Council of the European Union

Brussels, 16 May 2024
(OR. en)

10030/24
ADD 2

Interinstitutional File:
2024/0104(NLE)

PROBA 20
AGRI 417
WTO 71

COVER NOTE

From:	Secretary-General of the European Commission, signed by Ms Martine DEPREZ, Director
date of receipt:	8 May 2024
To:	Ms Thérèse BLANCHET, Secretary-General of the Council of the European Union
No. Cion doc.:	SWD(2024) 122 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 two methods of analyses and the IOC Trade Standard for olive oil and olive pomace oil

Delegations will find attached document SWD(2024) 122 final.

Encl.: SWD(2024) 122 final

EUROPEAN
COMMISSION

Brussels, 8.5.2024
SWD(2024) 122 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 two methods of analyses and the IOC Trade Standard for olive oil and olive pomace oil

{COM(2024) 194 final}

DRAFT DECISION No DEC-III.X/118-VI/2023

REVISING THE METHOD FOR THE ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OIL

DRAFT DECISION Nº DEC-III.X/118-VI/2023

REVISING THE METHOD FOR THE ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OIL

THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,

Having regard to the 2015 International Agreement on Olive Oil and Table Olives, in particular to Article 1 "Objectives of the Agreement" with respect to standardisation and research, as regards to achieving uniformity in national and international legislation, and in particular Chapter VI "Standardisation Provisions";

Having regard to Resolution No. RES-2/71-IV/94 of 17 November 1994 on the adoption by the IOC of method COI/T.20/Doc. No. 15 and Decision No. DEC-III.6/107-VI/2018 on the revision of the method "Organoleptic assessment of virgin olive oil" COI/T.20/Doc. No. 15/Rev.10;

Considering the decision of the organoleptic assessment experts and panel leaders recognised by the Council and appointed by the Members to revise document T.20/Doc. No. 15/Rev. 10 "Method for the organoleptic assessment of virgin olive oil";

Considering the proposal made by the Chemistry and Standardisation Committee at its 13th meeting with a view to revising the method;

Considering the unanimous position of the organoleptic experts at the meeting of 26 and 27 September 2023;

DECIDES

1. To revise the document T.20/Doc. No. 15/Rev. 10 "Method for the organoleptic assessment of virgin olive oil".
2. Method T.20/Doc. No. 15/Rev. 11 replaces and revokes method T.20/Doc. No. 15/Rev. 10.

Madrid (Spain), 23 November 2023

Mr Kaled Musa Al Henefat
Chair of the International Olive Council

INTERNATIONAL OLIVE COUNCIL

COI/T.20/Doc. No 15/Rev. ~~10~~ 11
June ~~2018~~ 2024

ENGLISH
Original: FRENCH

Príncipe de Vergara, 154 – 28002 Madrid – España Telef.: +34 915 903 638 Fax: +34 915 631 263 - e-mail: iooc@internationaloliveoil.org - http://www.internationaloliveoil.org/

SENSORY ANALYSIS OF OLIVE OIL

METHOD FOR THE ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OIL

1. PURPOSE

The purpose of this international method is to determine the procedure for assessing the organoleptic characteristics of virgin olive oil and to establish the method for its classification on the basis of those characteristics.

2. FIELD OF APPLICATION

The method described is only applicable to virgin olive oils and to the classification of such oils according to the intensity of the defects perceived and of the fruitiness, as determined by a group of tasters selected, trained and monitored as a panel.

It also provides indications for optional labelling.

3. GENERAL BASIC VOCABULARY FOR SENSORY ANALYSIS

Refer to the standard COI/T.20/Doc. no. 4 "Sensory Analysis: General Basic Vocabulary".

4. SPECIFIC VOCABULARY FOR VIRGIN OLIVE OIL

4.1. Negative attributes

Fusty/muddy

Sediment Characteristic flavour of oil obtained from olives piled or stored in such conditions as to have undergone an advanced stage of anaerobic fermentation, or of oil which has been left in contact with the sediment that settles in underground tanks and vats and which has also undergone a process of anaerobic fermentation.

Musty-humid-Earthy Characteristic flavour of oils obtained from fruit in which large numbers of fungi and yeasts have developed as a result of its being stored in humid conditions for several days or of oil obtained from olives that have been collected with earth or mud on them and which have not been washed.

Winey-vinegary Characteristic flavour of certain oils reminiscent of wine or vinegar.

Acid-sour This flavour is mainly due to a process of aerobic fermentation in the olives or in olive paste left on pressing mats which have not been properly cleaned and leads to the formation of acetic acid, ethyl acetate and ethanol.

Rancid Flavour of oils which have undergone an intense process of oxidation.

Frostbitten olives (wet wood) Characteristic flavour of oils extracted from olives which have been injured by frost while on the tree.

4.2. Other negative attributes

Heated or burnt Characteristic flavour of oils caused by excessive and/or prolonged heating during processing, particularly when the paste is thermally mixed, if this is done under unsuitable thermal conditions.

Hay–wood Characteristic flavour of certain oils produced from olives that have dried out.

Rough Thick, pasty mouthfeel sensation produced by certain old oils.

Greasy Flavour of oil reminiscent of that of diesel oil, grease or mineral oil.

Vegetable water Flavour acquired by the oil as a result of prolonged contact with vegetable water which has undergone fermentation processes.

Brine Flavour of oil extracted from olives which have been preserved in brine.

Metallic Flavour that is reminiscent of metals. It is characteristic of oil which has been in prolonged contact with metallic surfaces during crushing, mixing, pressing or storage.

Esparto Characteristic flavour of oil obtained from olives pressed in new esparto mats. The flavour may differ depending on whether the mats are made of green esparto or dried esparto.

Grubby Flavour of oil obtained from olives which have been heavily attacked by the grubs of the olive fly (*Bactrocera oleae*).

Cucumber Flavour produced when an oil is hermetically packed for too long, particularly in tin containers, and which is attributed to the formation of 2,6-nonadienal.

4.3. Positive attributes

Fruity Set of olfactory sensations characteristic of the oil which depends on the variety and comes from sound, fresh olives, either ripe or unripe. It is perceived directly and/or through the back of the nose.

Bitter Characteristic primary taste of oil obtained from green olives or olives turning colour. It is perceived in the circumvallate papillae on the "V" region of the tongue.

Pungent Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat.

4.4. OPTIONAL TERMINOLOGY FOR LABELLING PURPOSES

Upon request, the panel leader may certify that the oils which have been assessed comply with the definitions and ranges corresponding solely to the following expressions according to the intensity and perception of the attributes.

Positive attributes (fruity, bitter and pungent): According to the intensity of perception:

- ***Robust*,** when the median of the attribute is more than 6.0;
- ***Medium*,** when the median of the attribute is between 3.0 and 6.0;
- ***Delicate***, when the median of the attribute is less than 3.0.

Fruitiness Set of olfactory sensations characteristic of the oil which depends on the variety of olive and comes from sound, fresh olives in which neither green nor ripe fruitiness predominates. It is perceived directly and/or through the back of the nose.

Green fruitiness Set of olfactory sensations characteristic of the oil which is reminiscent of green fruit, depends on the variety of olive and comes from green, sound, fresh olives. It is perceived directly and/or through the back of the nose.

Ripe fruitiness Set of olfactory sensations characteristic of the oil which is reminiscent of ripe fruit, depends on the variety of olive and comes from sound, fresh olives. It is perceived directly and/or through the back of the nose.

Well balanced Oil which does not display a lack of balance, by which is meant the olfactory–gustatory and tactile sensation where the median of the bitter and/or pungent attributes is two points higher than the median of the fruitiness.

Mild oil Oil for which the median of the bitter and pungent attributes is 2.0 or less.

List of expressions according to the intensity of perception:

Expressions subject to production of an organoleptic test certificate	**Median of the attribute (Me)**
Fruitiness	-
Ripe fruitiness	-
Green fruitiness	-
Delicate fruitiness	≤ 3.0
Medium fruitiness	3.0 < Me ≤ 6.0
Robust fruitiness	> 6.0
Delicate ripe fruitiness	≤ 3.0
Medium ripe fruitiness	3.0 < Me ≤ 6.0
Robust ripe fruitiness	> 6.0
Delicate green fruitiness	≤ 3.0
Medium green fruitiness	3.0 < Me ≤ 6.0
Robust green fruitiness	> 6.0
Delicate bitterness	≤ 3.0
Medium bitterness	3.0 < Me ≤ 6.0
Robust bitterness	> 6.0
Delicate pungency	≤ 3.0
Medium pungency	3.0 < Me ≤ 6.0
Robust pungency	> 6.0
Well balanced oil	The median of the bitter attribute and the median of the pungent attribute are not more than 2.0 points above the median of the fruitiness.
Mild oil	The median of the bitter attribute and the median of the pungent attribute are 2.0 or less.

5. GLASS FOR OIL TASTING

Refer to the standard COI/T.20/Doc. no. 5, "Glass for Oil Tasting" (currently under revision).

6. TEST ROOM

Refer to the standard COI/T.20/Doc. No. 6, "Guide for the Installation of a Test Room" (currently under revision).

7. ACCESSORIES

The following accessories, which are required by tasters to perform their task properly, shall be supplied in each booth and shall be within easy reach:

- glasses (standardised) containing the samples, code numbered, covered with a watch-glass and kept at 28 °C ± 2 °C;
- profile sheet (see Figure 1) on hard copy, or on soft copy provided that the conditions of the profile sheet are met, together with the instructions for its use if necessary;
- pen or indelible ink;
- trays with slices of apple~~, and/or~~ water, (natural or sparkling ~~carbonated water~~), ~~and/or rusks;~~ bread, bread sticks or natural yoghourt;
- ~~glass of water at ambient temperature;~~
- sheet recalling the general rules listed in sections 9.4 and 10.1.1;
- spittoons.

8. PANEL LEADER AND TASTERS

8.1. Panel leader

The panel leader must be a suitably trained person with an expert knowledge of the kinds of oils which he or she will come across in the course of their work. They are the key figure in the panel and responsible for its organisation and running.

The work of the panel leader calls for basic training in the tools of sensory analysis, sensory skill, meticulousness in the preparation, organisation and performance of the tests and skill and patience to plan and execute the tests in a scientific manner.

They are the sole person responsible for selecting, training and monitoring the tasters in order to ascertain their level of aptitude. They are thus responsible for the appraisal of the tasters, which must always be objective and for which they must develop specific procedures based on tests and solid acceptance and rejection criteria. See standard ~~COI/T.20/Doc. no. 14~~ "Guide for the selection, training and monitoring of skilled virgin olive oil tasters" (COI/T.20/Doc. no. 14 currently under revision).

Panel leaders are responsible for the performance of the panel and hence for its evaluation, of which they must give reliable, objective proof. In any case, they must demonstrate at all times that the method and tasters are under control. Periodic calibration of the panel is recommended (COI/T.20/Doc. 14~~/ Rev 4 & 5~~ currently under revision revision).

They hold ultimate responsibility for keeping the records of the panel. These records must always be traceable. They must comply with the assurance and quality requirements laid down in international sensory analysis standards and ensure the anonymity of the samples at all times.

They shall be responsible for inventorying and ensuring that the apparatus and equipment needed to comply with the specifications of this method is properly cleaned and maintained and shall keep written proof thereof, as well as of the compliance with the test conditions.

They shall be in charge of the reception and storage of the samples upon their arrival at the laboratory as well as of their storage after being tested. When doing so, they shall ensure at all times that the samples remain anonymous and are properly stored, for which purpose they must develop written procedures in order to ensure that the entire process is traceable and affords guarantees.

In addition, they are responsible for preparing, coding and presenting the samples to the tasters according to an appropriate experimental design in line with pre-established protocols, as well as for assembling and statistically processing the data obtained by the tasters.

They shall be in charge of developing and drafting any other procedures that might be necessary to complement this standard and to ensure that the panel functions properly.

They must seek ways of comparing the results of the panel with those obtained by other panels undertaking the analysis of virgin olive oil in order to ascertain whether the panel is working properly.

It is the duty of the panel leader to motivate the panel members by encouraging interest, curiosity and a competitive spirit among them. To do so, they are strongly recommended to ensure a smooth two-way flow of information with the panel members by keeping them informed about all the tasks they carry out and the results obtained. In addition, they shall ensure that their opinion is not known and shall prevent possible leaders from asserting their criteria over the other tasters.

They shall summon the tasters sufficiently in advance and shall answer any queries regarding the performance of the tests, but shall refrain from suggesting any opinion to them on the sample.

8.1.1 Deputy panel leader

The panel leader may, on justified grounds, be replaced by a deputy panel leader who may stand in for duties regarding the performance of the tests. This substitute must have all the necessary skills required of a panel leader.

8.2. Tasters

The people acting as tasters in organoleptic tests carried out on olive oils must do so voluntarily. It is therefore advisable for candidates to submit an application in writing. Candidates shall be selected, trained and monitored by the panel leader in accordance with their skills in distinguishing between similar samples; it should be borne in mind that their accuracy will improve with training.

Tasters must act like real sensory observers, setting aside their personal tastes and solely reporting the sensations they perceive. To do so, they must always work in silence, in a relaxed, unhurried manner, paying the fullest possible sensory attention to the sample they are tasting.

Between 8 and 12 tasters are required for each test, although it is wise to keep some extra tasters in reserve to cover possible absences.

9. TEST CONDITIONS

9.1. Presentation of the sample

The oil sample for analysis shall be presented in standardised tasting glasses conforming to the standard COI/T.20/Doc. No 5 "Glass for oil tasting".

The glass shall contain 14–16 ml of oil, or between 12.8 and 14.6 g if the samples are to be weighed, and shall be covered with a watch-glass.

Each glass shall be marked with a code made up of digits or a combination of letters and digits chosen at random. The code will be marked by means of an odour-free system.

9.2. Test and sample temperature

The oil samples intended for tasting shall be kept in the glasses at 28° C ± 2° C throughout the test. This temperature has been chosen because it makes it easier to observe organoleptic differences than at ambient temperature and because at lower temperatures the aromatic compounds peculiar to these oils volatilise poorly while higher temperatures lead to the formation of volatile compounds peculiar to heated oils. See the standard COI/T.20/Doc. No 5 "Glass for Oil Tasting" for the method which has to be used for heating the samples when in the glass.

The test room must be at a temperature between 20° and 25° C (see COI/T.20/Doc. No 6).

9.3. Test times

The morning is the best time for tasting oils. It has been proved that there are optimum perception periods as regards taste and smell during the day. Meals are preceded by a period in which olfactory–gustatory sensitivity increases, whereas afterwards this perception decreases.

However, this criterion should not be taken to the extreme where hunger may distract the tasters, thus decreasing their discriminatory capacity; therefore, it is recommended to hold the tasting sessions between 10.00 in the morning and 12.00 noon.

9.4. Tasters: general rules of conduct

The following recommendations apply to the conduct of the tasters during their work.

When called by the panel leader to participate in an organoleptic test, tasters should be able to attend at the time set beforehand and shall observe the following:

- They shall not smoke or drink coffee at least 30 minutes before the time set for the test.
- They must not have used any fragrance, cosmetic or soap whose smell could linger until the time of the test. They must use an unperfumed soap to wash their hands which they shall then rinse and dry as often as necessary to eliminate any smell.
- They shall fast at least one hour before the tasting is carried out.
- Should they feel physically unwell, and in particular if their sense of smell or taste is affected, or if they are under any psychological effect that prevents them from concentrating on their work, the tasters shall refrain from tasting and shall inform the panel leader accordingly.
- When they have complied with the above, the tasters shall take up their place in the booth allotted to them in an orderly, quiet manner.
- They shall carefully read the instructions given on the profile sheet and shall not begin to examine the sample until fully prepared for the task they have to perform (relaxed and unhurried). If any doubts should arise, they should consult the panel leader in private.
- They must remain silent while performing their tasks.
- They must keep their mobile phone switched off at all times to avoid interfering with the concentration and work of their colleagues.

10. PROCEDURE FOR THE ORGANOLEPTIC ASSESSMENT AND CLASSIFICATION OF VIRGIN OLIVE OIL

10.1. Tasting technique

10.1.1. The tasters shall pick up the glass, keeping it covered with the watch-glass, and shall bend it gently; they shall then rotate the glass fully in this position so as to wet the inside as much as possible. Once this stage is completed, they shall remove the watch-glass and smell the sample, taking slow deep breaths to evaluate the oil. Smelling should not exceed 30 s. If no conclusion has been reached during this time, they shall take a short rest before trying again.

When the olfactory test has been performed, the tasters shall then evaluate the buccal sensations (overall retronasal olfactory, gustatory and tactile sensations). To do so, they shall take a small sip of approximately 3 ml of oil. It is very important to distribute the oil throughout the whole of the mouth cavity, from the front part of the mouth and tongue along the sides to the back part and to the palate support and throat, since it is a known fact that the perception of tastes and tactile sensations varies in intensity depending on the area of the tongue, palate and throat.

It should be stressed that it is essential for a sufficient amount of the oil to be spread very slowly over the back of the tongue towards the palate support and throat while the taster concentrates on the order in which the bitter and pungent stimuli appear. If this is not done, both of these stimuli may escape notice in some oils or else the bitter stimulus may be obscured by the pungentstimulus.

Taking short, successive breaths, drawing in air through the mouth, enables the taster not only to spread the sample extensively over the whole of the mouth but also to perceive the volatile aromatic compounds via the back of the nose by forcing the use of this channel.

N.B. When the tasters do not perceive fruitiness in a sample and the intensity of the classifying negative attribute is 3.5 or less the panel leader may decide to arrange for the tasters to analyse the sample again at ambient temperature (COI/T.20/Doc. No 6~~/Rev 1, September 2007~~ currently under revision , section 3 – General specifications for installation) while specifying the context and concept of ambient temperature. When the sample reaches room temperature, the tasters should re-assess it to check solely whether fruitiness is perceived. If it is, they should mark the intensity on the scale.

The tactile sensation of pungency should be taken into consideration. For this purpose, it is advisable to ingest the oil.

10.1.2. When organoleptically assessing a virgin olive oil, it is recommended that FOUR SAMPLES at the most be evaluated in each session with a maximum of three sessions per day, to avoid the contrast effect that could be produced by immediately tasting other samples.

As successive tastings produce fatigue or loss of sensitivity caused by the preceding samples, it is necessary to use a product that can eliminate the remains of the oil from the preceding tasting from the mouth.

The use of a small slice of apple is recommended which, after being chewed, can be disposed of in the spittoon. Then rinse out the mouth with a little water at ambient temperature. At least 15 minutes shall lapse between the end of one session and the start of the next.

10.2. Use of the profile sheet by tasters

The profile sheet intended for use by tasters is detailed in Figure 1 of this method.

Each taster on the panel shall smell and then taste [1/] the oil under consideration. They shall then enter the intensity with which they perceive each of the negative and positive attributes on the 10-cm scale shown in the profile sheetprovided.

Should the tasters perceive any negative attributes not listed in section 4, they shall record them under the "others" heading, using the term or terms that most accurately describes the attributes.

10.3. Use of the data by the panel leaders

The panel leader shall collect the profile sheets completed by each taster and shall review the intensities assigned to the different attributes. Should they find any anomaly, they shall invite the taster to revise his or her profile sheet and, if necessary, to repeat the test.

The panel leader shall enter the assessment data of each panel member in a computer program like that appended to this method with a view to statistically calculating the results of the analysis, based on the calculation of their median. See sections 10.4 and Annex 1 of this method. The data for a given sample shall be entered with the aid of a matrix comprising 9 columns representing the 9 sensory attributes and n lines representing the n panel members used.

When a defect is perceived and entered under the "others" heading by at least 50% of the panel, the panel leader shall calculate the median of the defect and shall arrive at the corresponding classification.

The value of the robust coefficient of variation which defines classification (defect with the strongest intensity and fruity attribute) must be no greater than 20.0%.

If the opposite is the case, the panel leader must repeat the evaluation of the specific sample in another tasting session.

If this situation arises often, the panel leader is recommended to give the tasters specific additional training (COI/T.20/Doc. No 14~~/Rev. 4, November 2012, ₤ 5~~ currently under revision) and to use the repeatability index and deviation index to check taster performance (COI/T.20/Doc. No 14~~/Rev. 4, November 2012, ₤ 6~~ currently under revision).

A method of calculation is illustrated in an example in the annex hereto.

1/ They may refrain from tasting an oil when they notice any extremely intense negative attribute by direct olfactory means, in which case they shall record this exceptional circumstance in the profile sheet.

10.4. Classification of the oil

The oil is graded as follows in line with the median of the defects and the median for the fruity attribute. The median of the defects is defined as the median of the defect perceived with the greatest intensity. The ***median*** of the defects and the median of the fruity attribute are expressed to one decimal place.

The oil is graded by comparing the median value of the defects and the median for the fruity attribute with the reference ranges given below. The error of the method has been taken into account when establishing the limits of these ranges, which are therefore considered to be absolute. The software packages allow the grading to be displayed as a table of statistics or a graph.

(a) Extra virgin olive oil: the median of the defects is 0.0 and the median of the fruity attribute is above 0.0;

(b) Virgin olive oil: the median of the defects is above 0.0 but not more than 3.5 and the median of the fruity attribute is above 0.0;

(c) Ordinary virgin olive oil: the median of the defects is above 3.5 but not more than 6.0, or the median of the defects is not more than 3.5 and the median of the fruity attribute is 0.0;

(d) Lampante virgin olive oil: the median of the defects is above 6.0.

Note 1: ~~When the median of the bitter and/or pungent attribute is more than 5.9, the panel elader shall state so on the test certificate.~~ Upon request, and depending on the positive **median of the** attributes ~~median~~ of the oils ~~which~~ **that** have been assessed, the panel leader may certify those attributes according to the expressions described in point 4.4 of this document.

For assessments intended to monitor compliance, one test shall be carried out. In the case of counter assessments, the analysis must be carried out in duplicate in different tasting sessions. The results of the duplicate analysis must be statistically homogenous. (See section 10.5). If not, the sample must be reanalysed twice again. The final value of the median of the classification attributes will be calculated using the average of both medians.

10.5 Criteria for the acceptance and rejection of duplicates

The normalised error, defined below, shall be used to determine whether the two results of a duplicate analysis are homogenous or statistically acceptable:

$$En = \frac{|x_1 - x_2|}{\sqrt[2]{U_1^2 + U_2^2}}$$

where x_1 and x_2 are the two values of the duplicate and U_1 and U_2 are the expanded uncertainties obtained for the two values, calculated as follows as specified in Annex I:

$$U_1 = c \times s^* \text{and } s^* = \frac{CV_r \times M_{e1}}{100}$$

For the expanded uncertainty, c = 1.96; hence:

$$U_1 = 0.0196 \times CV_r \times M_{e1}$$

where CV_r is the robust coefficient of variation and M_{e1} is the median of the first analysis.

For it to be stated that the two values obtained are not statistically different, E_n must be equal to or less than 1.0.

10.6 Verification of the declared category

The organoleptic characteristics of an oil shall be deemed consistent with the declared category if a panel recognized by the IOC or approved by the competent authorities at national level confirms it.
Should the panel not confirm the declared category as regards the organoleptic characteristics, the interested party may request the national authorities or their representatives to have carried out without any delay two independent counter-assessments by two other panels recognised by the IOC or approved by the competent authorities at national level. The characteristics concerned shall be deemed consistent with the characteristics declared if both counter- assessments confirm the declared category. If that is not the case, the interested party shall be responsible for the cost of the two counter-assessments.

Figure 1

PROFILE SHEET FOR VIRGIN OLIVE OIL INTENSITY OF

PERCEPTION OF DEFECTS

Fusty/muddy sediment _____

Musty/humid/earthy _____

Winey/vinegary acid/sour _____

Frostbitten olives (wet wood) _____

Rancid _____

Other negative attributes: _____

Descriptor:

☐ **Metallic** ☐ **Dry hay** ☐ **Grubby** ☐ **Rough**

☐ **Brine** ☐ **Heated or burnt** ☐ **Vegetable water**

☐ **Esparto** ☐ **Cucumber** ☐ **Greasy**

INTENSITY OF PERCEPTION OF POSITIVE ATTRIBUTES

Fruity

☐ **Green** ☐ **Ripe**

Bitter _____

Pungent _____

Name of taster: **Taster code:**

Sample code: **Signature**:

Date:

Comments:

Annex 1

METHOD FOR CALCULATING THE MEDIAN AND THE CONFIDENCE INTERVALS

Median

$$Me = [p\,(X < x_m) \leq ½ \wedge p\,(X \leq x_m) \geq ½]$$

The median is defined as the real number x_m characterised by the fact that the probability (p) that the distribution values (X) are below this number (x_m), is less than and equal to 0.5 and that simultaneously the probability (p) that the distribution values (X) are below or equal to x_m is greater than and equal to 0.5. A more practical definition is that the median is the 50th percentile of a distribution of numbers arranged in increasing order. In simpler terms, it is the midpoint of an ordered set of odd numbers, or the mean of two midpoints of an ordered set of even numbers.

Robust standard deviation

In order to arrive at a reliable estimate of the variability around the mean it is necessary to refer to the robust standard deviation as estimated according to Stuart and Kendall (4). The formula gives the asymptotic robust standard deviation, i.e. the robust estimate of the variability of the data considered where N is the number of observations and IQR is the interquartile range which encompasses exactly 50% of the cases of a given probability distribution:

$$s^* = \frac{1.25\ x\ \text{IQR}}{1.35\ x\ \sqrt{N}}$$

The interquartile range is calculated by calculating the magnitude of the difference between the 75th and 25th percentile.

IQR = 75th percentile - 25th percentile

Where the percentile is the value x_{pc} characterised by the fact that the probability (p) that the distribution values are less than x_{pc} is less than and equal to a specific hundredth and that simultaneously the probability (p) that the distribution values are less than or equal to x_{pc} is greater than and equal to that specific hundredth. The hundredth indicates the distribution fractile chosen. In the case of the median, it is equal to 50/100.

$$percentile = [p\,(X < x_{pc}) \leq \frac{n}{100} \wedge p\,(X \leq x_{pc}) \geq \frac{n}{100}]$$

For practical purposes, the percentile is the distribution value corresponding to a specific area subtended from the distribution or density curve. To give an example, the 25th percentile represents the distribution value corresponding to an area equal to 0.25 or 25/100.

In this method percentiles are computed on the basis of the real values which appear in the data matrix (percentiles computing procedure).

Robust coefficient of variation (%)

The *CVr%* represents a pure number which indicates the percentage variability of the set of numbers analysed. For this reason, it is very useful for checking the reliability of the panel assessors.

$$CV_r = \frac{s^*}{Me} 100$$

Confidence intervals of the median at 95%

The confidence intervals at 95% (value of the error of the first kind equal to 0.05 or 5%) represent the interval within which the value of the median could vary if it were possible to repeat an experiment an infinite number of times. In practice, it indicates the interval of variability of the test in the operating conditions adopted starting from the assumption that it is possible to repeat it many times. As with the *CVr%*, the interval helps to assess the reliability of the test.

$$C.I._{upper} = Me + (c \times s^*)$$

$$C.I._{lower} = Me - (c \times s^*)$$

where C = 1.96 for the confidence interval at the 95% level.

Explanation on the type of algorithm to compute the *25th and 75th percentiles*

IOC uses a unique algorithm (xxx-algorithm), presented below, for the computation of the 25th and 75th percentiles.

It is possible to calculate the statistics either manually or through any data acquisition system but the algorithm MUST BE the following.

The IOC provides an MS Excel worksheet in which the algorithm is applied successfully and monitors panel performance.

Percentiles computing procedure

There are several ways of calculating a percentile but let's describe and use the following algorithm, also implemented in a function {PERCENTILE*(array;k) k:0.25 and 0.75 or QUARTILE(array;q) q:1 and 3} in MS-Excel.

[*in French version of MS-Excel CENTILE(array;k) or QUARTIL(array;q)]

Step 1 - Sort the list of numbers into ascending order and score these numbers from 1 to n

Step 2 - Use the following formula to calculate the corresponding **ranking** (***split*** into an ***integer*** and a ***decimal***).

R=1+(P(n-1)/100)=I+D {ranks computation}

where:

P: percentile wanted
n: total number of values
I: integer part of ranking
D: decimal part of ranking

Step 3 - Use the following formula to **interpolate** between the necessary two numbers.

$p=Y_I+D(Y_{I+1}-Y_I)$ {interpolation}

Example (EVEN NUMBER OF TASTERS)

Computation of percentiles

Raw data

1.3
2.1
1.5
1.2
1.6
2.4
2.3
1.9

Ordered data

1.2
1.3
1.5
1.6
1.9
2.1
2.3
2.4

Median=1.8

Computing ranking

r=1+(P(n-1)/100) {ranks computation}

P: percentile (25 or 75)
n: number of cases
I: integer part of ranking
D: decimal part of ranking

1+((25*(8-1))/100)=2.75

I=2 (integer)
D=0.75 (decimal)

1+((75*(8-1))/100)=6.25

I=6 (integer)
D=0.25 (decimal)

Interpolating

$p=Y_I + D(Y_{I+1} – Y_I)$ {interpolation}

1.3+0.75*(1.5-1.3)=**1.45 (25th percentile)**

2.1+0.25*(2.3-2.1)=**2.15 (75th percentile)**

From excel {PERCENTILE(A2:A9;0.25 or 0.75)}

25th percentile=1.45
75th percentile=2.15

Computation of IQR

***IQR**= 75th percentile – 25th percentile*

IQR=2.15-1.45=0.7

Computations of s*

s= Coefficient * IQR / Square root (Number of Judges)*

s*=0.925*0.7/2.828=0.23

***CVr%** = (s* / Median) * 100*

CVr%=(0.23/1.75)=13.1%

The classification is reliable

Example (ODD NUMBER OF TASTERS)

Computation of percentiles

Raw data

1.3
2.1
1.5
1.2
1.6
2.4
2.3
1.9
1.6
1.8
2.7

Ordered data

1.2
1.3
1.5
1.6
1.6
1.8
1.9
2.1
2.3
2.4
2.7

Median=1.8

Computing ranking

r=1+(P(n-1)/100)

P: percentile (25 or 75)
n: number of cases
I: integer part of ranking
D: decimal part of ranking

1+((25*(11-1))/100)=3.5

I=3 (integer)
D=0.5 (decimal)

1+((75*(11-1))/100)=8.5

I=8 (integer)
D=0.5 (decimal)

Interpolating

$p=Y_l + D(Y_{l+1} – Y_l)$

1.5+0.5*(1.6-1.5)=**1.55 (25th percentile)**

2.1+0.5*(2.3-2.1)=**2.20 (75th percentile)**

From Excel {PERCENTILE(A2:A9;0.25 or 0.75)}

25th percentile=1.55
75th percentile=2.20

Computation of IQR

***IQR**= 75th percentile – 25th percentile*

IQR=2.20-1.55=0.65

Computations of s*

***s**= Coefficient * IQR / Square root (Number of Judges)*

s*=0.925*0.65/3.317=0.18

***CVr%** = (s* / Median) * 100*

CVr%=(0.18/1.80)=10.0%

The classification is reliable

Function PERCENTILE in different languages

Language	Function "PERCENTILE"
Czechoslovak	PERCENTIL
Danish	FRAKTIL
Finnish	PROSENTTIPISTE
French	CENTILE
Norwegian	PERSENTIL
Dutch	PERCENTIEL
Polish	PERCENTYL
Portuguese	PERCENTIL
Russian	ПЕРСЕНТИЛЬ
Spanish	PERCENTIL
Swedish	PERCENTIL
German	QUANTIL
Turkish	YÜZDEBIRLIK
Hungarian	PERCENTILIS

Bibliography

(1) Wilkinson, L. 1990. Systat: The system for statistics. Evanston, IL.SYSTAT Inc.

(2) Cicchitelli, G. 1984. Probabilità e Statistica. Maggioli Editore, Rimini.

(3) Massart, D.L.; Vandeginste, B.G.M.; Deming, Y.; Michotte, L. 1988. Chemometrics. A textbook. Elsevier. Amsterdam.

(4) Kendall, M.G.; Stuart, A. 1967. The advanced theory of statistics. Vol. 1. Hafner Publishing Co.

(5) McGill, R.; Tukey, J.W.; Larsen, W.A. 1978. Variation of Box Plots. The American Statistician, 32, (2), 12-16.

(6) COI/T.28/Doc. No 1 September 2007 “Guidelines for the accreditation of sensory testing laboratories with particular reference to virgin olive oil according to standard ISO/IEC 17025:2005”

(7) COI/T.20/Doc. No 14/~~Rev. 3 November 2011~~ currently under revision

(8) ISO/IEC 17025:05 currently under revision

DRAFT DECISION No DEC III.X/118-VI/ 2023

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

INTERNATIONAL OLIVE COUNCIL | CONSEJO OLEICOLA INTERNACIONAL | CONSEIL OLEICOLE INTERNATIONAL | CONSIGLIO OLEICOLO INTERNAZIONALE | المجلس الدولي للزيتون

DRAFT DECISION Nº DEC-III.X/118-VI/2023

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

THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,

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

Having regard to the recommendation made by the Chemistry and Standardisation Committee at its 13th meeting during the 118th session of the Council of Members;

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

Considering the unanimous position of the chemistry experts appointed by Members at their meeting on 5 and 6 October 2023, and the recommendation to incorporate the modification into document COI/T.20/Doc. No 28/Rev. 3 and subsequently issue a corrigendum for the latter;

DECIDES

To adopt the corrigendum for revision 3 of the document COI/T.20/Doc. No 28/Rev. 3 Corr. 1 on the method of analysis for the determination of the content of waxes and fatty acid ethyl esters by capillary gas chromatography.

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

Document COI/T.20/Doc. No. 28/Rev. 3 Corr. 1 November 2023 replaces and repeals document COI/T.20/Doc. No 28/Rev. 3 November 2022.

Madrid (Spain), 23 November 2023

Mr Kaled Musa Al Henefat
Chair of the International Olive Council

INTERNATIONAL
OLIVE
COUNCIL

COI/T.20/Doc. No 28/Rev. 3 Corr 1
November 2023

ENGLISH
Original: ENGLISH

Príncipe de Vergara, 154 – 28002 Madrid – Spain Tel.: +34 915 903 638 Fax: +34 915 631 263 - E-mail: iooc@internationaloliveoil.org – http://www.internationaloliveoil.org/

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 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-

---

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.

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 0.02% (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

---

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

sources of heat. Store in a corrosive-free environment. Do not use damaged or unlabelled bottles. Do not inhale. Use solely for technical purposes.

4.9. 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) *(Note 4)* and collect 150 ml at a flow of about 15 drops every 10 seconds.

(**This fraction contains ethyl esters and waxes)** *(Note 5)***.**

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

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.

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 20 °C/min

80 °C at first (1') 240 °C 325 °C (6') 340 ºC (10') 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.

---

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.

Note 6 . Due to the high final temperature, positive drift is allowed but may not exceed more than 10% of 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 g).

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

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

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:

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 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.~~

-Results should be expressed to the nearest mg/kg.

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.

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

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 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

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.

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.

4.9. **Auxiliary gases:**

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

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

WARNING

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

5. 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 a 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 *(Note [5]).*

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

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.

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 methyl and ethyl esters diluted with 0.5-1 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 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.

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

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

- Detector temperature: 350 °C.
- Amount injected: 1 µl of n-heptane solution (0.5-1ml).

Note [6]. Due to the high final temperature, positive drift is allowed but may not exceed more than 10% of 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.

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.

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 g).

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

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.~~

Results should be expressed to the nearest mg/kg.

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.

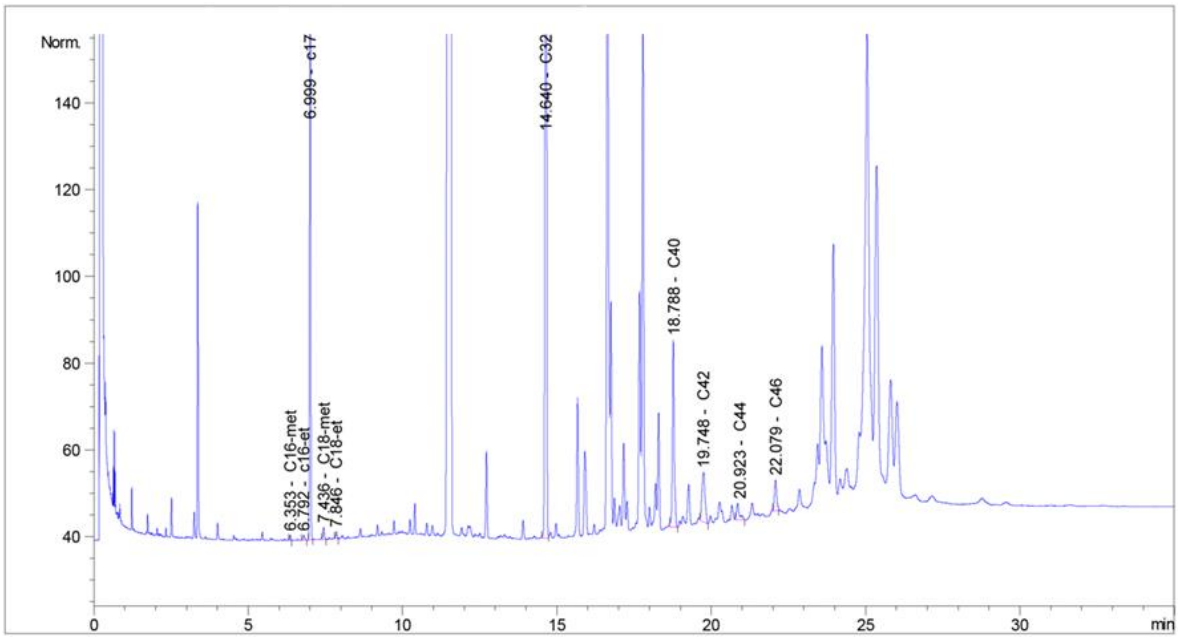


Figure 1: Chromatogram of FAEE and waxes of EVOO using method A (15 g).

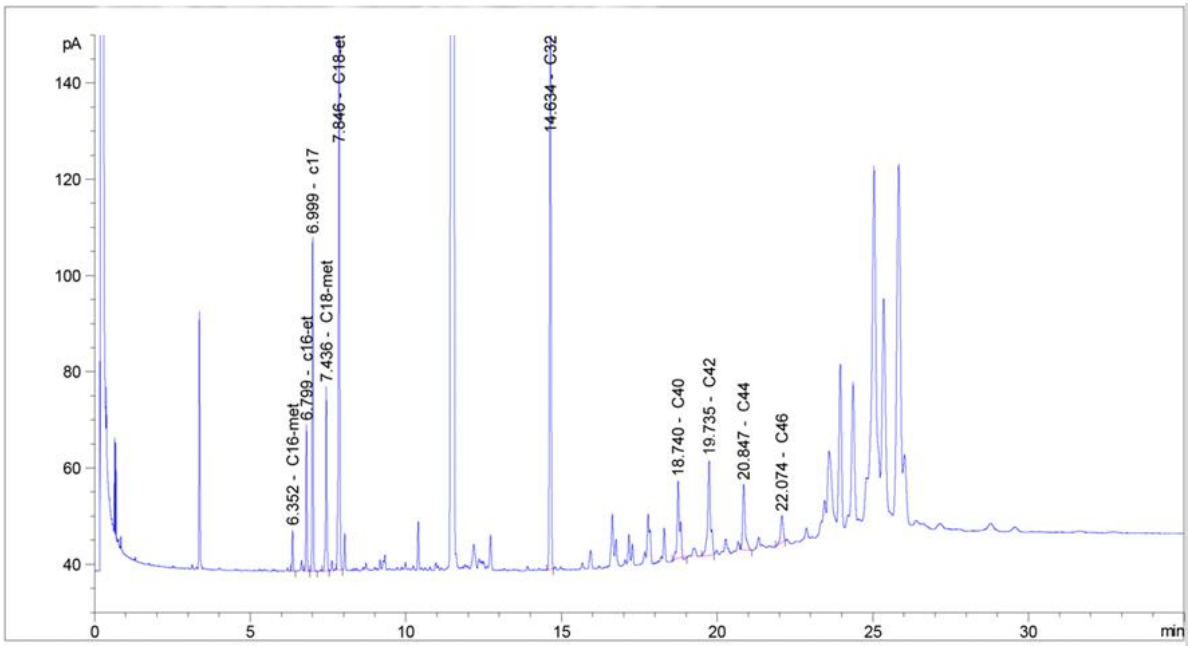


Figure 2: Chromatogram of FAEE and waxes of Lampante olive oil using method A (15 g).

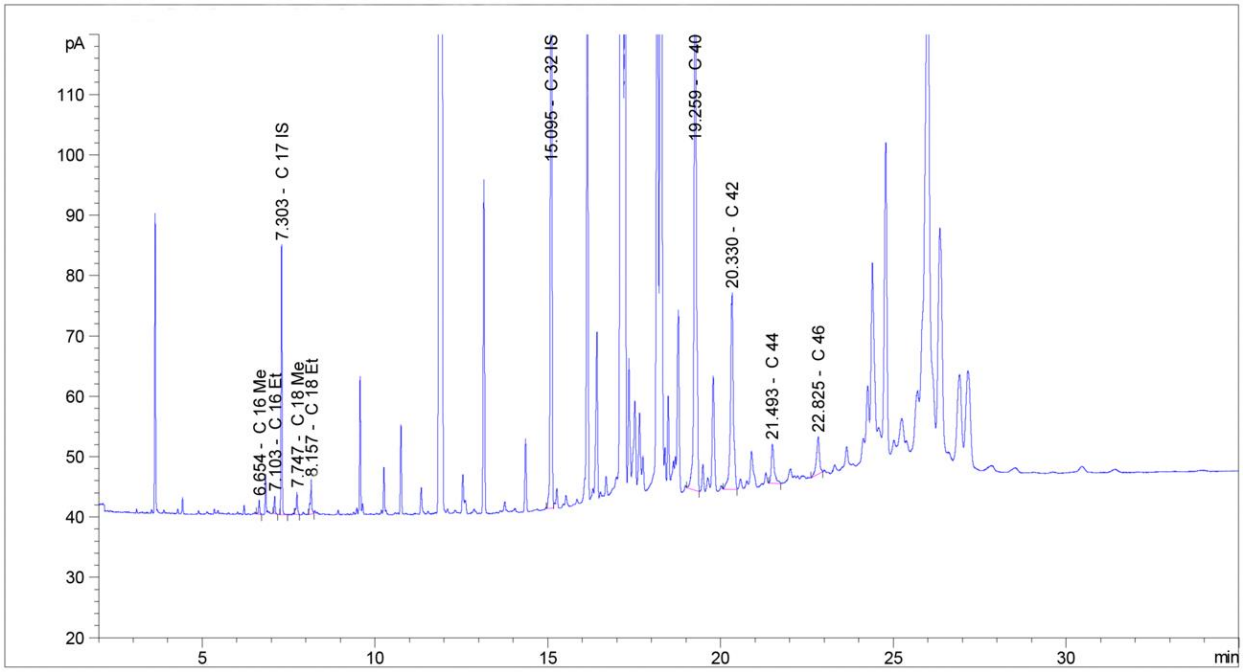


Figure 3: Chromatogram of FAEE and waxes of EVOO using method B (3 g).

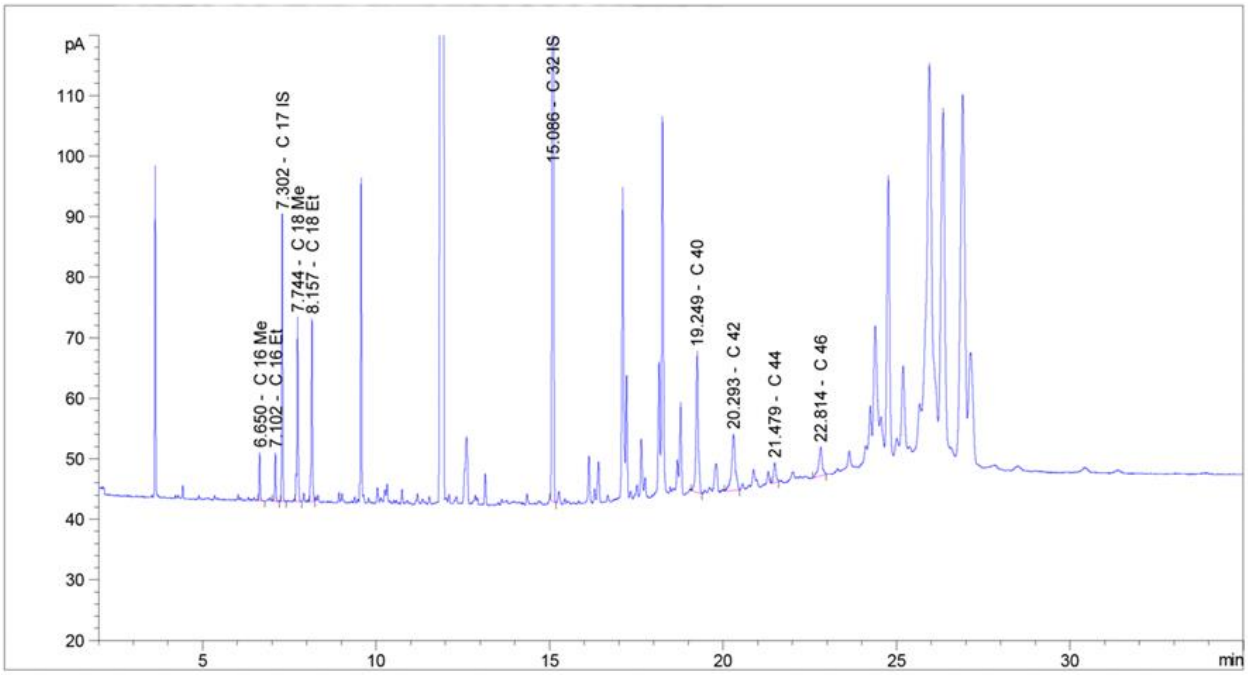


Figure 4: Chromatogram of FAEE and waxes of VOO using method B (3 g).

ANNEX B

Determination of linear gas speed

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

DECISION Nº. xxx/2024

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

INTERNATIONAL OLIVE COUNCIL

COI/T.15/NC No 3/Rev. ~~19~~ 20
June 2024

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							≥ 120°C		
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~~ 11, "Organoleptic assessment of virgin olive oil".

11.10. Determination of the free acidity

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

11.11. Determination of the peroxide value

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

11.12. Determination of the absorbency in ultra-violet

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

11.13. Determination of the moisture and volatile matter

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

11.14. Determination of the insoluble impurities in light petroleum

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

11.15. Determination of the flash point

According to the FOSFA International method.

11.16. Detection of trace metals

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

11.17. Determination of the alpha-tocopherol

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

11.18. Determination of traces of heavy metals

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

- Determination of arsenic: according to AOAC 952.13 or AOAC 942.17 or AOAC 985.16.

11.19. Detection of traces of halogenated solvents

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

11.20. Determination of the content of waxes and alkyl esters

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

11.21. Determination of biophenols

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

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

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

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

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