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PROPOSAL

From:	Secretary-General of the European Commission, signed by Mr Jordi AYET PUIGARNAU, Director
date of receipt:	28 May 2019
To:	Mr Jeppe TRANHOLM-MIKKELSEN, Secretary-General of the Council of the European Union
No. Cion doc.:	SWD(2019) 192 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 in the Council of Members of the International Olive Council (IOC) in connection with trade standards

Delegations will find attached document SWD(2019) 192 final.

Encl.: SWD(2019) 192 final

EUROPEAN
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Brussels, 28.5.2019
SWD(2019) 192 final

LIMITED

COMMISSION STAFF WORKING DOCUMENT
Accompanying the document

Proposal for a
COUNCIL DECISION

on the position to be taken on behalf of the European Union in the Council of Members of the International Olive Council (IOC) in connection with trade standards

{COM(2019) 247 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 in the Council of Members of the International Olive Council (IOC) in connection with trade standards

The present Commission Staff Working Document is accompanying a proposal for a Council Decision on the position to be taken on behalf of the European Union in the Council of Members of the International Olive Council (IOC) in connection with trade standards. It includes in its annexes the text of the decisions transmitted by the Executive Secretariat of the IOC with the view to adopt them during the 109th session in June 2019.

Annex 1:
Draft Decision NO DEC-.../109-VI/2019 concerning the method for spectrophotometric investigation in the ultraviolet

DRAFT DECISION No DEC-.../109-VI/2019

CONCERNING THE METHOD FOR SPECTROPHOTOMETRIC INVESTIGATION IN THE ULTRAVIOLET

THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,

Having regard to the 2015 International Agreement on Olive Oil and Table Olives, 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, with a view to regrouping international standards that allow the quality control of products, their international trade and development, the protection of consumer rights and the prevention of fraudulent and misleading practices and falsification; and Chapter VI "Standardisation Provisions";

Having regard to the recommendations made by the Chemistry and Standardisation Committee at its 4th meeting, during the 108th session of the Council of Members, concerning removing the absolute value of point 6.2 of the method COI/T.20/Doc. No 19 Rev. 4;

Considering the unanimous decision of the chemistry experts at their meeting on 4 and 5 October 2018;

DECIDES

To revise point 6.2 of the method COI/T.20/Doc. No 19/Rev. 4 for spectrophotometric investigation in the ultraviolet. The method COI/T.20/Doc. No 19/Rev. 5 replaces and revokes method COI/T.20/Doc. no 19/Rev. 4 and shall be listed in the IOC trade standard.

Madrid (Spain), XX 2019

COI/T.20/Doc. No 19/Rev. 5

2019

Original: ENGLISH

SPECTROPHOTOMETRIC INVESTIGATION IN THE ULTRAVIOLET

FOREWORD

Spectrophotometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about by technological processes.

The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems resulting from oxidation processes and/or refining practices. These absorptions are expressed as specific extinctions E1% (the extinction of 1% w/v solution of the fat in the specified solvent, in a 10 mm cell) conventionally indicated by K (also referred to as "extinction coefficient").

1. SCOPE

The method describes the procedure for performing a spectrophotometric examination of olive oil in the ultraviolet region.

2. PRINCIPLE OF THE METHOD

A sample is dissolved in the required solvent and the absorbance of the solution is measured at the specified wavelengths with reference to pure solvent.

The specific extinctions at 232 nm and 268 nm in iso-octane or 232 nm and 270 nm in cyclohexane are calculated for a concentration of 1% w/v in a 10 mm cell.

3. EQUIPMENT

3.1. A spectrophotometer suitable for measurements at ultraviolet wavelengths (220 nm to 360 nm), with the capability of reading individual nanometric units. A regular check is recommended for the accuracy and reproducibility of the absorbance and wavelength scales as well as for stray light.

3.1.1. *Wavelength scale*: This may be checked using a reference material consisting of an optical glass filter containing holmium oxide or a holmium oxide solution (sealed or not) that has distinct absorption bands. The reference materials are designed for the verification and calibration of the wavelength scales of visible and ultraviolet spectrophotometers having nominal spectral bandwidths of 5 nm or less. The measurements are carried out against an air blank over the wavelength range of 640 to 240 nm, according to the instructions enclosed

with the reference materials. A baseline correction is performed with an empty beam path at every slit width alteration.

The wavelengths of the standard are listed in the certificate of the reference material.

3.1.2. *Absorbance scale*: This may be checked using commercially available sealed reference materials consisting of acidic potassium dichromate solutions, in certain concentrations and certified values of absorbance at its λmax (of 4 solutions of potassium dichromate in perchloric acid sealed in four UV quartz cells to measure the linearity and photometric accuracy reference in the UV). The potassium dichromate solutions are measured against a blank of the acid used, after baseline correction, according to the instructions enclosed with the reference material. The absorbance values are listed in the certificate of the reference material.

Another possibility in order to check the response of the photocell and the photomultiplier is to proceed as follows: weigh 0.2000 g of pure potassium chromate for spectrophotometry and dissolve in 0.05N potassium hydroxide solution in a 1000 ml graduated flask and make up to the mark. Take precisely 25 ml of the solution obtained, transfer to a 500 ml graduated flask and dilute up to the mark using the same potassium hydroxide solution.

Measure the extinction of the solution so obtained at 275 nm, using the potassium hydroxide solution as a reference. The extinction measured using a 1 cm cuvette should be 0.200 ± 0.005.

3.2. Rectangular quartz cuvettes, with covers, suitable for measurements at the ultraviolet wavelengths (220 to 360 nm) having an optical path-length of 10 mm. When filled with water or other suitable solvent the cuvettes should not show differences between them of more than 0.01 extinction units.

3.3. One- mark volumetric flasks, capacity 25 ml, class A.

3.4. Analytical balance, capable of being read to the nearest 0.0001 g

4. REAGENTS

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

Solvent: Iso-octane (2,2,4 trimethylpentane) for the measurements at 232 nm and 268 nm and cyclohexane for the measurements at 232 nm and 270 nm, having an absorbance less than 0.12 at 232 nm and less than 0.05 at 270 nm against distilled water, measured in a 10 mm cell.

5. PROCEDURE

5.1. The sample must be perfectly homogeneous and without suspended impurities. If not, it must be filtered through paper at a temperature of approximately 30°C.

5.2. Weigh accurately approximately 0.25 g (to the nearest 1 mg) of the sample so prepared into a 25 ml graduated flask, make up to the mark with the specified solvent and homogenize

(1)[1]. The resulting solution must be perfectly clear. If opalescence or turbidity is present, filter quickly through paper.

5.3. If necessary, correct the baseline (220-290 nm) with solvent in both quartz cells (sample and reference), then fill the sample quartz cell with the test solution and measure the extinctions at 232, 268 or 270 nm against the solvent used as a reference.

The extinction values recorded must lie within the range 0.1 to 0.8 or within the range of linearity of the spectrophotometer which should be verified. If not, the measurements must be repeated using more concentrated or more dilute solutions as appropriate.

5.4. After measuring the absorbance at 268 or 270 nm, measure the absorbance at λ_{max}, λ_{max+4} and λ_{max-4}. These absorbance values are used to determine the variation in the specific extinction (ΔK).

NOTE: λ_{max} is considered to be 268 nm for isooctane used as solvent and 270 nm for cyclohexane.

6. EXPRESSION OF THE RESULTS

6.1. Record the specific extinctions (extinction coefficients) at the various wavelengths calculated as follows:

$$K\lambda = \frac{E\lambda}{c * s}$$

where:

K_ = specific extinction at wavelength λ;

E_ = extinction measured at wavelength λ;

c = concentration of the solution in g/100 ml;

s = path length of the quartz cell in cm;

expressed to two decimal places.

6.2. Variation of the specific extinction (ΔK)

The variation of the extinction (ΔK) is given by:

$$\Delta K = K_m - \left(\frac{K_{m-4} + K_{m+4}}{2} \right)$$

[1] Generally, a mass of 0.25 -0.30 g is sufficient for absorbance measurements of virgin and extra virgin olive oils at 268 nm and 270 nm. For measurements at 232 nm, 0.05 g of sample are usually required, so two distinct solutions are usually prepared. For absorbance measurements of olive pomace oils, refined olive oils and adulterated olive oils, a smaller portion of sample, e.g. 0.1 g is usually needed due to their higher absorbance.

where Km is the specific extinction at the wavelength for maximum absorption at 270 nm and 268nm depending on the solvent used.

expressed to two decimal places.

PRECISION VALUES OF THE METHOD

Analysis of the collaborative test results

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

Twenty one laboratories holding IOC recognition at the time took part in the collaborative test arranged by the Executive Secretariat in 2009. The laboratories were from thirteen countries.

The test was performed on five samples:

A: extra virgin olive oil

B: second centrifugation olive oil

C: refined olive-pomace oil

D: virgin olive oil + rapeseed oil + high oleic sunflower oil

E: olive oil + refined soybean oil

The results of the collaborative test organised by the IOC Executive Secretariat have been 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' test to the laboratory results for each determination (replicates a and b) and each sample. The results are summarized in Tables A.1 to A.6

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

RDS_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

RDS_R (%) Reproducibility coefficient of variation (S_R x 100/mean)

Table A.1 — UV extinction at 232 nm in isooctane

	A	B	C	D	E
n	21	22	22	22	22
outliers	1	4	4	1	5
mean	1.76	2.10	3.81	3.85	2.82
r	0.072	0.035	0.043	0.101	0.054
S_r	0.026	0.013	0.016	0.036	0.019
RSD_r(%)	1.5	0.6	0.4	0.9	0.7
R	0.216	0.194	0.488	0.582	0.194
S_R	0.077	0.069	0.174	0.211	0.069
RSD_R(%)	4.4	3.3	4.6	5.5	2.5

Table A.2 — UV extinction at 232 nm in cyclohexane

	A	B	C	D	E
n	21	21	21	21	21
outliers	3	1	1	0	0
mean	1.76	2.12	3.83	3.86	2.79
r	0.070	0.060	0.119	0.113	0.093
S_r	0.025	0.0216	0.0423	0.0405	0.0332
RSD_r(%)	1.4	1.0	1.1	1.1	1.2
R	0.138	0.204	0.424	0.386	0.279
S_R	0.049	0.073	0.151	0.138	0.100

RSD_R(%)	2.8	3.4	4.0	3.6	3.6

Table A.3 — UV extinction at 268 nm in isooctane

	A	B	C	D	E
n	21	22	22	22	22
outliers	1	4	2	5	2
mean	0.12	0.43	1.14	0.45	0.60
r	0.014	0.014	0.043	0.018	0.018
S_r	0.005	0.005	0.016	0.007	0.007
RSD_r(%)	4.0	1.2	1.4	1.5	1.1
R	0.028	0.045	0.083	0.038	0.094
S_R	0.010	0.016	0.030	0.013	0.034
RSD_R(%)	8.0	3.8	2.6	3.0	5.6

Table A.4 — UV extinction at 270 nm in cyclohexane

	A	B	C	D	E
n	21	21	21	21	21
outliers	1	2	1	1	4
mean	0.13	0.43	1.12	0.45	0.59
r	0.014	0.023	0.029	0.033	0.018
S_r	0.005	0.008	0.010	0.012	0.006
RSD_r(%)	4.0	1.9	0.9	2.6	1.1
R	0.031	0.044	0.074	0.04	0.042

S_R	0.011	0.016	0.027	0.014	0.015
RSD_R(%)	8.5	3.7	2.4	3.2	2.5

Table A.5 — Variation of the specific extinction ΔK at (270 ± 4) nm in cyclohexane

	A	B	C	D	E
n	20	21	21	21	21
outliers	1	1	2	1	3
mean	-0.00	0.00	0.09	0.04	0.05
r	0.002	0.002	0.003	0.003	0.004
S_r	0.001	0.001	0.001	0.001	0.001
RSD_r(%)	28.9	21.6	1.1	2.9	2.9
R	0.008	0.004	0.012	0.007	0.011
S_R	0.003	0.001	0.004	0.003	0.004
RSD_R(%)	147.5	52.0	5.1	7.6	8.1

Table A.6 — Variation of the specific extinction ΔK at (268 ± 4) nm in isooctane

	A	B	C	D	E
n	21	21	22	22	22
outliers	0	3	1	2	2
mean	-0.00	0.00	0.08	0.03	0.04
r	0.003	0.001	0.005	0.004	0.002
S_r	0.001	0.001	0.002	0.001	0.001
RSD_r(%)	36.4	121.1	2.3	4.4	1.7

R	0.011	0.003	0.023	0.011	0.013
S_R	0.004	0.001	0.008	0.004	0.005
RSD_R(%)	148.2	234.8	10.0	12.6	10.6

Normative 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: 1994 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

Annex 2:
Draft Decision NO DEC-.../109-VI/2019 concerning the precision values of the methods of analysis adopted by the international olive council

DRAFT DECISION No DEC-.../109-VI/2019

CONCERNING THE PRECISION VALUES OF THE METHODS OF ANALYSIS ADOPTED BY THE INTERNATIONAL OLIVE COUNCIL

THE COUNCIL OF MEMBERS OF THE INTERNATIONAL OLIVE COUNCIL,

Having regard to the 2015 International Agreement on Olive Oil and Table Olives, 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, with a view to regrouping international standards that allow the quality control of products, their international trade and development, the protection of consumer rights and the prevention of fraudulent and misleading practices and falsification; and Chapter VI "Standardisation Provisions";

Having regard to the recommendations made by the Chemistry and Standardisation Committee at its 4th meeting, during the 108th session of the Council of Members, concerning the discussions on the revision of methods COI/T.20/Doc. n.19 and COI/T.20/Doc. n. 26;

Considering the unanimous decision of the chemistry experts at their meeting on 4 and 5 October 2018;

DECIDES

To revise the method COI/T.20/Doc. n. 42-2/Rev. 2 on Precision values of the methods of analysis adopted by the International Olive Council. The revision refers to an update of the precision values related to the methods COI/T.20/Doc. n.19 and COI/T.20/Doc. n. 26 in their latest Revision.

The method COI/T.20/Doc. n. 42-2/Rev. 3 replaces and revokes method COI/T.20/Doc. n. 42-2/Rev. 2.

Madrid (Spain), XX 2019

COI/T.20/Doc. no. 42-2/Rev. 3

2019

Original: ENGLISH

PRECISION VALUES OF THE METHODS OF ANALYSIS ADOPTED BY THE INTERNATIONAL OLIVE COUNCIL

In compliance with the proposal of the group of chemical experts of the International Olive Council (IOC), the Executive Secretariat set up a electronic working group which was given the brief of reviewing expression of results and the precision values of the physico–chemical testing methods drawn up and adopted by the IOC and inserted in the trade standard for olive oils and olive-pomace oils..

The electronic working group drew up this document for approval at the 106th IOC session to be held in Madrid, Spain, on 21-24 November 2017.

The document reports the precision values for the following methods bearing the reference COI/T.20:

Reference	Method
COI/T.20/Doc. no 18/ Rev.3	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
COI/T.20/Doc. n° 20/ Rev.4	Determination of the difference between actual and theoretical content of triacylglycerols with ECN42
COI/T.20/Doc. n° 11/ Rev.3	Determination of stigmastadienes in vegetable oils
COI/T.20/Doc. n° 16/ Rev.2	Determination of sterenes in refined vegetable oils
COI/T.20/Doc. n° 26/ Rev.4	Determination of the sterol composition and content and alcoholic compounds by capillary gas chromatography
COI/T.20/Doc. n° 19/ Rev.5	Spectrophotometric analysis in the ultraviolet
COI/T.20/Doc. n° 23/ Rev.1	Determination of the percentage of 2-glyceryl monopalmitate
COI/T.20/Doc. n° 29/ Rev.1	Determination of biophenols in olive oils by HPLC
COI/T.20/Doc. n° 34/ Rev.1	Determination of free acidity, cold method
COI/T.20/Doc. n° 35/ Rev.1	Determination of the peroxide value
COI/T.20/Doc. n° 33/ Rev.1	Determination of fatty acid methyl esters by gas chromatography

The precision values for the following methods :

- COI/T.20/Doc. no. 16 – Determination of sterenes in refined vegetable oils;

- COI/T.20/Doc. no. 33 – Determination of fatty acid methyl esters by gas chromatography (relative solely to heptadecanoic acid and heptadecenoic acid)

have been calculated from the data for 2000–2017 supplied by the laboratories of many countries for earning entitlement under the IOC recognition scheme. The results underwent statistical analysis according to ISO 5725 “Accuracy (trueness and precision) of measurement methods and results” and with the aid of the AOAC Statistical Manual (W.J. Youden, E.H. Steiner). Outliers were detected by applying the Ranking, Cochran and Grubbs tests to the laboratory results for all the samples (replicates a and b).

The tables on the next pages report the following data for each parameter studied:.

n number of laboratories which participated in the test

outliers number of laboratories with outlying values

mean mean of the accepted results

r repeatability

S_r repeatability standard deviation

RSD_r(%) repeatability coefficient of variation (S_r x 100/ mean)

R reproducibility

S_R reproducibility standard deviation

RSD_R(%) reproducibility coefficient of variation (S_R x 100/ mean)

Table	**1**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	Waxes - Ring Test COI 1999
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil

B: virgin olive oil + refined sunflower oil

C: virgin olive oil + refined olive-pomace oil

D: virgin olive oil + refined soybean oil + refined sunflower oil

E: refined olive oil + refined olive-pomace oil + refined soybean oil + lampante virgin olive oil

	A	B	C	D	E
n	19	19	19	19	19
outliers	5	5	4	3	5
mean	120	123	222	174	346
r	9.5	12.6	10.5	12.2	14.9
S_r	3.4	4.5	3.8	4.7	5.3
RSD_r(%)	2.8	3.6	1.7	2.7	1.5
R	38.8	48.9	58.9	25.7	44.4
S_R	13.9	17.5	21.0	9.2	15.9
RSD_R(%)	11.5	14.2	9.5	5.3	4.6

Table	**2**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	Waxes - Ring Test COI 2008
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive retail Italy

B: extra virgin olive retail Italy

C: extra virgin olive retail + refined

D: extra virgin olive oil + lampante

E: extra virgin olive oil + retail Germany

	A	**B**	**C**	**D**	**E**
n	20	18	19	18	18
outliers	2	1	0	0	0
mean	125	181	199	142	174
r	9.8	13.0	20.1	17.6	12.2
S_r	3.3	4.4	6.8	5.9	4.1
RSD_r(%)	2.7	2.4	3.4	4.2	2.4
R	87.3	75.4	67.9	82.7	44.0
S_R	29.5	25.6	23.0	27.8	14.8
RSD_R(%)	23.7	11.8	11.6	19.6	8.5

Table	**3**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	FAEE (Ethyl C16+C18) - Ring Test COI 2010
Unit	mg/kg
Final result rounded to	no decimal

A: high quality extra virgin year 2001

B: high quality extra virgin year 1991

C: extra virgin supermarket year 2010

D: extra virgin supermarket year 2010

E: extra virgin supermarket year 2010

	A	**B**	**C**	**D**	**E**

n	15	17	17	17	17
outliers	1	2	1	2	2
mean	5	137	276	96	28
r	2.14	5.36	7.6	6.66	2.66
S_r	0.76	1.91	2.71	2.38	0.95
RSD_r(%)	14.8	1.4	1.0	2.5	3.4
R	6.71	38.82	95.91	29.23	15.50
S_R	2.40	13.86	34.25	10.44	5.54
RSD_R(%)	46.5	10.1	12.4	10.9	19.7

Table	**4**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	FAME (Methyl C16+C18) - Ring Test COI 2010
Unit	mg/kg
Final result rounded to	no decimal

A: high quality extra virgin year 2001
B: high quality extra virgin year 1991
C: extra virgin supermarket year 2010
D: extra virgin supermarket year 2010
E: extra virgin supermarket year 2010

	A	**B**	**C**	**D**	**E**
n	15	17	17	17	17
outliers	2	2	1	1	3
mean	33	69	74	44	16
r	5.67	10.1	5.09	7.69	2.71
S_r	2.02	3.61	1.82	2.75	0.97
RSD_r(%)	6.1	5.2	2.5	6.2	6.1
R	13.38	26.85	29.48	18.44	10.52
S_R	4.78	9.59	10.53	6.58	3.76
RSD_R(%)	14.3	13.8	14.2	14.9	23.6

Table	**5**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	FAAE (SUM Methyl + Ethyl) - Ring Test COI 2010
Unit	mg/kg
Final result rounded to	no decimal

A: high quality extra virgin year 2001
B: high quality extra virgin year 1991
C: extra virgin supermarket year 2010
D: extra virgin supermarket year 2010
E: extra virgin supermarket year 2010

	A	B	C	D	E
n	15	17	17	17	17
outliers	2	1	2	2	2
mean	38	212	350	139	43
r	6.80	16.83	6.29	7.21	4.09
S_r	2.43	6.01	2.25	2.58	1.46
RSD_r(%)	6.3	2.8	0.6	1.9	3.4
R	17.91	77.26	112.95	38.47	14.12
S_R	6.39	27.59	40.34	13.74	5.04
RSD_R(%)	16.6	13.0	11.5	9.9	11.7

Table	**6**
Analysis	Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography
Method	COI/T.20/Doc.n°18/Rev.3
Parameter	RATIO (FAEE/FAME) - Ring Test COI 2010
Unit	-
Final result rounded to	1 decimal place

A: high quality extra virgin year 2001

B: high quality extra virgin year 1991

C: extra virgin supermarket year 2010

D: extra virgin supermarket year 2010

E: extra virgin supermarket year 2010

	A	**B**	**C**	**D**	**E**
n	15	17	17	17	17
outliers	0	1	1	1	1
mean	0.2	2.0	3.8	2.2	1.8
r	0.08	0.21	0.30	0.35	0.42
S_r	0.03	0.08	0.11	0.13	0.15
RSD_r(%)	18.2	3.8	2.8	5.7	8.5
R	0.23	0.57	1.56	0.68	1.38
S_R	0.08	0.20	0.56	0.24	0.49
RSD_R(%)	51.5	10.1	14.7	11.0	28.2

Table	**7**
Analysis	Determination of the difference between actual and theoretical content of triacylglycerols with ECN42
Method	COI/T.20/Doc.n°20/Rev.4
Parameter	ΔECN42 determined with acetone and acetonitrile - Ring Test COI 1999
Unit	%
Final result rounded to	2 decimal place

A: extra virgin olive oil

B: virgin olive oil + refined sunflower oil

C: virgin olive oil + refined olive-pomace oil

D: virgin olive oil + refined soybean oil + refined sunflower oil

E: refined olive oil + refined olive-pomace oil + refined soybean oil + lampante virgin olive oil

	A	B	C	D	E
n	19	19	19	19	19
outliers	1	0	0	0	3
mean	0.04	1.66	0.04	0.18	0.82
r	0.08	0.12	0.09	0.11	0.11
S_r	0.02	0.04	0.03	0.04	0.04
RSD_r(%)	82.2(not sig.)	2.8	76.1(not sig.)	22.5	5.1
R	0.12	0.25	0.16	0.22	0.24
S_R	0.05	0.09	0.05	0.08	0.08
RSD_R(%)	127.6(not sig.)	5.4	132.2(not sig.)	46.2	10.9

Table	**8**
Analysis	Determination of the difference between actual and theoretical content of triacylglycerols with ECN42
Method	COI/T.20/Doc.n°20/Rev.4
Parameter	ΔECN42 determined with proprionitrile
Unit	%
Final result rounded to	2 decimal place

A: 70% virgin olive oil + 10% refined olive-pomace oil + 20% high oleic sunflower oil

B: 80% high campesterol virgin olive oil + 20% palm olein

C: 100% virgin olive oil

D: 70% virgin olive oil + 15% refined olive-pomace oil + 15% high oleic sunflower oil

	A	**B**	**C**	**D**
n	16	16	11	11
outliers	0	2	0	0
mean	1.07	0.10	0.06	0.84
r	0.05	0.02	0.06	0.06
S_r	0.02	0.01	0.02	0.02
RSD_r(%)	1.6	7.9	36.6	2.7
R	0.33	0.11	0.12	0.35
S_R	0.12	0.04	0.04	0.12
RSD_R(%)	11.2	36.8	$78.6_{(not\ sig.)}$	14.8

Table	**9**
Analysis	Determination of stigmastadienes in vegetable oils
Method	COI/T.20/Doc.n°11/Rev.3
Parameter	Stigmastadienes - Ring Test COI 1999
Unit	mg/kg
Final result rounded to	2 decimal place

A: extra virgin olive oil

B: virgin olive oil + refined sunflower oil

C: virgin olive oil + refined olive-pomace oil

D: virgin olive oil + refined soybean oil + refined sunflower oil

E: refined olive oil + refined olive-pomace oil + refined soybean oil + lampante virgin olive oil

	A	B	C	D	E
n	19	19	19	19	19
outliers	3	5	7	2	5
mean	0.01	0.80	9.49	0.22	7.55
r	0.01	0.08	0.39	0.05	0.48
S_r	0.00	0.03	0.14	0.01	0.17
RSD_r(%)	32.4(not sig.)	3.7	1.5	8.4	2.3
R	0.03	0.15	1.66	0.06	1.59
S_R	0.01	0.05	0.59	0.03	0.57
RSD_R(%)	98.6(not sig.)	6.7	6.3	11.5	7.6

Table	**10**
Analysis	Determination of sterenes in refined vegetable oils
Method	COI/T.20/Doc.n°16/Rev.2
Parameter	Sterenes - Results from recognition 2000-2006
Unit	mg/kg
Final result rounded to	1 decimal place

	A	**B**	**C**	**D**	**E**
n	31	31	31	31	31
outliers	4	4	5	4	4
mean	9.5	31.0	46.0	9.0	11.4
r	0.2	1.0	1.0	0.3	0.5
S_r	0.1	0.3	0.4	0.1	0.2
RSD_r(%)	0.8	1.0	0.9	1.1	1.6
R	2.0	5.0	12.0	1.0	1.0
S_R	0.6	1.7	4.2	0.5	0.5
RSD_R(%)	6.1	5.3	9.1	5.9	4.4

Table	**11**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Total sterols - Ring Test COI 2016-1 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**

n	14	14	14	14	14
outliers	1	0	0	1	1
mean	1572	1742	1679	2830	3181
r	84.9	134.8	144.7	246.2	307.3
S_r	30.3	48.1	51.7	87.9	109.7
RSD_r(%)	1.9	2.8	3.1	3.1	3.5
R	291.3	495.9	321.6	346.4	610.4
S_R	104.0	177.1	114.8	123.7	218.0
RSD_R(%)	6.6	10.2	6.8	4.4	6.9

Table	**12**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Total sterols - Ring Test COI 2016-1 – Separation by HPLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	1	1	1	0
mean	1583	1754	1730	2897	3216
r	74.0	93.5	95.0	59.01	181.9
S_r	264.4	33.4	33.9	21.1	65.0
RSD_r(%)	1.7	1.9	2.0	0.7	2.0
R	315.0	190.2	156.6	230.2	480.2
S_R	112.5	67.9	55.9	82.2	171.5
RSD_R(%)	7.1	3.9	3.2	2.8	5.3

Table	**13**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Cholesterol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	1	0	0
mean	0.1	0.3	0.1	0.1	0.1
r	0.03	0.10	0.05	0.07	0.03
S_r	0.01	0.04	0.02	0.03	0.01
RSD_r(%)	8.3	13.6	14.2	20.2	8.6
R	0.06	0.28	0.09	0.14	0.07
S_R	0.02	0.10	0.03	0.05	0.02
RSD_R(%)	15.9	37.5	22.5	40.3	23.4

Table	**14**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Cholesterol - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	0	0
mean	0.1	0.3	0.1	0.1	0.1
r	0.03	0.10	0.07	0.03	0.04
S_r	0.01	0.04	0.02	0.01	0.01
RSD_r(%)	7.6	15.0	17.7	9.3	10.8
R	0.06	0.24	0.09	0.11	0.06
S_R	0.02	0.08	0.03	0.04	0.02
RSD_R(%)	16.5	34.3	23.2	35.4	16.7

Table	**15**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Brassicasterol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	1	0	0	1	1
mean	0.0	0.0	0.0	0.0	0.1
r	0.02	0.03	0.03	0.02	0.02
S_r	0.01	0.01	0.01	0.01	0.01
RSD_r(%)	68.1	23.6	39.3	25.3	14.7
R	0.03	0.11	0.09	0.07	0.15
S_R	0.01	0.04	0.03	0.03	0.05
RSD_R(%)	103.7	90.5	105.3	94.5	90.7

Table	**16**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Brassicasterol - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	0	0
mean	0.1	0.3	0.1	0.1	0.1
r	0.03	0.10	0.07	0.03	0.04
S_r	0.01	0.04	0.02	0.01	0.01
RSD_r(%)	7.6	15.0	17.7	9.3	10.8
R	0.06	0.24	0.09	0.11	0.06
S_R	0.02	0.08	0.03	0.04	0.02
RSD_R(%)	16.5	34.3	23.2	35.4	16.7

Table	**17**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Campesterol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	2	0	0	0	1
mean	3.1	3.2	3.9	8.3	3.1
r	0.22	0.15	0.26	0.18	0.15
S_r	0.08	0.06	0.09	0.06	0.05
RSD_r(%)	2.6	1.7	2.4	0.8	1.7
R	0.25	0.39	0.45	0.78	0.27
S_R	0.09	0.13	0.16	0.28	0.10
RSD_R(%)	2.9	4.3	4.1	3.4	3.1

Table	**18**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Campesterol - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	1	0	0	0
mean	3.0	3.3	3.9	8.4	3.2
r	0.12	0.13	0.18	0.22	0.11
S_r	0.04	0.05	0.06	0.09	0.04
RSD_r(%)	1.5	1.4	1.6	0.9	1.3
R	0.48	0.59	0.37	0.52	0.28
S_R	0.17	0.21	0.13	0.18	0.10
RSD_R(%)	5.7	6.5	3.4	2.2	3.2

Table	**19**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Stigmasterol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	1	0
mean	1.1	2.4	2.0	7.2	1.3
r	0.07	0.16	0.25	0.12	0.09
S_r	0.02	0.06	0.09	0.04	0.03
RSD_r(%)	2.1	2.4	4.5	0.6	2.5
R	0.18	0.29	0.41	0.62	0.11
S_R	0.06	0.10	0.15	0.22	0.04
RSD_R(%)	5.9	4.3	7.4	3.1	3.0

Table	**20**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Stigmasterol - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	1	0
mean	1.1	2.4	2.0	7.2	1.3
r	0.07	0.14	0.08	0.21	0.16
S_r	0.03	0.05	0.03	0.08	0.06
RSD_r(%)	2.3	2.1	1.5	1.1	4.4
R	0.15	0.22	0.11	0.45	0.19
S_R	0.06	0.08	0.04	0.16	0.07
RSD_R(%)	5.1	3.3	2.0	2.2	5.3

Table	**21**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Δ7 Stigmastenol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	0	1
mean	0.3	0.4	3.2	16.0	0.5
r	0.06	0.08	0.53	1.08	0.06
S_r	0.02	0.03	0.19	0.39	0.02
RSD_r(%)	7.5	6.4	5.9	2.4	4.4
R	0.15	0.19	0.83	1.52	0.19
S_R	0.05	0.07	0.30	0.54	0.07
RSD_R(%)	18.7	16.0	9.4	3.4	13.5

Table	**22**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Δ7 Stigmastenol - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	0	0	0	0
mean	0.32	0.46	3.22	16.09	0.52
r	0.10	0.12	0.38	0.75	0.08
S_r	0.037	0.041	0.13	0.267	0.029
RSD_r(%)	11.4	9.0	4.2	1.7	5.6
R	0.13	0.24	0.75	1.95	0.16
S_R	0.045	0.087	0.269	0.696	0.058
RSD_R(%)	14.2	18.8	8.3	4.3	11.0

Table	**23**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Apparent β-sitosterol - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	1	1	1
mean	94.4	92.6	89.0	61.1	94.0
r	0.45	0.37	1.43	1.43	0.33
S_r	0.16	0.13	0.51	0.51	0.12
RSD_r(%)	0.17	0.14	0.57	0.84	0.13
R	0.76	1.31	1.79	4.00	0.63
S_R	0.27	0.47	0.63	1.43	0.23
RSD_R(%)	0.29	0.51	0.72	2.34	0.24

Table	**24**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Apparent β-sitosterol - Ring Test COI 2016-1– Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	14
outliers	0	0	1	1	1
mean	94.4	92.5	88.7	60.7	94.1
r	0.38	0.45	1.15	1.08	0.50
S_r	0.13	0.16	0.41	0.39	0.18
RSD_r(%)	0.14	0.17	0.46	0.63	0.19
R	0.81	1.11	1.41	4.04	0.99
S_R	0.29	0.40	0.51	1.44	0.35
RSD_R(%)	0.31	0.43	0.57	2.38	0.38

Table	**25**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol + uvaol (% total sterols) - Ring Test COI 2016-1 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	1	0	1	0	0
mean	2.1	3.8	1.2	2.5	17.2
r	0.32	0.34	0.19	0.27	0.76
S_r	0.12	0.12	0.07	0.10	0.27
RSD_r(%)	5.4	3.2	5.4	3.9	1.6
R	0.80	0.85	0.53	1.09	4.68
S_R	0.29	0.30	0.19	0.39	1.67
RSD_R(%)	13.3	8.0	15.3	15.5	9.7

Table	**26**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol + uvaol (% total sterols) - Ring Test COI 2016-1 – Separation by HPLC
Unit	%
Final result rounded to	1 decimal place

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	0	0
mean	2.2	3.8	1.4	2.0	17.2
r	0.40	0.32	0.24	0.16	0.73
S_r	0.14	0.11	0.09	0.06	0.26
RSD_r(%)	6.5	3.0	6.1	2.8	1.5
R	0.52	0.57	0.46	0.62	3.66
S_R	0.19	0.20	0.17	0.22	1.31

RSD_R(%)	8.4	5.3	11.8	10.9	7.6

Table	**27**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol absolute - Ring Test COI 2016-1 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	1	1	1	1
mean	31	61	17	52	598
r	2.8	4.4	1.5	4.0	71.0
S_r	1.0	1.6	0.5	1.4	25.3
RSD_r(%)	3.3	2.6	3.1	2.7	4.2
R	6.0	21.0	10.2	9.4	148.3
S_R	2.1	2.6	3.6	3.3	53.0
RSD_R(%)	7.0	12.4	20.8	6.5	8.9

Table	**28**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol absolute - Ring Test COI 2016-1 – Separation by HPLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	1	0	0	1
mean	32	60	18	51	605
r	3.0	8.8	2.5	5.6	36.5
S_r	1.1	3.1	0.9	2.0	13.0
RSD_r(%)	3.3	5.2	5.1	4.0	2.2
R	7.3	23.1	5.6	5.8	152.5
S_R	2.6	8.2	2.0	2.0	54.5
RSD_R(%)	8.1	13.7	11.4	4.1	9.0

Table	**29**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Uvaol absolute - Ring Test COI 2016-1 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	2	1	1	1	2
mean	3.0	8	4	20	65
r	0.83	1.5	3.3	3.5	12.5
S_r	0.30	0.55	1.2	1.2	4.5
RSD_r(%)	10.1	6.8	27.8	6.2	6.8
R	4.6	9.0	4.1	3.5	23.1
S_R	1.6	3.2	1.5	1.2	8.3
RSD_R(%)	55.6	40.0	34.0	6.2	12.7

Table	**30**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Uvaol absolute - Ring Test COI 2016-1 – Separation by HPLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	1	1	0	1
mean	5	8	5	20.0	65
r	1.6	1.3	0.80	2.8	6.5
S_r	0.55	0.48	0.29	1.0	2.3
RSD_r(%)	10.2	5.7	5.7	5.2	3.6
R	4.2	6.8	3.4	3.5	15.5
S_R	1.5	2.4	1.2	1.3	5.5
RSD_R(%)	27.3	28.6	24.0	6.4	8.5

Table	**31**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Total aliphatic alcohols (C22 + C24 + C26 + C28) - Ring Test COI 2016-1– Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	B	C	D	E
n	14	14	14	14	14
outliers	0	1	0	0	0
mean	143	420	62	78	1512
r	5.5	23	4.5	5.1	70
S_r	1.9	8.2	1.6	1.8	24.9
RSD_r(%)	1.4	2.0	2.6	2.3	1.7
R	25	67	10	10	95
S_R	8.9	23.8	3.7	3.6	34.7
RSD_R(%)	6.2	5.7	6.1	4.7	2.3

Table	**32**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Total aliphatic alcohols (C22 + C24 + C26 + C28) - Ring Test COI 2016-1 – Separation by HPLC
Unit	mg/kg
Final result rounded to	no decimal

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

	A	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	0	1
mean	139	423	62	78	1495
r	6.9	15	6.2	5.9	46
S_r	2.5	5.3	2.2	2.1	16.2
RSD_r(%)	1.8	1.3	3.6	2.7	1.1
R	23	36	9.0	11	86
S_R	8.4	12.9	3.2	3.8	30.8
RSD_R(%)	6.0	3.1	5.2	4.9	2.1

Table	**33**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol + uvaol (% total sterols) - Ring Test COI 2016-2 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3.13% / 49.26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	B	C	D	E
n	17	17	17	17	17
outliers	1	3	3	2	3
mean	3.3	4.3	3.5	22.8	22.7
r	0.30	0.70	0.20	1.5	1.5
S_r	0.10	0.20	0.10	0.50	0.50
RSD_r(%)	6.5	3.0	6.1	2.8	1.5
R	3.1	5.7	2.3	2.3	2.4
S_R	2.6	1.1	0.70	3.0	3.3
RSD_R(%)	0.9	0.4	0.2	1.1	1.2

Table	**34**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol + Uvaol content - Ring Test COI 2016-2 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3.13% / 49.26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	**B**	**C**	**D**	**E**
n	16	16	17	16	16
outliers	3	4	3	2	3
mean	59	50	52	772	745
r	6.5	6.7	4.7	40	100
S_r	2.3	2.4	1.7	14.2	35.6
RSD_r(%)	3.9	4.8	3.3	1.8	4.8
R	9.9	14	31	146	183
S_R	3.5	4.8	11.1	52.1	65.4
RSD_R(%)	6.0	9.8	21.5	6.8	8.8

Table	**35**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol (% total sterols) - Ring Test COI 2016-2 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3,13% / 49,26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	B	C	D	E
n	17	17	16	17	17
outliers	1	3	1	2	3
mean	3.1	3.9	3.4	18.8	18.7
r	0.30	0.60	0.20	1.2	1.5
S_r	0.10	0.20	0.10	0.40	0.50
RSD_r(%)	3.1	5.3	2.4	2.3	2.9
R	0.80	1.0	0.60	2.8	2.8
S_R	0.30	0.40	0.20	1.0	1.0
RSD_R(%)	8.9	9.1	6.6	5.3	5.3

Table	**36**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Erythrodiol content - Ring Test COI 2016-2 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3.13% / 49.26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	**B**	**C**	**D**	**E**
n	16	16	16	16	16
outliers	1	3	2	0	2
mean	53	46	48	638	635
r	5.1	13	4.1	41	78
S_r	1.8	4.6	1.5	14.5	27.7
RSD_r(%)	3.4	9.9	3.1	2.3	4.4
R	13	16	12	125	130
S_R	4.7	5.7	4.1	44.6	46.4
RSD_R(%)	8.8	12.2	8.6	7.0	7.3

Table	**37**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Uvaol (% total sterols) - Ring Test COI 2016-2 – Separation by TLC
Unit	%
Final result rounded to	1 decimal place

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3,13% / 49,26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	B	C	D	E
n	16	16	16	16	16
outliers	1	1	3	1	0
mean	0.4	0.4	0.1	4.0	4.1
r	0.10	0.30	0.10	0.40	0.30
S_r	0.00	0.10	0.00	0.10	0.10
RSD_r(%)	11.4	23.3	35.8	3.4	2.4
R	0.70	0.20	0.10	0.60	0.70
S_R	0.20	0.10	0.00	0.20	0.30
RSD_R(%)	55.4	22.5	86.9	5.5	6.2

Table	**38**
Analysis	Determination of the sterol content and alcoholic compounds
Method	COI/T.20/Doc.n°26/Rev.4
Parameter	Uvaol content - Ring Test COI 2016-2 – Separation by TLC
Unit	mg/kg
Final result rounded to	no decimal

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3,13% / 49,26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded)

E: Pomace Olive oil (traded)

	A	**B**	**C**	**D**	**E**
n	15	14	15	14	13
outliers	2	1	3	0	1
mean	8	5	1	136	138
r	2.5	2.6	1.0	12	22
S_r	0.90	0.90	0.30	4.4	8.0
RSD_r(%)	11.6	20.0	43.3	3.2	5.8
R	11	3.3	2.2	31	36
S_R	3.9	1.2	0.80	11.0	12.8
RSD_R(%)	51.6	25.8	99.0	8.1	9.2

Table	**39**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	K270 using cyclohexane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	21	21	21	21	21

outliers	1	2	1	1	4
mean	0.13	0.43	1.12	0.45	0.59
r	0.014	0.023	0.029	0.033	0.018
S_r	0.005	0.008	0.010	0.012	0.006
RSD_r(%)	4.0	1.9	0.9	2.6	1.1
R	0.031	0.044	0.074	0.04	0.042
S_R	0.011	0.016	0.027	0.014	0.015
RSD_R(%)	8.5	3.7	2.4	3.2	2.5

Table	**40**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	K268 using isooctane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	21	22	22	22	22
outliers	1	4	2	5	2
mean	0.12	0.43	1.14	0.45	0.60
r	0.014	0.014	0.043	0.018	0.018
S_r	0.005	0.005	0.016	0.007	0.007
RSD_r(%)	4.0	1.2	1.4	1.5	1.1
R	0.028	0.045	0.083	0.038	0.094
S_R	0.010	0.016	0.030	0.013	0.034
RSD_R(%)	8.0	3.8	2.6	3.0	5.6

Table	**41**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	K232 using cyclohexane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	21	21	21	21	21
outliers	3	1	1	0	0
mean	1.76	2.12	3.83	3.86	2.79
r	0.070	0.060	0.119	0.113	0.093
S_r	0.025	0.0216	0.0423	0.0405	0.0332
RSD_r(%)	1.4	1.0	1.1	1.1	1.2
R	0.138	0.204	0.424	0.386	0.279

S_R	0.049	0.073	0.151	0.138	0.100
RSD_R(%)	2.8	3.4	4.0	3.6	3.6

Table	**42**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	K232 using isooctane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	21	22	22	22	22
outliers	1	4	4	1	5
mean	1.76	2.10	3.81	3.85	2.82
r	0.072	0.035	0.043	0.101	0.054
S_r	0.026	0.013	0.016	0.036	0.019
RSD_r(%)	1.5	0.6	0.4	0.9	0.7
R	0.216	0.194	0.488	0.582	0.194
S_R	0.077	0.069	0.174	0.211	0.069
RSD_R(%)	4.4	3.3	4.6	5.5	2.5

Table	**43**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	ΔK using cyclohexane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	20	21	21	21	21
outliers	1	1	2	1	3
Mean	-0.00	0.00	0.09	0.04	0.05
r	0.002	0.002	0.003	0.003	0.004
S_r	0.001	0.001	0.001	0.001	0.001
RSD_r(%)	28.9	21.6	1.1	2.9	2.9
R	0.008	0.004	0.012	0.007	0.011

S_R	0.003	0.001	0.004	0.003	0.004
RSD_R(%)	147.5	52.0	5.1	7.6	8.1

Table	**44**
Analysis	Spectrophotometric analysis in the ultraviolet
Method	COI/T.20/Doc.n°19/Rev.5
Parameter	ΔK using isooctane - Ring Test COI 2009
Unit	-
Final result rounded to	2 decimal place

	A	**B**	**C**	**D**	**E**
n	21	21	22	22	22
outliers	0	3	1	2	2
mean	-0.00	0.00	0.08	0.03	0.04
r	0.003	0.001	0.005	0.004	0.002
S_r	0.001	0.001	0.002	0.001	0.001
RSD_r(%)	36.4	121.1	2.3	4.4	1.7
R	0.011	0.003	0.023	0.011	0.013
S_R	0.004	0.001	0.008	0.004	0.005
RSD_R(%)	148.2	234.8	10.0	12.6	10.6

Table	**45**
Analysis	Determination of the percentage of 2-glyceryl monopalmitate
Method	COI/T.20/Doc.n°23/Rev.1
Parameter	2-glyceryl monopalmitate
Unit	%
Final result rounded to	1 decimal place

A: Extra virgin olive oil

B: Lampante virgin olive oil

C: Refined olive oil

D: Refined olive oil + re-esterified oil (90:10)

E: Refined olive oil + re-esterified oil (80:20)

	A	**B**	**C**	**D**	**E**
n	12	12	12	12	12
outliers	0	0	0	0	0

mean	0.5	0.8	0.9	1.8	2.8
r	0.11	0.11	0.17	0.10	0.26
S_r	0.04	0.04	0.06	0.04	0.09
RSD_r(%)	8.9	5.4	6.8	2.0	3.3
R	0.14	0.27	0.26	0.56	0.86
S_R	0.05	0.10	0.09	0.20	0.31
RSD_R(%)	11.1	12.7	10.2	11.1	10.9

Table	**46**
Analysis	Determination of biophenols in olive oils by HPLC
Method	COI/T.20/Doc.n°29/Rev.1
Parameter	Biophenols - Ring Test COI 2008
Unit	mg/kg
Final result rounded to	no decimal

A: Extra virgin olive oil (Italy)

B : Extra virgin olive oil (Spain)

C : Extra virgin olive oil (Tunisia)

D : Extra virgin olive oil (Slovenia)

E : Extra virgin olive oil (Greece)

R : Extra virgin olive oil (Italy)

	A	**B**	**C**	**D**	**E**	**R**
n	17	17	17	17	17	17
outliers	3	3	1	2	2	2
mean	694	573	153	343	297	301
r	29	36	18	24	22	17
S_r	10.4	12.7	6.4	8.7	7.7	6.2
RSD_r(%)	1.5	2.2	4.2	2.5	2.6	2.1
R	100.8	83.7	59.6	62.7	77.0	32.2
S_R	36.0	29.9	21.3	22.4	27.5	11.5
RSD_R(%)	5.2	5.2	14.0	6.5	9.3	3.8

Table	**47**
Analysis	Determination of free acidity, cold method
Method	COI/T.20/Doc.n°34/Rev.1
Parameter	Acidity - Ring Test COI 2014-2015
Unit	% of oleic acid
Final result rounded to	2 decimal place if ≤ 1 ; 1 decimal place if > 1

A: Crude olive pomace oil

B : Refined olive pomace oil

C : Refined olive oil

D : Extra virgin olive oil (Mario Solinas 2011)

E : Extra virgin olive oil (Mario Solinas 2014)

F : : 70% Lampante olive oil + 30% Grape Seed oil

G : Extra virgin olive oil from late harvest

H : 90% Lampante olive oil + 10% palm olein

	A	B	C	D	E	F	G	H
n	22	22	22	22	22	20	20	20
outliers	1	1	2	2	0	2	3	2
mean	6.3	0.11	0.07	0.13	0.15	1.4	0.50	0.69
r	0.144	0.019	0.018	0.011	0.021	0.015	0.018	0.022
S_r	0.052	0.007	0.006	0.004	0.007	0.005	0.006	0.008
RSD_r(%)	0.8	6.1	9.3	3.2	4.8	0.4	1.3	1.1
R	0.535	0.074	0.043	0.053	0.100	0.121	0.074	0.085
S_R	0.191	0.027	0.015	0.019	0.036	0.043	0.026	0.030
RSD_R(%)	3.0	24.2	22.7	14.7	23.3	3.1	5.3	4.4

Table	**48**
Analysis	Determination of the peroxide value
Method	COI/T.20/Doc.n°35/Rev.1
Parameter	Peroxide value - Ring Test COI 2016
Unit	meqO_2/kg
Final result rounded to	1 decimal place if ≤ 20 ; no decimal if > 20

A: 70% Lampante Olive Oil + 30% Grape Seed Oil

B : 90% Lampante olive oil + 10% palm olein

C : Extra virgin olive oil

D : Olive oil

E : 50% Extra virgin olive oil + 10% refined sunflower oil

F : Extra virgin olive oil (ripe fruitiness)

G : Extra virgin olive oil

	A	B	C	D	E	F	G
n	16	20	15	14	14	19	15
outliers	0	0	0	0	0	0	0
mean	11.7	24	7.8	2.8	4.9	14.3	8.2
r	0.26	1.31	0.41	0.27	0.19	0.52	0.33
S_r	0.09	0.47	0.15	0.1	0.07	0.18	0.12
RSD_r(%)	0.8	1.9	1.9	3.4	1.4	1.3	1.4
R	1.86	4.00	1.55	1.09	1.19	3.18	2.81
S_R	0.66	1.43	0.55	0.39	0.43	1.14	1.0
RSD_R(%)	5.7	5.9	7.1	13.8	8.6	8.0	12.3

Table	**49**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Myristic acid C14:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	0	1	1	3
mean	0.01	0.01	0.01	0.01	0.02
r	0.005	0.007	0.012	0.011	0.006
S_r	0.002	0.003	0.004	0.004	0.002
RSD_r(%)	20	20	36	38	11
R	0.011	0.017	0.017	0.013	0.016
S_R	0.004	0.006	0.006	0.005	0.006
RSD_R(%)	45	47	52	42	32

Table	**50**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Palmitic acid C16:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	15
outliers	2	3	1	0	0
mean	7.96	10.32	10.35	10.51	9.67
r	0.12	0.18	0.42	0.29	0.38
S_r	0.04	0.06	0.15	0.1	0.14
RSD_r(%)	0.5	0.6	1.5	1.000	1.4
R	0.68	0.44	0.93	1.3	1.3
S_R	0.24	0.16	0.33	0.46	0.45
RSD_R(%)	3.0	1.5	3.2	4.4	4.7

Table	**51**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Palmitoleic acid C16:1- Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	2	0	1	1
mean	0.50	0.68	0.74	0.91	0.64
r	0.041	0.027	0.074	0.034	0.040
S_r	0.014	0.01	0.026	0.012	0.014
RSD_r(%)	2.9	1.4	3.6	1.3	2.3
R	0.966	0.077	0.132	0.123	0.128
S_R	0.034	0.027	0.047	0.44	0.046
RSD_R(%)	6.8	4.1	6.4	4.9	7.2

Table	**52**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Heptadecanoic acid C17:0 - from 2000-2006 recognition data
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	25	25	25	25	25
outliers	1	1	1	2	2
mean	0.18	0.06	0.11	0.14	0.12
r	0.013	0.011	0.010	0.009	0.009
S_r	0.005	0.004	0.004	0.003	0.003
RSD_r(%)	2.7	6.9	3.1	2.3	2.7
R	0.020	0.021	0.024	0.021	0.027
S_R	0.007	0.007	0.009	0.008	0.010
RSD_R(%)	4.1	12.6	7.7	5.2	7.8

Table	**53**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Heptadecenoic acid C17:1 - from 2000-2006 recognition data
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	29	29	29	29	29
outliers	3	2	2	3	2
mean	0.26	0.09	0.24	0.22	0.19
r	0.010	0.010	0.014	0.013	0.012
S_r	0.004	0.004	0.005	0.005	0.004
RSD_r(%)	1.4	3.8	2.0	2.2	2.2
R	0.031	0.027	0.041	0.030	0.031
S_R	0.011	0.010	0.015	0.011	0.011
RSD_R(%)	4.2	10.6	6.1	4.9	5.8

Table	**54**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Stearic acid C18:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	15
outliers	2	0	0	0	1
mean	2.88	2.49	2.62	3.49	3.12
r	0.089	0.034	0.084	0.094	0.107
S_r	0.032	0.012	0.030	0.034	0.038
RSD_r(%)	1.1	0.5	1.1	1.0	1.2
R	0.171	0.259	0.246	0.367	0.328
S_R	0.061	0.092	0.088	0.131	0.117
RSD_R(%)	2.1	3.7	3.4	3.8	3.8

Table	**55**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Oleic acid C18:1 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	0	0	1	1	0
mean	79.42	74.55	75.55	76.14	75.8
r	0.42	0.30	0.39	0.23	0.46
S_r	0.15	0.11	0.14	0.08	0.16
RSD_r(%)	0.2	0.2	0.2	0.1	0.2
R	1.37	1.26	1.26	1.33	1.80
S_R	0.49	0.45	0.45	0.47	0.64
RSD_R(%)	0.6	0.6	0.6	0.6	0.9

Table	**56**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Linoleic acid C18:2 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
N	15	15	15	15	15
Outliers	2	1	0	1	0
Mean	7.33	9.66	8.52	7.18	8.75
r	0.07	0.08	0.17	0.12	0.13
S_r	0.02	0.03	0.06	0.04	0.05
RSD_r(%)	0.3	0.3	0.7	0.6	0.6
R	0.34	0.52	0.50	0.45	0.59
S_R	0.12	0.19	0.18	0.16	0.21
RSD_R(%)	1.7	1.9	2.1	2.2	2.4

Table	**57**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Linolenic acid C18:3 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	2	0	0	0	4
mean	0.73	0.90	0.86	0.74	0.75
r	0.036	0.049	0.029	0.039	0.055
S_r	0.013	0.017	0.010	0.014	0.020
RSD_r(%)	1.8	1.9	1.2	1.9	2.6
R	0.08	0.1	0.101	0.079	0.115
S_R	0.029	0.041	0.036	0.028	0.041
RSD_R(%)	3.9	4.6	4.2	3.8	5.4

Table	**58**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Arachidic acid C20:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	15
outliers	1	0	0	1	0
mean	0.39	0.44	0.44	0.42	0.43
r	0.041	0.050	0.037	0.037	0.053
S_r	0.015	0.018	0.013	0.013	0.019
RSD_r(%)	3.8	4.0	3.0	3.1	4.4
R	0.080	0.089	0.086	0.117	0.102
S_R	0.029	0.032	0.031	0.042	0.036
RSD_R(%)	7.3	7.2	7.0	9.8	8.6

Table	**59**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Eicosenoic acid C20:1 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	1	1	1	0	1
mean	0.37	0.39	0.37	0.28	0.30
r	0.026	0.032	0.036	0.047	0.073
S_r	0.009	0.011	0.013	0.017	0.026
RSD_r(%)	7.8	3.0	3.5	6.0	8.9
R	0.082	0.095	0.064	0.079	0.077
S_R	0.029	0.034	0.023	0.028	0.027
RSD_R(%)	7.9	8.7	6.2	10.0	9.3

Table	**60**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Behenic acid C22:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	15
outliers	0	1	1	1	3
mean	0.11	0.14	0.14	0.12	0.19
r	0.022	0.036	0.039	0.045	0.036
S_r	0.008	0.013	0.014	0.016	0.013
RSD_r(%)	7.0	9.6	10.0	14.0	6.9
R	0.038	0.044	0.050	0.056	0.043
S_R	0.014	0.016	0.018	0.020	0.015
RSD_R(%)	12.0	12.0	13.0	17.0	8.3

Table	**61**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	Lignoceric acid C24:0 - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	1	0	0	0	3
mean	0.04	0.06	0.06	0.05	0.08
r	0.017	0.015	0.033	0.033	0.040
S_r	0.006	0.005	0.012	0.012	0.014
RSD_r(%)	15.0	8.9	20.0	24.0	19.0
R	0.055	0.073	0.072	0.054	0.04
S_R	0.020	0.026	0.026	0.019	0.014
RSD_R(%)	49.0	42.0	45.0	39.0	19.0

Table	**62**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	C18:1 trans - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	**B**	**C**	**D**	**E**
n	15	15	15	15	15
outliers	1	1	1	1	2
mean	0.01	0.01	0.01	0.01	0.12
r	0.011	0.013	0.008	0.013	0.044
S_r	0.004	0.005	0.003	0.005	0.016
RSD_r(%)	38.0	46.0	27.0	45.0	13.2
R	0.027	0.028	0.030	0.032	0.157
S_R	0.010	0.010	0.011	0.011	0.056
RSD_R(%)	96.0	86.0	100.0	89.0	48.0

Table	**63**
Analysis	Determination of fatty acid methyl esters by gas chromatography
Method	COI/T.20/Doc. n° 33/ Rev.1
Parameter	C18:2 trans + C18:3 trans - Ring Test COI 2015
Unit	%
Final result rounded to	2 decimal place

A: Extra virgin olive oil

B: Virgin olive oil

C: Lampante olive oil

D: Olive oil

E: Crude olive pomace oil

	A	B	C	D	E
n	15	15	15	15	15
outliers	3	3	4	2	3
mean	0.01	0.01	0.01	0.01	0.03
r	0.013	0.014	0.006	0.029	0.017
S_r	0.005	0.005	0.002	0.010	0.006
RSD_r(%)	84.0	50.0	28.0	115.0	24.0
R	0.019	0.022	0.018	0.032	0.059
S_R	0.007	0.008	0.006	0.012	0.021
RSD_R(%)	123.0	79.0	81.0	130.0	83.0

3. References

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

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

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

AOAC - Statistical Manual of the Association of Official Analytical Chemists. W.J. Youden. E.H. Steiner

Annex 3:
Draft Decision NO DEC-.../109-VI/2019 concerning the method for the determination of the composition and content of sterols, triterpenic dialcohols and aliphatic alcohols by capillary column gas chromatography

DRAFT DECISION No DEC-.../109-VI/2019

CONCERNING THE METHOD FOR THE DETERMINATION OF THE STEROL COMPOSITION AND CONTENT AND ALCOHOLIC COMPOUNDS 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, in particular article 1 "Objectives of the Agreement" as regards standardisation and research, concerning the achievement of 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;

Having regard to the current method for the determination of aliphatic and triterpenic alcohols and considering that triterpenic alcohols are markers of the presence of the pomace olive oil and second-phase virgin olive oil, contributing to fraud control;

Having regard to the recommendation made by the Chemistry and Standardisation Committee at its 2nd meeting, during the 106th session of the Council of Members, concerning the revision of the precision values of method COI/T.20/Doc. No 26 Rev. 1;

Considering the unanimous position of the chemistry experts at their meeting on 25 and 26 September 2017 and their proposal to revise the title, the precision and figure margins and the chromatograms formulated at the meeting on 4 and 5 October 2018;

DECIDES

To revise the title, the precision and figure margins and the chromatograms of the method COI/T.20/Doc. No 26/Rev. 3 (*Determination of the composition and content of sterols, triterpenic dialcohols and aliphatic alcohols by capillary column*

gas chromatography). The revised method COI/T.20/Doc. No 26/Rev.4 (*Determination of the sterol composition and content and alcoholic compounds by capillary gas chromatography*) shall replace and revoke method COI/T.20/Doc. No 26/Rev.3 and shall be listed in the IOC trade standard.

Madrid (Spain), XX 2019

COI/T.20/ Doc. No 26/Rev. 4

Original: ENGLISH

METHOD OF ANALYSIS

DETERMINATION OF THE STEROL COMPOSITION AND CONTENT AND ALCOHOLIC COMPOUNDS BY CAPILLARY GAS CHROMATOGRAPHY

1. SCOPE

The method describes a procedure for determining the individual and total alcoholic compound content of olive oils and olive pomace oils as well as of blends of these two oils.

The alcoholic compounds in olive and olive pomace oils comprise aliphatic alcohols, sterols and triterpenic dialcohols.

2. PRINCIPLE

The oils, with added α-cholestanol and 1-eicosanol as internal standards, are saponified with potassium hydroxide in ethanolic solution and the unsaponifiable matter is then extracted with ethyl ether.

The different alcoholic compounds fractions are separated from the unsaponifiable matter either by thin-layer chromatography on a basic silica gel plate (reference method) or by HPLC with a silica gel column. The fraction recovered from the silica gel separation is transformed into trimethylsilyl ethers and then analysed by capillary column gas chromatography.

PART 1. PREPARATION OF THE UNSAPONIFIABLE MATTER

1. SCOPE

This part describes the preparation and extraction of the unsaponifiable matter. It includes the preparation and extraction of the unsaponifiable matter from olive and olive-pomace oils.

2. PRINCIPLE

A test portion is saponified by boiling under reflux with an ethanolic potassium hydroxide solution. The unsaponifiable matter is extracted with diethyl ether.

3. APPARATUS

The usual laboratory equipment and in particular the following:

3.1. Round bottomed flask fitted with a reflux condenser with ground-glass joints, 250 mL.

3.2. Separating funnel, 500 mL.

3.3. Flasks, 250 mL.

3.4. Microsyringes, 100 μL and 500 μL.

3.5. Cylindrical filter funnel with a G3 porous septum (porosity 15-40 μm) of diameter approximately 2 cm and a depth of 5 cm, suitable for filtration under vacuum with male ground-glass joint.

3.6. Conical flask with ground-glass female joint, 50 mL which can be fitted to the filter funnel (3.5).

3.7. Test tube with a tapering bottom and a sealing glass stopper, 10 mL.

3.8. Calcium dichloride desiccator.

4. REAGENTS

4.1. Potassium hydroxide minimum titre 85 %.

4.2. Potassium hydroxide ethanolic solution, approximately 2 M.

Dissolve 130 g of potassium hydroxide (4.1) with cooling in 200 ml of distilled water and then make up to one litre with ethanol (4.7). Keep the solution in well-stoppered dark glass bottles and stored max. 2 days.

4.3. Ethyl ether, for analysis quality.

4.4. Anhydrous sodium sulphate, for analysis quality.

4.5. Acetone, for chromatography quality.

4.6. Ethyl ether, for chromatography quality.

4.7. Ethanol of analytical quality.

4.8. Ethyl acetate of analytical quality.

4.9. Internal standard, α-cholestanol, purity more than 99% (purity must be checked by GC analysis).

4.10. Internal standard solution of α-cholestanol, 0.2% solution (m/V) in ethyl acetate (4.8).

4.11. Phenolphthalein solution, 10 g/L in ethanol (4.7).

4.12. A 0.1% (m/v) solution of 1-eicosanol in ethyl acetate (internal standard).

5. PROCEDURE

Using a 500 μL micro-syringe (3.4) introduce into the 250 mL flask (3.1) a volume of the α-cholestanol internal standard solution (4.10) and a volume of 1-eicosanol (4.12) containing an amount of cholestanol and eicosanol corresponding to approximately 10% of the sterol and alcohol content of the sample. For example, for 5 g of olive oil sample add 500 μL of the α-cholestanol solution (4.10) and 250 μL of 1-eicosanol solution (4.12). For pomace olive oils add 1500 μL of both α-cholestanol solution (4.10) and 1-eicosanol (4.12). Evaporate until dryness with a gentle current of nitrogen in a warm water bath. After cooling the flask, weigh 5.00 ± 0.01 g of the dry filtered sample into the same flask.

Note 1: Animal or vegetable oils and fats containing appreciable quantities of cholesterol may show a peak having a retention time identical to cholestanol. If this case occurs that the sterol fraction will have to be analysed in duplicate with and without internal standard.

Add 50 mL of 2M ethanolic potassium hydroxide solution (4.2) and some pumice, fit the reflux condenser and heat to gentle boiling until saponification takes place (the solution becomes clear). Continue heating for a further 20 minutes, then add 50 mL of distilled water

from the top of the condenser, detach the condenser and cool the flask to approximately 30 ºC.

Transfer the contents of the flask quantitatively into a 500 mL separating funnel (3.2) using several portions of distilled water (50 mL). Add approximately 80 ml of ethyl ether (4.6), shake vigorously for approximately 60 seconds, periodically releasing the pressure by inverting the separating funnel and opening the stopcock. Allow standing until there is complete separation of the two phases (Note 2). Then draw off the soap solution as completely as possible into a second separating funnel. Perform two further extractions on the water-alcohol phase in the same way using 60 to 70 mL of ethyl ether (4.6).

Note 2: Any emulsion can be destroyed by adding small quantities of ethanol (4.7).

Combine the three ether extracts in one separating funnel containing 50 mL of water. Continue to wash with water (50 mL) until the wash water no longer gives a pink colour on the addition of a drop of phenolphthalein solution (4.11). When the wash water has been removed, filter on anhydrous sodium sulphate (4.4) into a previously weighed 250 mL flask, washing the funnel and filter with small quantities of ethyl ether (4.6).

Evaporate the solvent by distillation in a rotary evaporator at 30 ºC under vacuum. Add 5mL of acetone (4.5) and remove the volatile solvent completely in a gentle current of nitrogen. Dry the residue in the oven at 103 ± 2 ºC for 15 min. Cool in desiccators and weigh to the nearest 0.1 mg.

PART 2. SEPARATION OF THE ALCOHOLIC COMPOUNDS FRACTIONS

1. SCOPE

The unsaponifiable matter prepared in Part 1 is fractionated in the different alcoholic compounds, aliphatic alcohols, sterols and triterpenic dialcohols (erythrodiol and uvaol).

2. PRINCIPLE

The unsaponifiable matter can be fractionated using basic thin layer chromatography (reference method), revealed and the corresponding bands scratched and extracted. As an alternative method of separation, HPLC using a silica gel column and UV detector and the different fractions collected. The aliphatic and triterpenic alcohols as well as the sterol and triterpenic dialcohols are isolated together.

3. APPARATUS

The usual laboratory equipment and in particular the following:

3.1. Complete apparatus for analysis by thin-layer chromatography using 20 x 20 cm glass plates.

3.2. Ultraviolet lamp with a wavelength of 366 or 254 nm.

3.3. Microsyringes, 100 μL and 500 μL.

3.4. Cylindrical filter funnel with a G3 porous septum (porosity 15-40 μm) of diameter approximately 2 cm and a depth of 5 cm, suitable for filtration under vacuum with male ground-glass joint.

3.5. Conical flask with ground-glass female joint, 50 mL which can be fitted to the filter funnel (3.4).

3.6. Test tube with a tapering bottom and a sealing glass stopper, 10 mL.

3.7. Calcium dichloride desiccator.

3.8. HPLC system, consisting of:

3.8.1. Binary pump

3.8.2. Manual or automatic injector equipped with 200 μL injection loop.

3.8.3. In-line degasser.

3.8.4. UV-VIS or IR detector

3.9 HPLC column (25 cm x 4 mm i.d.) with silica gel 60 (5 μm particle size).

3.10. Syringe filter, 0.45 μm.

3.11. Conical flask 25 mL.

4. REAGENTS

4.1. Potassium hydroxide minimum titre 85 %.

4.2. Potassium hydroxide ethanolic solution, approximately 2 M.

Dissolve 130 g of potassium hydroxide (4.1) with cooling in 200 ml of distilled water and then make up to one litre with ethanol (4.9). Keep the solution in well-stoppered dark glass bottles and stored max. 2 days.

4.3. Ethyl ether, for analysis quality.

4.4. Potassium hydroxide ethanolic solution, approximately 0.2 M.

Dissolve 13 g of potassium hydroxide (4.1) in 20 ml of distilled water and make up to one litre with ethanol (4.9).

4.5. Glass 20x20 plates coated with silica gel, without fluorescence indicator, thickness 0.25 mm (commercially available ready for use).

4.6. Acetone, for chromatography quality.

4.7. n-Hexane, for chromatography quality.

4.8. Ethyl ether, for chromatography quality.

4.9. Ethanol of analytical quality.

4.10. Ethyl acetate of analytical quality.

4.11. Reference solution for thin-layer chromatography: cholesterol, phytosterols, alcohols and Erythrodiol 5% solution in Ethyl acetate (4.10).

4.12. Solution of 2,7-dichlorofluorescein, 0.2% in ethanolic solution. Make slightly basic by adding a few drops of 2 M alcoholic potassium hydroxide solution (4.2).

4.13. *n*-Hexane (4.7)/ethyl ether (4.8) mixture 65:35 (V/V).

4.14. HPLC mobile phase *n*-hexane (4.7)/ethyl ether (4.8) (1:1) (V/V).

5. REFERENCE METHOD: SEPARATION OF THE ALCOHOLIC COMPOUNDS BY BASIC TLC.

Preparation of the basic thin layer chromatography plates. Immerse or dip the silica gel plates (4.5) about 4 cm in the 0.2 M ethanolic potassium hydroxide solution (4.4) for 10 seconds,

then allow to dry in a fume cupboard for two hours and finally place in an oven at 100º C for one hour.

Remove from the oven and keep in a calcium chloride desiccator (3.7) until required for use (plates treated in this way must be used within 15 days).

Place hexane/ethyl ether mixture (4.13) (Note 3) into the development chamber, to a depth of approximately 1 cm. Close the chamber with the appropriate cover and leave thus for at least half an hour, in a cool place, so that liquid-vapour equilibrium is established strips of filter paper dipping into the eluent may be placed on the internal surfaces of the chamber. This reduces developing time by approximately one-third and brings about more uniform and regular elution of the components.

Note 3: The developing mixture should have replaced for every test, in order to achieve perfectly reproducible elution conditions, alternative solvent 50:50 (V/V) *n*-hexane/ethyl ether may be used.

Prepare an approximately 5% solution of the unsaponifiable prepared in PART 1 in ethyl acetate (4.10) and, using the 100 μL microsyringe (3.3), depose 0.3 ml of the solution on a narrow and uniform streak on the lower end (2 cm) of the chromatographic plate (4.5). In line with the streak, place 2 to 3 μL of the material reference solution (4.11), so that the sterol, triterpene dialcohols and alcohols bands can be identified after developing.

Place the plate in the developing chamber (3.1). The ambient temperature should be maintained between 15 and 20 ºC (Note 4). Immediately close the chamber with the cover and allow eluting until the solvent front reaches approximately 1 cm from the upper edge of the plate. Remove the plate from the developing chamber and evaporate the solvent in a flow of hot air or by leaving the plate for a short while, under a hood.

Note 4: Higher temperature could worsen the separation.

Spray the plate lightly and uniformly with the 2,7-dichlorofluorescein solution (4.12) and then

leave to dry. When the plate is observed under ultraviolet lamp (3.2), the sterols, triterpene dialcohols and alcohols bands can be identified through being aligned with the spots obtained from the reference solution (4.11). Mark the limits of the bands along the edges of the fluorescence with a black pencil (see TLC plate Figure 1).

By using a metal spatula, scrape off the silica gel of the marked area. Place the finely comminuted material removed into the filter funnel (3.4). Add 10 mL of hot ethyl acetate (4.10), mix carefully with the metal spatula and filter (under vacuum if necessary), collecting the filtrate in the conical flask (3.5.) attached to the filter funnel.

Wash the residue in the flask three times with ethyl ether (4.3) (approximately 10 mL each time), collecting the filtrate in the same flask attached to the funnel, evaporate the filtrate to a volume of 4 to 5 mL, transfer the residual solution to the previously weighed 10 mL test tube (3.6), evaporate to dryness by mild heating, in a gentle flow of nitrogen, make up again using a few drops of acetone (4.6), evaporate again to dryness, The residue contained in the test tube consists of the sterol and triterpene dialcohols or the alcohols and triterpenic alcohols fractions.

6. SEPARATION OF THE ALCOHOLIC FRACTION BY HPLC.

The unsaponifiable obtained from PART 1 is dissolved in 3 mL of the mobile phase (4.14), filter the solution with a syringe filter (3.10) and reserve.

Inject 200 μL of the filtered unsaponifiable solution in the HPLC (3.8).

Run the HPLC separation at 0.8 mL/min, discard the first 5 min. and collect in 25 mL conical flasks (3.11) between the 5 and 10 min. for aliphatic and triterpenic alcohols and between 11 and 25 min for sterols and erythrodiol and uvaol (Note 5).

The separation can be monitored with an UV detector at 210 nm wavelengths or a refractive

index detector. (see Figure 6 in Annexes).

The fractions are evaporated until dryness and prepared for chromatographic analysis.

Note 5. Carefully control the pressure of the HPLC pump, the ethyl ether can increase the pressure, adjust the flow to keep the pressure under control.

PART 3. GAS CHROMATOGRAPHIC ANALYSIS OF THE ALCOHOLIC COMPOUNDS FRACTIONS.

1. SCOPE

This part gives general guidance for the application of capillary column gas chromatography to determine the qualitative and quantitative composition of the alcoholic compounds isolated in accordance with the method specified in PART 2 of this method.

2. PRINCIPLE

The fractions collected from the unsaponifiable matter using TLC or HPLC are derivatized into trimethylsilyl ethers and analysed by capillary column gas chromatography with split injection and flame ionization detector.

3. APPARATUS

The usual laboratory equipment and in particular the following:

3.1. Test tube with a tapering bottom and a sealing glass stopper, 10 mL.

3.2. Gas chromatograph suitable for use with a capillary column with split injection system, consisting of:

3.2.1. A thermostatic chamber for columns capable of maintaining the desired temperature with an accuracy of ± 1ºC;

3.2.2. A temperature-adjustable injection unit with a persilanised glass vaporising element and split system;

3.2.3. A flame ionisation detector (FID);

3.2.4. Data acquisition system suitable for use with the FID detector (3.10.3.), capable of

manual integration.

3.3. Fused-silica capillary column of length 20 to 30 m, internal diameter 0.25 to 0.32 mm, coated with 5 % Diphenyl - 95 % Dimethylpolysiloxane (SE-52 or SE-54 stationary phase or equivalent), to a uniform thickness between 0.10 and 0.30 μm.

3.4. Microsyringe, of 10 μL capacity, for gas chromatography, with cemented needle suitable for split injection.

4. REAGENTS

4.1. Anhydrous pyridine, for chromatography quality.

4.2. Hexamethyl disilazane of analytical quality.

4.3. Trimethylchlorosilane of analytical quality.

4.4. Sample solutions of sterol trimethylsilyl ethers. To be prepared at the time of use from sterols and erythrodiol obtained from oils containing them.

4.5. Standard solutions of trimethylsilyl ethers of aliphatic alcohols from C20 to C28. They may be prepared from mixtures of pure alcohols at the time they are required for use.

4.6. Carrier gas: hydrogen or helium, gas-chromatographic purity.

4.7. Auxiliary gases: hydrogen, helium, nitrogen and air, of gas-chromatographic purity.

4.8. Silylation reagent, consisting of a 9:3:1 (V/V/V) mixture of pyridine/hexamethyl disilazane/trimethylchlorosilane.

4.9. n-Hexane, for chromatography quality.

5. PREPARATION OF THE TRIMETHYLSILYL ETHERS.

Add the silylation reagent (4.8) (Note 6), in the ratio of 50 μl for every milligram of alcoholic compound, in the test tube (3.1) containing the alcoholic compound fraction, avoiding any uptake of moisture (Note 7).

Note 6: Ready for use solutions are available commercially. Other silylation reagents, such as, for example, bistrimethylsilyl trifluor acetamide + 1% trimethylchlorosilane, which has to be diluted with an equal volume of anhydrous pyridine, are also available. Pyridine can be replaced by the same amount of acetonitrile.

Note 7: The slight opalescence, which may form, is normal and does not cause any anomaly. The formation of a

white flock or the appearance of a pink colour is indicative of the presence of moisture or deterioration of the reagent. If these occur the test must be repeated (only if hexamethyldisilazane/trimethylchlorosilane is used).

Stopper the test tube (3.1), shake carefully (without overturning) until the compounds are completely dissolved. Leave to stand for at least 15 minutes at ambient temperature and then centrifuge for a few minutes. The clear solution is ready for gas chromatographic analysis.

6. GAS CHROMATOGRAPHIC ANALYSIS.

6.1. Preliminary operations, capillary column conditioning.

Fit the column (3.3) in the gas chromatograph, by attaching the inlet end to the split injector and the outlet end to the detector.

Carry out general checks on the gas chromatograph unit (leaks from the gas circuits, detector efficiency, efficiency of the splitting system and recording system, etc.).

If the column is being used for the first time, it is recommended that it should be subjected to conditioning: passing a gentle flow of gas through the column itself, then switch on the gas chromatography unit and begin a gradual heating, up to a temperature of at least 20 ºC above the operating temperature (Note 8). Hold this temperature for at least two hours, then place the entire unit in operating mode (adjustment of gas flows and splitting, ignition of the flame, connection with the computing system, adjustment of the column, detector and injector temperature, etc.) and then record the signal with a sensitivity at least two times greater than that one intended for the analysis. The course of the base line must be linear, without peaks of any kind, and must not show drift. A negative straight-line drift indicates leakage from the column connections; a positive drift indicates inadequate conditioning of the column.

Note 8: The conditioning temperature must always be at least 20ºC less than the maximum temperature specified for the stationary phase used.

6.2. Operating conditions.

Optimize the temperature programme and the carrier gas flow so that chromatograms similar to Figure 2 to 5 are obtained.

The following parameters were tested and found useful:

6.2.1. Aliphatic alcohols

Oven Program	180 ºC (8 min.) → 260 ºC (at 5ºC/min) → 260 ºC (15 min)
Injector Temperature	280 ºC
Detector Temperature	290 ºC
Linear Velocity of Carrier gas	Helium (20 to 30 cm/s); Hydrogen (30 to 50 cm/s)
Split Ratio	1:50 to 1:100
Volume Injected	0.5 to 1 µL of TMSE solution

6.2.2. Sterol and triterpenic dialcohols

Oven Program	260 ± 5 ºC Isothermal
Injector Temperature	280 – 300 ºC
Detector Temperature	280 – 300 ºC
Linear Velocity of Carrier gas	Helium (20 to 30 cm/s); Hydrogen (30 to 50 cm/s)
Split Ratio	1:50 to 1:100
Volume Injected	0.5 to 1 µL of TMSE solution

These conditions may be changed according to the characteristics of the column and gas chromatograph, so as to obtain chromatograms, which meet the following requirements:

- Alcohol C26 retention time shall be 18 ± 5 minutes.
- Alcohol C22 peak shall be 80 ± 20% of the full-scale value for olive oil and 40 ± 20% of the full-scale value for olive-pomace oil.
- The retention time for the β-sitosterol peak should be at 20 ± 5 min.
- The campesterol peak should be: for olive oil (mean content 3 %) 20 ± 5 % of full scale.

– All the present sterols must be separated. In addition to being separated the peaks, they must also be completely resolved, i.e. the peak trace should return to the base line before leaving for the next peak. Incomplete resolution is, however, tolerated, provided that the peak at RRT 1.02 (Sitostanol) can be quantified using the perpendicular.

6.3. Analytical procedure

By using the 10 µl microsyringe (3.4), take 1 µl of hexane, draw in 0.5 µl of air and then 0.5 to 1 µl of the sample solution. Raise the plunger of the syringe further, so the needle is emptied. Push the needle through the membrane of the injector and after one to two seconds, inject rapidly, and then slowly remove the needle after around five seconds. An automatic injector can be used as well.

Carry out the recording until the TMSE of the corresponding alcoholic compounds present are completely eluted. The base line must continue to meet the requirements of the corresponding operating conditions (6.2.1 or 6.2.2).

6.4. Peak identification:

Identify individual peaks on the basis of retention times and by comparison with the mixture of the aliphatic and triterpenic alcohols or the sterol and triterpene dialcohols TMSE, analysed under the same conditions. A chromatogram of the aliphatic and triterpenic alcohols fraction is showed in Figure 4 and the corresponding chromatogram for sterols and triterpenic dialcohols are showed in Figure 2.

The aliphatic alcohols are eluted in the following order: C20-ol (I.S.), C22-ol, C23-ol, C24-ol, C25-ol, C26-ol, C27-ol and C28-ol.

The sterols and triterpene dialcohols are eluted in the following order: cholesterol, brassicasterol, ergosterol, 24-methylen-cholesterol, campesterol, campestanol, stigmasterol, Δ7-campesterol, Δ5,23-stigmastadienol, clerosterol, β-sistosterol, sitostanol, Δ5-avenasterol, Δ5,24-stigmastadienol, Δ7-stigmastenol, Δ7-avenasterol, erythrodiol and uvaol.

6.5. Quantitative evaluation.

The peak areas of 1-eicosanol and of the aliphatic alcohols C22, C24, C26, C28 are calculated by a data acquisition system. The response factor for 1-eicosanol should be considered equal to 1.

Calculate the areas of the α-cholestanol and the sterol and triterpene dialcohols peaks by using the computing system. Ignore peaks for any compound, which are not included (ergosterol must not be calculated) among those listed in Table 1. The response factor for α-cholestanol should be considered equal to 1.

Calculate the concentration of each individual alcoholic compound, in mg/kg of fatty material, as follows:

$$Alcoholic\ compound\ x = \frac{A_x \times m_s}{A_s \times m} \times 1000$$

where:

A_x = Peak area for alcoholic compound x, in computing system counts.

A_s = Area of the 1-eicosanol/α-cholestanol peak, in computing system counts.

m_s = Mass of added 1-eicosanol/α-cholestanol, in milligrams.

m = Mass of the sample used for determination, in grams.

7. EXPRESSION OF THE RESULTS

Report individual aliphatic and triterpenic alcohols concentrations as mg/kg of fatty material and their sum as "total aliphatic alcohol content". The total content is the sum of C22, C24, C26 and C28.

The composition of each of the individual alcoholic compound should be expressed to one decimal point.

Total sterol concentration should be expressed without any decimal point.

Calculate the percentage of each individual sterol from the ratio of the relevant peak area to the total peak area for sterols:

$$Sterol\ x = \frac{A_x}{\Sigma A} \times 100$$

where:

A_x = Peak area for sterol x.

ΣA = Total peak area for sterols.

Apparent β-sitosterol: Δ5-23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5-24-stigmastadienol.

Calculate the percentage of erythrodiol and uvaol:

$$Erythrodiol + Uvaol = \frac{A_{Er} + A_{Uv}}{\Sigma A_T} \times 100$$

where:

A_{Er} = Area of Erythrodiol in computing system counts.

A_{Uv} = Area of Uvaol in computing system counts.

ΣA_T = Sum area for sterol + erythrodiol + uvaol in computing system counts.

Besides the calculation of relative percentage of single sterols and triterpenic dialcohols and the total concentration of sterols, the concentration of erythrodiol and of uvaol and their sum, in mg/kg of fatty material must be calculated, according the following expressions:

$$Erythrodiol\ = \frac{A_{Er} \times m_s}{A_s \times m} \times 1000$$

$$Uvaol = \frac{A_{Uv} \times m_s}{A_s \times m} \times 1000$$

where:

A_{Er} = Peak area of Erythrodiol, in computing system counts.

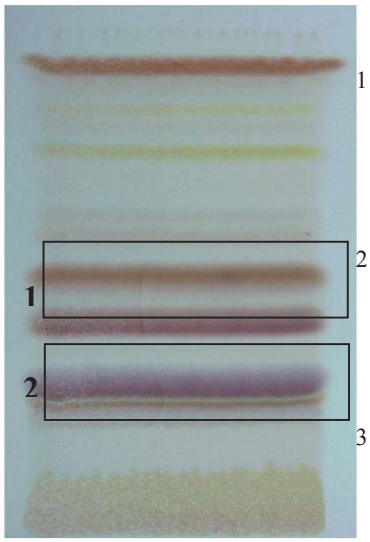
A_{Uv} = Area of Uvaol in computing system counts.

A_s = Area of the α-cholestanol peak, in computing system counts.

m_s = Mass of added α-cholestanol, in milligrams.

m = Mass of the sample used for determination, in grams.

APPENDIX



1 Hydrocarbons

2 α-Tocopherol

3 Prenols

4 Triterpenic Alcohols

5 Aliphatic Alcohols

Figure 1. Thin-layer chromatography plate of the unsaponifiable fraction from olive pomace oil eluted twice with hexane:diethyl ether (65:35), developed with SO_4H_2 (50%) and heated. The bands that should be scrapped are the contained within the rectangle, 1 are the bands for aliphatic alcohols and 2 for the sterols and triterpenic dialcohols.

TABLE I

RELATIVE RETENTION TIMES FOR STEROLS

Peak	Identification		Relative retention Time	
			SE 54 column	SE 52 Column
1	Cholesterol	Δ-5-cholesten-3*β*-ol	0.67	0.63
2	Cholestanol	5*α*-cholestan-3*β*-ol	0.68	0.64
3	Brassicasterol	[24S]-24-methyl-Δ-5,22-cholestadien-3*β*-ol	0.73	0.71
*	Ergosterol	[24S] 24 methyl-Δ-5,7,22-cholestatrien-3*β*-ol	0,78	0,76
4	24-methylene-cholesterol	24-methylene-Δ-5,24-cholestadien-3*β*-o1	0.82	0.80
5	Campesterol	(24R)-24-methyl-Δ-5-cholesten-3*β*-ol	0.83	0.81
6	Campestanol	(24R)-24-methyl-cholestan-3*β*-ol	0.85	0.82
7	Stigmasterol	(24S)-24-ethyl-Δ-5,22-cholestadien-3*β*-ol	0.88	0.87
8	Δ-7-campesterol	(24R)-24-methyl-Δ-7-cholesten-3*β*-ol	0.93	0.92
9	Δ-5,23-stigmastadienol	(24R,S)-24-ethyl-Δ-5,23-choIestadien-3*β*-ol	0.95	0.95
10	Clerosterol	(24S)-24-ethyl-Δ-5,25-cholestadien-3*β*-ol	0.96	0.96
11	*β*-sitosterol	(24R)-24-ethyl-Δ-5-cholesten-3*β*-ol	1.00	1.00
12	Sitostanol	24-ethyl-cholestan-3*β*-ol	1.02	1.02
13	Δ-5-avenasterol	(24Z)-24-ethylidene-Δ-cholesten-3*β*-ol	1.03	1.03
14	Δ-5-24-stigmastadienol	(24R,S)-24-ethyl-Δ-5,24-cholestadien-3*β*-ol	1.08	1.08
15	Δ-7-stigmastenol	(24R,S)-24-ethyl-Δ-7-cholesten-3*β*-ol	1.12	1.12
16	Δ-7-avenasterol	(24Z)-24-ethylidene-Δ-7-cholesten-3*β*-ol	1.16	1.16
17	Erythrodiol	5α olean-12en-3β28 diol	1,41	1,41
18	Uvaol	Δ12-ursen-3β28 diol	1,52	1,52

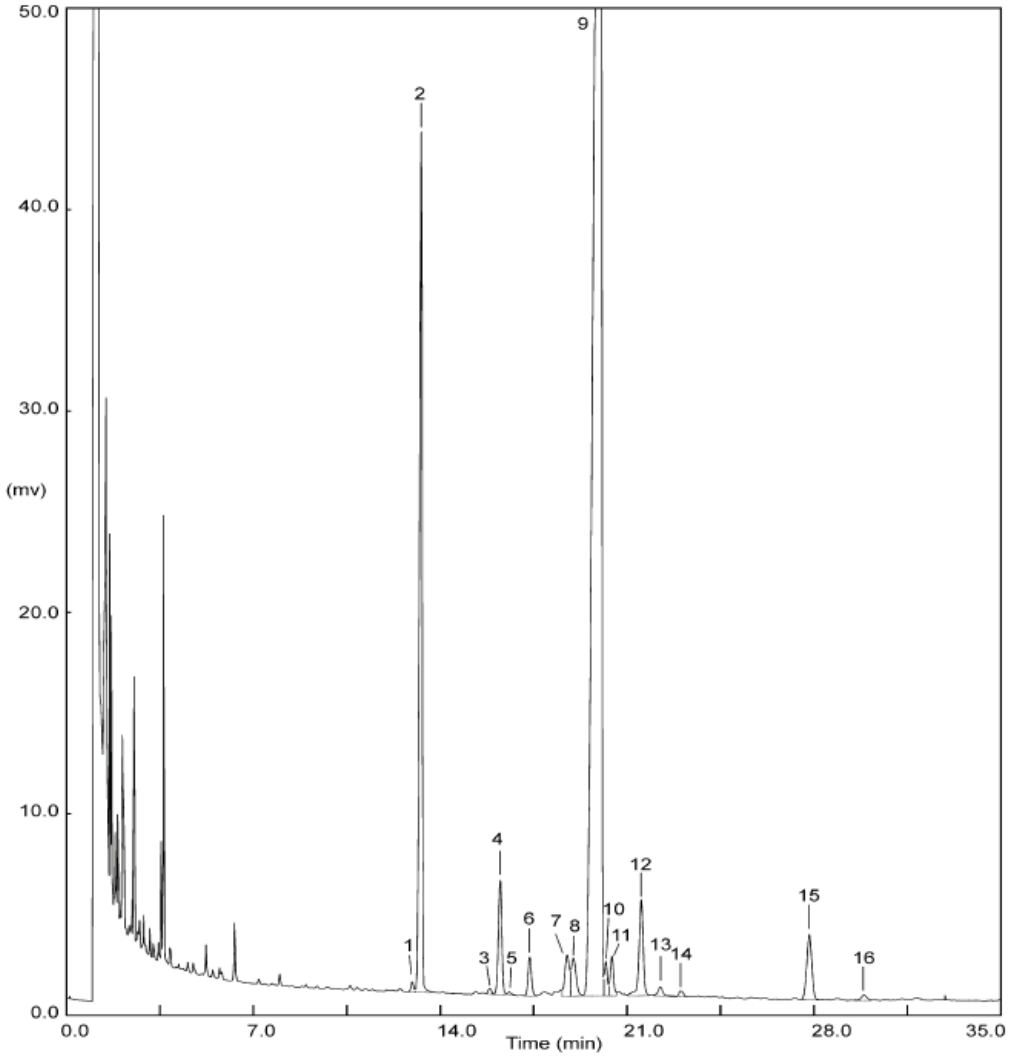


Figure 2. GC-FID chromatographic profile of the sterol and triterpenic dialcohols from refined olive oil. (1) Cholesterol, (2) α-Cholestanol (I.S.), (3) 24-Methylencholesterol, (4) Campesterol, (5) Campestanol, (6) Stigmasterol, (7) Δ5,23-Stigmastadienol, (8) Clerosterol, (9) β-Sitosterol, (10) Sitostanol, (11) Δ5-Avenasterol, (12) Δ5,24-Stigmastadienol, (13) Δ7-Stigmastenol, (14) Δ7-Avenasterol, (15) Erythrodiol, (16) Uvaol.

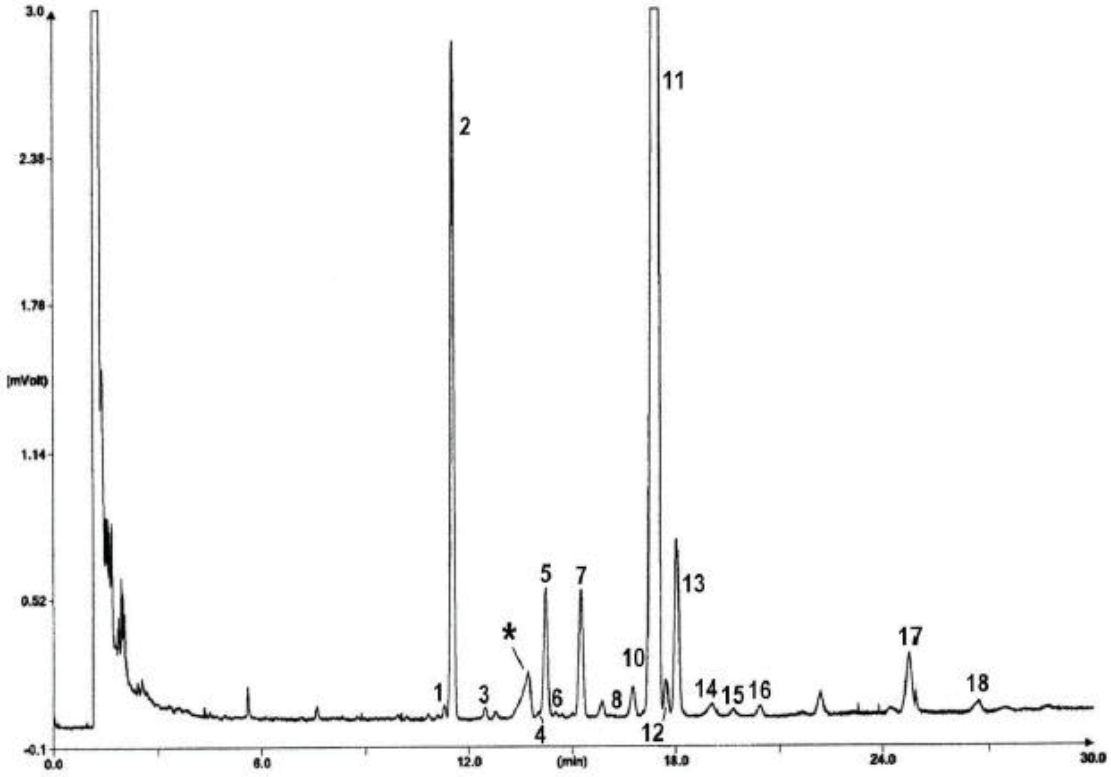


Figure 3. GC-FID chromatographic profile of the sterol and triterpenic dialcohols from a lampante olive oil. (1) Cholesterol, (2) α-Cholestanol, (3) Brassicasterol, (4) 24-Methylencholesterol, (5) Campesterol, (6) Campestanol, (7) Stigmasterol, (8) Δ7-Campesterol, (10) Clerosterol, (11) β-Sitosterol, (12) Sitostanol, (13) Δ5-Avenasterol, (14) Δ5,24-Stigmastadienol, (15) Δ7-Stigmastenol, (16) Δ7-Avenasterol, (17) Erythrodiol, (18) Uvaol.

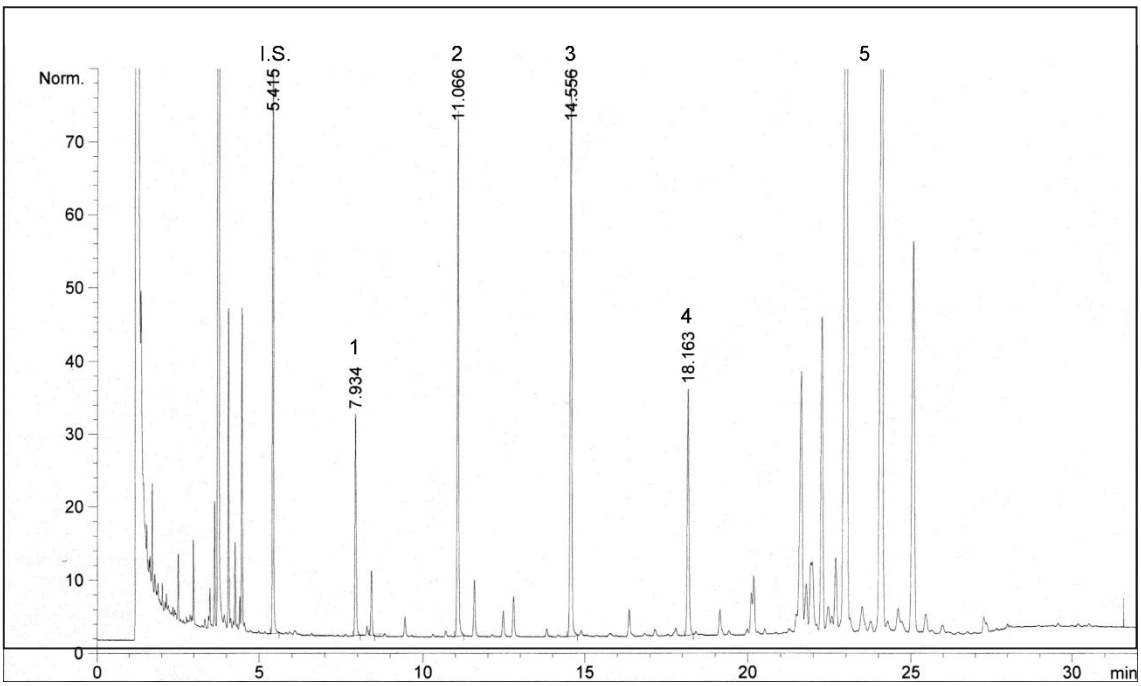


Figure 4. GC-FID chromatographic profile of aliphatic alcohols and triterpenic alcohols of olive oil. (I.S.) C20-ol, (1) C22-ol, (2) C24-ol, (3) C26-ol, (4) C28-ol, (5) triterpenic alcohols.

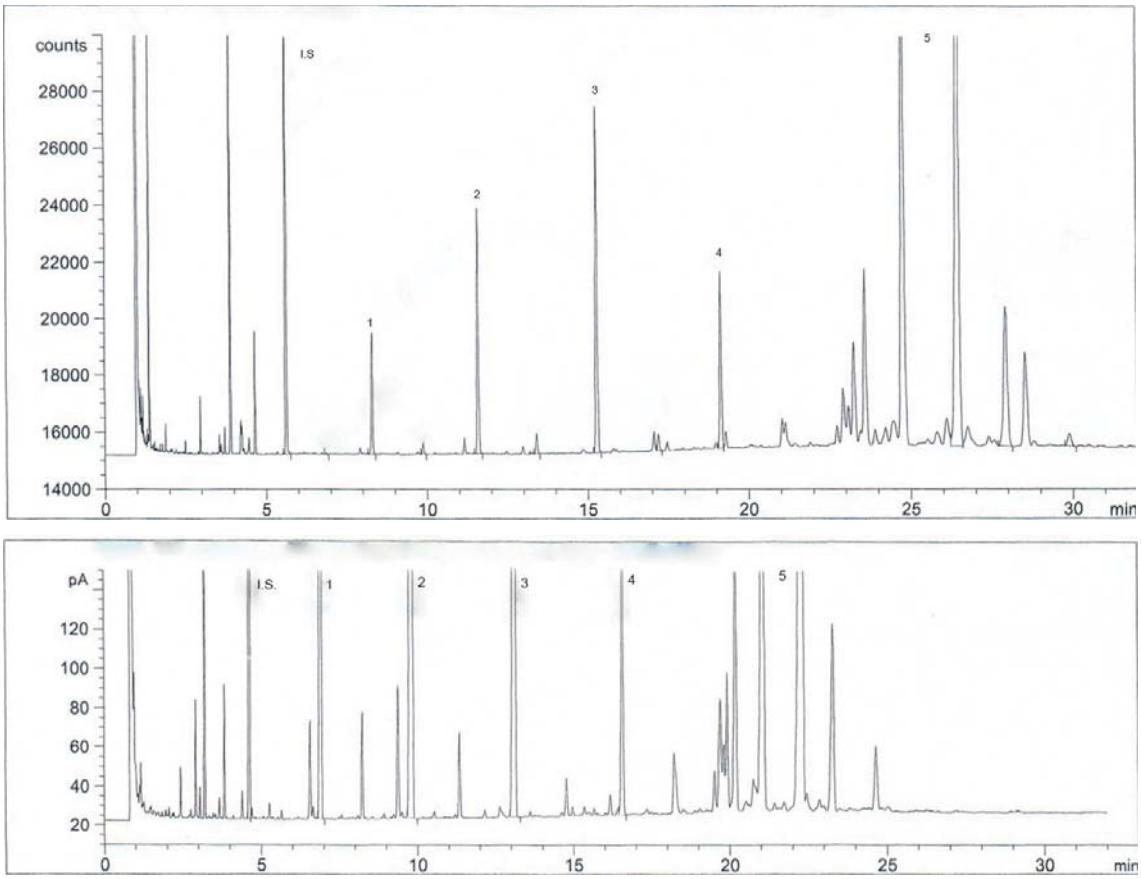


Figure 5- GC-FID chromatographic profile of aliphatic alcohols and triterpenic alcohols of a refined olive oil and a second centrifugation olive oil. (I.S.) C20-ol, (1) C22-ol, (2) C24-ol, (3) C26-ol, (4) C28-ol, (5) triterpenic alcohols.

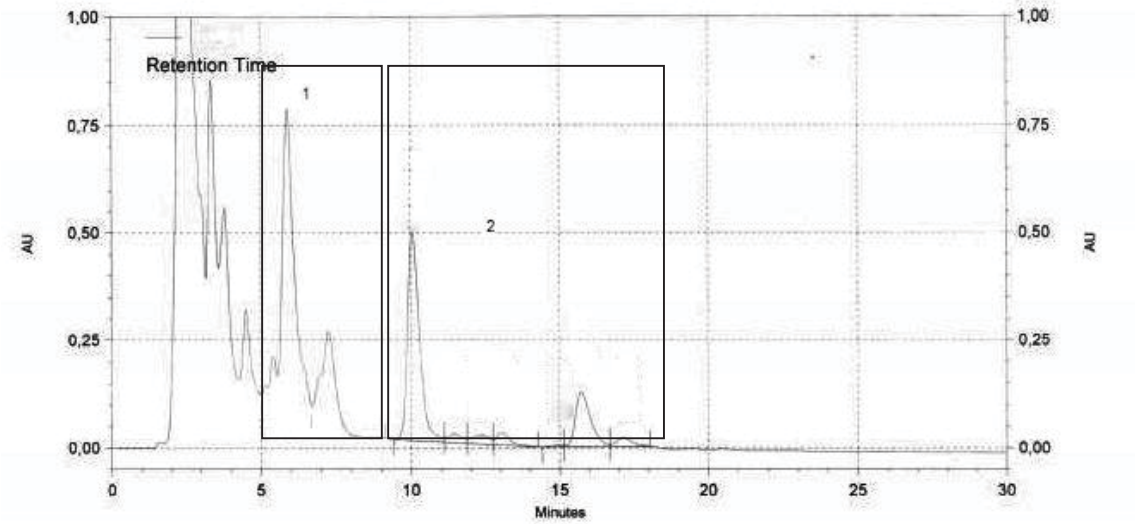


Figure 6. HPLC Chromatogram of an olive oil unsaponifiable separated by HPLC using a UV detector. (1) Aliphatic and triperpenic alcohols; (2) Sterols and triterpenic dialcohols.

PRECISION VALUES OF THE METHOD

1. Analysis of the collaborative test results for sterol content and alcoholic compounds:

The precision values of the method are given in the table on the next page for each parameter studied:

n number of laboratories which participated in the test

outliers number of laboratories with outlying values

mean mean of the accepted results

r repeatability

S_r repeatability standard deviation

RSD_r(%) repeatability coefficient of variation (S_r x 100/ mean)

R reproducibility

S_R reproducibility standard deviation

RSD_R(%) reproducibility coefficient of variation (S_R x 100/ mean)

Results of interlaboratory test to separate the unsaponifiable fraction by TLC and HPLC of the sterol and alcohol fraction. Evaluation of the absolute content of erythrodiol and uvaol. An interlaboratory test was carried out in 2016 in accordance with ISO 5725. The results are summarised in Tables A.1 to A.22.

A: extra virgin olive oil from picual variety

B: lampante olive oil

C: olive oil + 10% sunflower oil

D: high oleic sunflower + 50 mg/kg erythrodiol + 20 mg/kg uvaol

E: virgin olive oil + refined olive pomace oil

Table A.1— Cholesterol by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	1	0	0
mean (%)	0.1	0.3	0.1	0.1	0.1
r	0.03	0.10	0.05	0.07	0.03
S_r	0.01	0.04	0.02	0.03	0.01
RSD_r(%)	8.3	13.6	14.2	20.2	8.6
R	0.06	0.28	0.09	0.14	0.07
S_R	0.02	0.10	0.03	0.05	0.02
RSD_R(%)	15.9	37.5	22.5	40.3	23.4

Table A.2— Cholesterol by HPLC

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	0	0
mean (%)	0.1	0.3	0.1	0.1	0.1
r	0.03	0.10	0.07	0.03	0.04
S_r	0.01	0.04	0.02	0.01	0.01
RSD_r(%)	7.6	15.0	17.7	9.3	10.8
R	0.06	0.24	0.09	0.11	0.06
S_R	0.02	0.08	0.03	0.04	0.02
RSD_R(%)	16.5	34.3	23.2	35.4	16.7

Table A.3— Brassicasterol by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	1	0	0	1	1
mean (%)	0.0	0.0	0.0	0.0	0.1

r	0.02	0.03	0.03	0.02	0.02
S_r	0.01	0.01	0.01	0.01	0.01
RSD_r(%)	68.1	23.6	39.3	25.3	14.7
R	0.03	0.11	0.09	0.07	0.15
S_R	0.01	0.04	0.03	0.03	0.05
RSD_R(%)	103.7	90.5	105.3	94.5	90.7

Table A.4— Brassicasterol by HPLC

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	1	0	1	0
mean (%)	0.0	0.0	0.0	0.0	0.0
r	0.01	0.02	0.02	0.02	0.03
S_r	0.004	0.01	0.01	0.01	0.01
RSD_r(%)	23.9	19.9	30.0	35.6	22.5
R	0.04	0.06	0.05	0.04	0.09
S_R	0.02	0.02	0.02	0.01	0.03
RSD_R(%)	90.3	68.0	88.7	83.3	79.8

Table A.5— Campesterol by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	2	0	0	0	1
mean (%)	3.1	3.2	3.9	8.3	3.1
r	0.22	0.15	0.26	0.18	0.15
S_r	0.08	0.06	0.09	0.06	0.05
RSD_r(%)	2.6	1.7	2.4	0.8	1.7
R	0.25	0.39	0.45	0.78	0.27
S_R	0.09	0.13	0.16	0.28	0.10
RSD_R(%)	2.9	4.3	4.1	3.4	3.1

Table A.6— Campesterol by HPLC

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	1	0	0	0
mean (%)	3.0	3.3	3.9	8.4	3.2

r	0.12	0.13	0.18	0.22	0.11
S_r	0.04	0.05	0.06	0.08	0.04
RSD_r(%)	1.5	1.4	1.6	0.9	1.3
R	0.48	0.59	0.37	0.52	0.28
S_R	0.17	0.21	0.13	0.18	0.10
RSD_R(%)	5.7	6.5	3.4	2.2	3.2

Table A.7— Stigmasterol by Reference Method (TLC)

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	1	0
mean (%)	1.1	2.4	2.0	7.2	1.3
r	0.07	0.16	0.25	0.12	0.09
S_r	0.02	0.06	0.09	0.04	0.03
RSD_r(%)	2.1	2.4	4.5	0.6	2.5
R	0.18	0.29	0.41	0.62	0.11
S_R	0.06	0.10	0.15	0.22	0.04
RSD_R(%)	5.9	4.3	7.4	3.1	3.0

Table A.8— Stigmasterol by HPLC

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	1	0
mean (%)	1.1	2.4	2.0	7.2	1.3
r	0.07	0.14	0.08	0.21	0.16
S_r	0.03	0.05	0.03	0.08	0.06
RSD_r(%)	2.3	2.1	1.5	1.1	4.4
R	0.15	0.22	0.11	0.45	0.19
S_R	0.06	0.08	0.04	0.16	0.07
RSD_R(%)	5.1	3.3	2.0	2.2	5.3

Table A.9— Apparent □-Sitosterol by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	1	1	1
mean (%)	94.4	92.6	89.0	61.1	94.0
r	0.45	0.37	1.43	1.43	0.33
S_r	0.16	0.13	0.51	0.51	0.12
RSD_r(%)	0.17	0.14	0.57	0.84	0.13
R	0.76	1.31	1.79	4.00	0.63
S_R	0.27	0.47	0.63	1.43	0.23
RSD_R(%)	0.29	0.51	0.72	2.34	0.24

Table A.10 — Apparent □ -Sitosterol by HPLC

Sample	A	B	C	D	E
n	15	15	15	15	14
outliers	0	0	1	1	1
mean (%)	94.4	92.5	88.7	60.7	94.1
r	0.38	0.45	1.15	1.08	0.50
S_r	0.13	0.16	0.41	0.39	0.18
RSD_r(%)	0.14	0.17	0.46	0.63	0.19
R	0.81	1.11	1.41	4.04	0.99
S_R	0.29	0.40	0.51	1.44	0.35
RSD_R(%)	0.31	0.43	0.57	2.38	0.38

Table A.11 — □7-Stigmastenol by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	0	1
mean (%)	0.3	0.4	3.2	16.0	0.5
r	0.06	0.08	0.53	1.08	0.06

S_r	0.02	0.03	0.19	0.39	0.02
RSD_r(%)	7.5	6.4	5.9	2.4	4.4
R	0.15	0.19	0.83	1.52	0.19
S_R	0.05	0.07	0.30	0.54	0.07
RSD_R(%)	18.7	16.0	9.4	3.4	13.5

Table A.12 — □7-Stigmastenol by HPLC

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	0	0	0	0
mean (%)	0.32	0.46	3.22	16.09	0.52
r	0.10	0.12	0.38	0.75	0.08
S_r	0.037	0.041	0.13	0.267	0.029
RSD_r(%)	11.4	9.0	4.2	1.7	5.6
R	0.13	0.24	0.75	1.95	0.16
S_R	0.045	0.087	0.269	0.696	0.058
RSD_R(%)	14.2	18.8	8.3	4.3	11.0

Table A.13 — Total Sterols by Reference Method (TLC)

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	0	0	1	1
mean (mg/kg)	1572	1742	1679	2830	3181
r	84.9	134.8	144.7	246.2	307.3
S_r	30.3	48.1	51.7	87.9	109.7
RSD_r(%)	1.9	2.8	3.1	3.1	3.5
R	291.3	495.9	321.6	346.4	610.4
S_R	104.0	177.1	114.8	123.7	218.0
RSD_R(%)	6.6	10.2	6.8	4.4	6.9

Table A.14 — Total Sterols by HPLC

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	1	1	1	1	0
mean (mg/kg)	1583	1754	1730	2897	3216
r	74.0	93.5	95.0	59.01	181.9
S_r	264.4	33.4	33.9	21.1	65.0
RSD_r(%)	1.7	1.9	2.0	0.7	2.0
R	315.0	190.2	156.6	230.2	480.2
S_R	112.5	67.9	55.9	82.2	171.5
RSD_R(%)	7.1	3.9	3.2	2.8	5.3

Table A.15 — Erythrodiol + uvaol (% total sterols) by Reference Method (TLC)

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	1	0	1	0	0
mean (%)	2.1	3.8	1.2	2.5	17.2
r	0.32	0.34	0.19	0.27	0.76
S_r	0.12	0.12	0.07	0.10	0.27
RSD_r(%)	5.4	3.2	5.4	3.9	1.6
R	0.80	0.85	0.53	1.09	4.68
S_R	0.29	0.30	0.19	0.39	1.67
RSD_R(%)	13.3	8.0	15.3	15.5	9.7

Table A.16 — Erythrodiol + uvaol (% total sterols) by HPLC

Sample	A	B	C	D	E
n	14	14	14	14	14
outliers	0	0	0	0	0
mean (%)	2.2	3.8	1.4	2.0	17.2
r	0.40	0.32	0.24	0.16	0.73
S_r	0.14	0.11	0.09	0.06	0.26
RSD_r(%)	6.5	3.0	6.1	2.8	1.5
R	0.52	0.57	0.46	0.62	3.66

S_R	0.19	0.20	0.17	0.22	1.31
RSD_R(%)	8.4	5.3	11.8	10.9	7.6

Table A.17 — Erythrodiol absolute by Reference Method (TLC)

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	1	1	1	1
mean (mg/kg)	31	61	17	52	598
r	2.8	4.4	1.5	4.0	71.0
S_r	1.0	1.6	0.5	1.4	25.3
RSD_r(%)	3.3	2.6	3.1	2.7	4.2
R	6.0	21.0	10.2	9.4	148.3
S_R	2.1	2.6	3.6	3.3	53.0
RSD_R(%)	7.0	12.4	20.8	6.5	8.9

Table A.18 — Uvaol absolute by Reference Method (TLC)

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	2	1	1	1	2
mean (mg/kg)	3.0	8	4	20	65
r	0.83	1.5	3.3	3.5	12.5
S_r	0.30	0.55	1.2	1.2	4.5
RSD_r(%)	10.1	6.8	27.8	6.2	6.8
R	4.6	9.0	4.1	3.5	23.1
S_R	1.6	3.2	1.5	1.2	8.3
RSD_R(%)	55.6	40.0	34.0	6.2	12.7

Table A.19 — Erythrodiol absolute by HPLC

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	1	1	0	0	1

mean (mg/kg)	32	60	18	51	605
r	3.0	8.8	2.5	5.6	36.5
S_r	1.1	3.1	0.9	2.0	13.0
RSD_r(%)	3.3	5.2	5.1	4.0	2.2
R	7.3	23.1	5.6	5.8	152.5
S_R	2.6	8.2	2.0	2.0	54.5
RSD_R(%)	8.1	13.7	11.4	4.1	9.0

Table A.20 — Uvaol absolute by HPLC

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	1	1	0	1
mean (mg/kg)	5	8	5	20.0	65
r	1.6	1.3	0.80	2.8	6.5
S_r	0.55	0.48	0.29	1.0	2.3
RSD_r(%)	10.2	5.7	5.7	5.2	3.6
R	4.2	6.8	3.4	3.5	15.5
S_R	1.5	2.4	1.2	1.3	5.5
RSD_R(%)	27.3	28.6	24.0	6.4	8.5

Table A.21 — Aliphatic alcohols by Reference Method (TLC)

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	1	0	0	0
mean (mg/kg)	143	420	62	78	1512
r	5.5	23	4.5	5.1	70
S_r	1.9	8.2	1.6	1.8	24.9
RSD_r(%)	1.4	2.0	2.6	2.3	1.7
R	25	67	10	10	95
S_R	8.9	23.8	3.7	3.6	34.7
RSD_R(%)	6.2	5.7	6.1	4.7	2.3

Table A.22 — Aliphatic alcohols by HPLC

Sample	**A**	**B**	**C**	**D**	**E**
n	14	14	14	14	14
outliers	0	0	0	0	1
mean (mg/kg)	139	423	62	78	1495
r	6.9	15	6.2	5.9	46
S_r	2.5	5.3	2.2	2.1	16.2
RSD_r(%)	1.8	1.3	3.6	2.7	1.1
R	23	36	9.0	11	86
S_R	8.4	12.9	3.2	3.8	30.8
RSD_R(%)	6.0	3.1	5.2	4.9	2.1

2. Analysis of the collaborative test results for triterpenic dialcohols content:

Results of interlaboratory test to separate the unsaponifiable fraction by TLC of the triterpenic dialcohols fraction. Evaluation of the % and absolute content of erythrodiol and uvaol. An interlaboratory test was carried out in 2016 in accordance with ISO 5725. The results are summarised in Tables B.1 to B.6 for each parameter studied:

n number of laboratories which participated in the test

outliers number of laboratories with outlying values

mean mean of the accepted results

r repeatability

S_r repeatability standard deviation

RSD_r(%) repeatability coefficient of variation (S_r x 100/ mean)

R reproducibility

S_R reproducibility standard deviation

RSD_R(%) reproducibility coefficient of variation (S_R x 100/ mean)

A: Lampante olive oil

B: refined olive oil (from sample 1)

C: Desterolysed high oleic sunflower oil + 3,13% / 49,26 mg/kg of standard erythrodiol

D: Pomace Olive oil (traded) same as D

E: Pomace Olive oil (traded) same as E

Table B.1 Erythrodiol + uvaol (% total sterols) by Reference Method (TLC)

Sample	A	B	C	D	E
n	17	17	17	17	17

outliers	1	3	3	2	3
mean (%)	3.3	4.3	3.5	22.8	22.7
r	0.30	0.70	0.20	1.5	1.5
$\mathbf{S_r}$	0.10	0.20	0.10	0.50	0.50
$\mathbf{RSD_r(\%)}$	6.5	3.0	6.1	2.8	1.5
R	3.1	5.7	2.3	2.3	2.4
$\mathbf{S_R}$	2.6	1.1	0.70	3.0	3.3
$\mathbf{RSD_R(\%)}$	0.9	0.4	0.2	1.1	1.2

Table B.2 Erythrodiol + uvaol content (mg/kg) by Reference Method (TLC)

	A	B	C	D	E
n	16	16	17	16	16
outliers	3	4	3	2	3
mean (mg/kg)	59	50	52	772	745
r	6.5	6.7	4.7	40	100
S_r	2.3	2.4	1.7	14.2	35.6
RSD_r(%)	3.9	4.8	3.3	1.8	4.8
R	9.9	14	31	146	183
S_R	3.5	4.8	11.1	52.1	65.4
RSD_R(%)	6.0	9.8	21.5	6.8	8.8

Table B.3 Erythrodiol (% total sterols) by Reference Method (TLC)

	A	B	C	D	E
n	17	17	16	17	17
outliers	1	3	1	2	3
mean (%)	3.1	3.9	3.4	18.8	18.7
r	0.30	0.60	0.20	1.2	1.5
S_r	0.10	0.20	0.10	0.40	0.50
RSD_r(%)	3.1	5.3	2.4	2.3	2.9
R	0.80	1.0	0.60	2.8	2.8
S_R	0.30	0.40	0.20	1.0	1.0
RSD_R(%)	8.9	9.1	6.6	5.3	5.3

Table B.4: Erythrodiol absolute by Reference Method (TLC)

	A	B	C	D	E
n	16	16	16	16	16
outliers	1	3	2	0	2
mean (mg/kg)	53	46	48	638	635
r	5.1	13	4.1	41	78

S_r	1.8	4.6	1.5	14.5	27.7
RSD_r(%)	3.4	9.9	3.1	2.3	4.4
R	13	16	12	125	130
S_R	4.7	5.7	4.1	44.6	46.4
RSD_R(%)	8.8	12.2	8.6	7.0	7.3

Table B.5 Uvaol (% total sterols) by Reference Method (TLC)

	A	**B**	**C**	**D**	**E**
n	16	16	16	16	16
outliers	1	1	3	1	0
mean (%)	0.4	0.4	0.1	4.0	4.1
r	0.10	0.30	0.10	0.40	0.30
S_r	0.00	0.10	0.00	0.10	0.10
RSD_r(%)	11.4	23.3	35.8	3.4	2.4
R	0.70	0.20	0.10	0.60	0.70
S_R	0.20	0.10	0.00	0.20	0.30
RSD_R(%)	55.4	22.5	86.9	5.5	6.2

Table B.6 Uvaol absolute by Reference Method (TLC)

	A	**B**	**C**	**D**	**E**
n	15	14	15	14	13
outliers	2	1	3	0	1
mean (mg/kg)	8	5	1	136	138
r	2.5	2.6	1.0	12	22
S_r	0.90	0.90	0.30	4.4	8.0
RSD_r(%)	11.6	20.0	43.3	3.2	5.8
R	11	3.3	2.2	31	36
S_R	3.9	1.2	0.80	11.0	12.8
RSD_R(%)	51.6	25.8	99.0	8.1	9.2