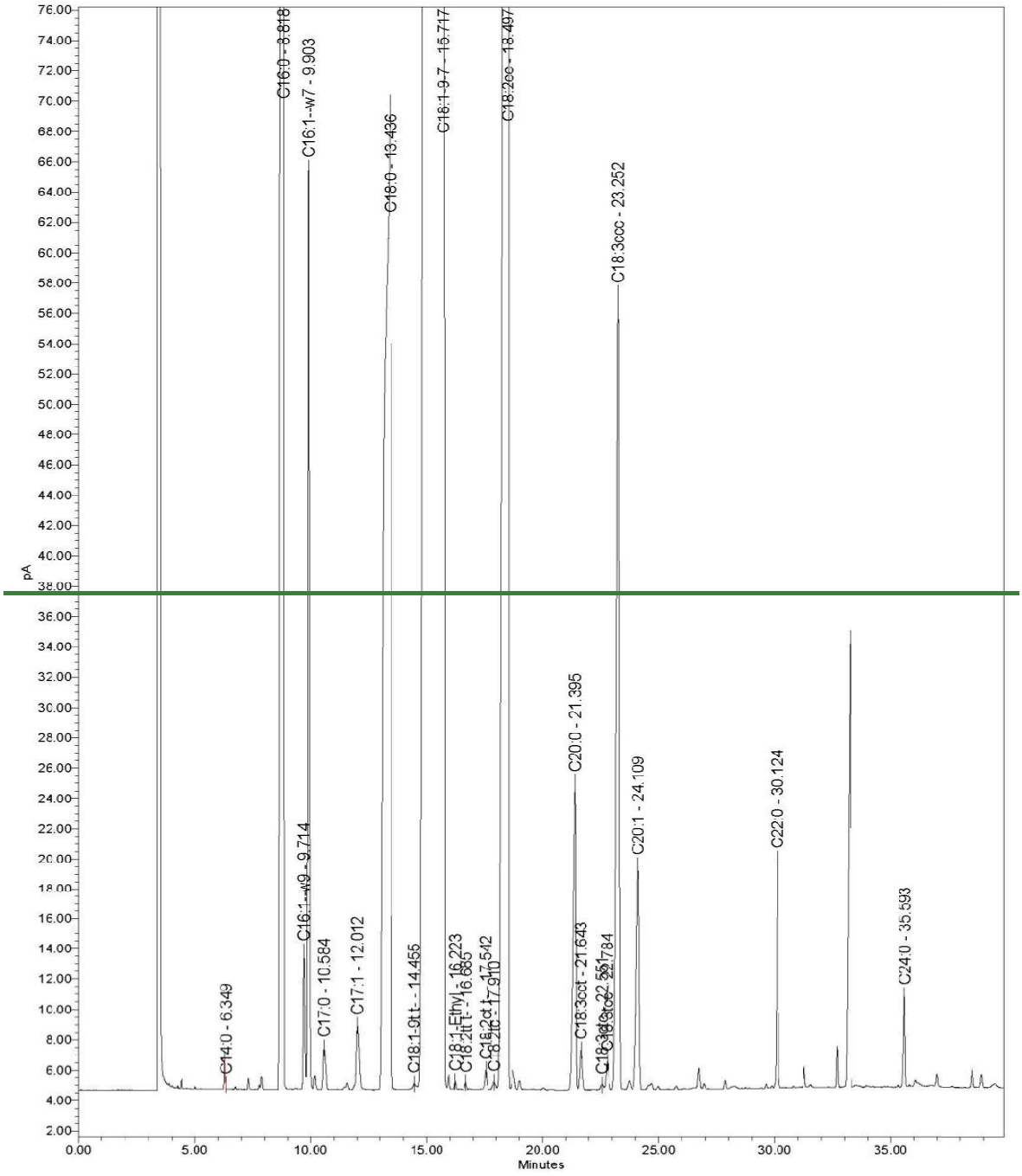


Figure 2: Gas chromatographic profile obtained by the cold methylation method f~~rom~~ for virgin olive oil. T~~he c~~Chromatographic peaks correspond to ~~the~~ methyl and ethyl esters, except where otherwise indicated.

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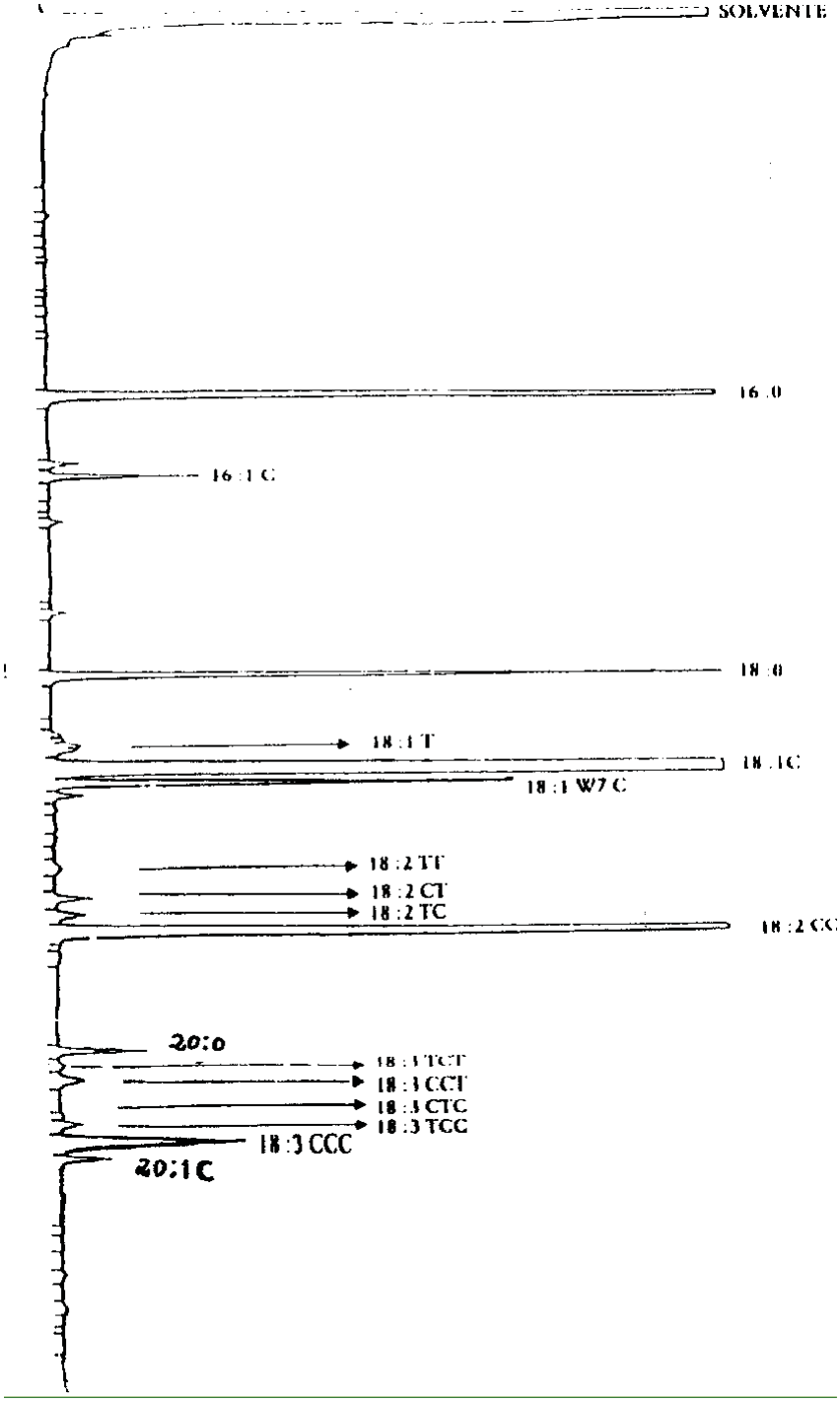


Figure 3: Specimen chromatogram obtained ~~from~~ by capillary column analysis of the trans fatty acids.

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ANNEX C

Precision values of the method

A: Extra virgin olive oil B: Virgin olive oil

C: Lampante olive oil D: Olive oil

E: Crude olive pomace oil

Table 1: Myristic acid C14:0 from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	0	1	1	3
mean	0,01	0,01	0,01	0,01	0,02
r	0,005	0,007	0,012	0,011	0,006
S_r	0,002	0,003	0,004	0,004	0,002
RSD_r(%)	20	20	36	38	11
R	0,011	0,017	0,017	0,013	0,016
S_R	0,004	0,006	0,006	0,005	0,006
RSD_R(%)	45	47	52	42	32

Table 2: Palmitic acid C16:0 from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	2	3	1	0	0
mean	7,96	10,32	10,35	10,51	9,67
r	0,12	0,18	0,42	0,29	0,38
S_r	0,04	0,06	0,15	0,1	0,14
RSD_r(%)	0,5	0,6	1,5	1,000	1,4
R	0,68	0,44	0,93	1,3	1,3
SR	0,24	0,16	0,33	0,46	0,45
RSDR (%)	3,0	1,5	3,2	4,4	4,7

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Table 3: Palmitoleic acid C16:1 from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	2	0	1	1
mean	0,50	0,68	0,74	0,91	0,64
r	0,041	0,027	0,074	0,034	0,040
S_r	0,014	0,01	0,026	0,012	0,014
RSD_r (%)	2,9	1,4	3,6	1,3	2,3
R	0,966	0,077	0,132	0,123	0,128
S_R	0,034	0,027	0,047	0,44	0,046
RSD_R (%)	6,8	4,1	6,4	4,9	7,2

Table 4: Heptadecanoic acid C17:0 - from 2000-2006 recognition data

	A	B	C	D	E
n	25	25	25	25	25
outliers	1	1	1	2	2
mean	0,18	0,06	0,11	0,14	0,12
r	0,013	0,011	0,010	0,009	0,009
Sr	0,005	0,004	0,004	0,003	0,003
RSD_r (%)	2,7	6,9	3,1	2,3	2,7
R	0,020	0,021	0,024	0,021	0,027
SR	0,007	0,007	0,009	0,008	0,010
RSD_R (%)	4,1	12,6	7,7	5,2	7,8

Table 5: Heptadecenoic acid C17:1 - from 2000-2006 recognition data

	A	B	C	D	E
n	29	29	29	29	29
outliers	3	2	2	3	2
mean	0,26	0,09	0,24	0,22	0,19
r	0,010	0,010	0,014	0,013	0,012
S_r	0,004	0,004	0,005	0,005	0,004
RSD_r (%)	1,4	3,8	2	2,2	2,2
R	0,031	0,027	0,041	0,030	0,031
S_R	0,011	0,010	0,015	0,011	0,011
RSD_R (%)	29	29	29	29	29

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Table 6: Stearic acid C18:0 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	2	0	0	0	1
mean	2,88	2,49	2,62	3,49	3,12
r	0,089	0,034	0,084	0,094	0,107
S_r	0,032	0,012	0,030	0,034	0,038
RSD_r (%)	1,1	0,5	1,1	1,0	1,2
R	0,171	0,259	0,246	0,367	0,328
S_R	0,061	0,092	0,088	0,131	0,117
RSD_R (%)	2,1	3,7	3,4	3,8	3,8

Table 7: Oleic acid C18:1 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	0	1	1	0
mean	79,42	74,55	75,55	76,14	75,8
r	0,42	0,30	0,39	0,23	0,46
S_r	0,15	0,11	0,14	0,08	0,16
RSD_r (%)	0,2	0,2	0,2	0,1	0,2
R	1,37	1,26	1,26	1,33	1,80
S_R	0,49	0,45	0,45	0,47	0,64
RSD_R (%)	0,6	0,6	0,6	0,6	0,9

Table 8: Linoleic acid C18:2 - from Ring Test COI 2015

	A	B	C	D	E
N	15	15	15	15	15
Outliers	2	1	0	1	0
Mean	7,33	9,66	8,52	7,18	8,75
r	0,07	0,08	0,17	0,12	0,13
S_r	0,02	0,03	0,06	0,04	0,05
RSD_r (%)	0,3	0,3	0,7	0,6	0,6
R	0,34	0,52	0,50	0,45	0,59
S_R	0,12	0,19	0,18	0,16	0,21
RSD_R (%)	1,7	1,9	2,1	2,2	2,4

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Table 9: Linolenic acid C18:3 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	2	0	0	0	4
mean	0,73	0,90	0,86	0,74	0,75
r	0,036	0,049	0,029	0,039	0,055
S_r	0,013	0,017	0,010	0,014	0,020
RSD_r (%)	1,8	1,9	1,2	1,9	2,6
R	0,08	0,1	0,101	0,079	0,115
S_R	0,029	0,041	0,036	0,028	0,041
RSD_R (%)	3,9	4,6	4,2	3,8	5,4

Table 10: Arachidic acid C20:0 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	1	0	0	1	0
mean	0,39	0,44	0,44	0,42	0,43
r	0,041	0,050	0,037	0,037	0,053
S_r	0,015	0,018	0,013	0,013	0,019
RSD_r (%)	3,8	4	3	3,1	4,4
R	0,080	0,089	0,086	0,117	0,102
S_R	0,029	0,032	0,031	0,042	0,036
RSD_R (%)	7,3	7,2	7	9,8	8,6

Table 11: Eicosenoic acid C20:1 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	1	1	1	0	1
mean	0,37	0,39	0,37	0,28	0,30
r	0,026	0,032	0,036	0,047	0,073
S_r	0,009	0,011	0,013	0,017	0,026
RSD_r (%)	7,8	3,0	3,5	6,0	8,9
R	0,082	0,095	0,064	0,079	0,077
S_R	0,029	0,034	0,023	0,028	0,027
RSD_R (%)	7,9	8,7	6,2	10,0	9,3

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Table 12: Behenic acid C22:0 - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	1	1	1	3
mean	0,11	0,14	0,14	0,12	0,19
r	0,022	0,036	0,039	0,045	0,036
S_r	0,008	0,013	0,014	0,016	0,013
RSD_r (%)	7,0	9,6	10,0	14,0	6,9
R	0,038	0,044	0,050	0,056	0,043
S_R	0,014	0,016	0,018	0,020	0,015
RSD_R (%)	12,0	12,0	13,0	17,0	8,3

Table 13: Lignoceric acid C24:0 - from Ring Test COI 2015

	A	B	C	D	E
	15	15	15	15	15
outliers	1	0	0	0	3
mean	0,04	0,06	0,06	0,05	0,08
r	0,017	0,015	0,033	0,033	0,040
S_r	0,006	0,005	0,012	0,012	0,014
RSD_r (%)	15,0	8,9	20,0	24,0	19,0
R	0,055	0,073	0,072	0,054	0,04
S_R	0,020	0,026	0,026	0,019	0,014
RSD_R (%)	49,0	42,0	45,0	39,0	19,0

Table 14: C18:1 trans - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	1	1	1	1	2
mean	0,01	0,01	0,01	0,01	0,12
r	0,011	0,013	0,008	0,013	0,044
S_r	0,004	0,005	0,003	0,005	0,016
RSD_r (%)	38,0	46,0	27,0	45,0	13,2
R	0,027	0,028	0,030	0,032	0,157
S_R	0,010	0,010	0,011	0,011	0,056
RSD_R (%)	96,0	86,0	100,0	89,0	48,0

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Table 15: C18:2 trans + C18:3 trans - from Ring Test COI 2015

	A	B	C	D	E
n	15	15	15	15	15
outliers	3	3	4	2	3
mean	0,01	0,01	0,01	0,01	0,03
r	0,013	0,014	0,006	0,029	0,017
S_r	0,005	0,005	0,002	0,010	0,006
RSD_r (%)	84,0	50,0	28,0	115,0	24,0
R	0,019	0,022	0,018	0,032	0,059
S_R	0,007	0,008	0,006	0,012	0,021
RSD_R (%)	123,0	79,0	81,0	130,0	83,0

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