COMMISSION REGULATION (EC) No 796/2002

of 6 May 2002

amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis and the additional notes in the Annex to Council Regulation (EEC) No 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Regulation No 136/66/EEC of 22 September 1966 on the establishment of a common organisation of the market in oils and fats [1], as last amended by Regulation (EC) No 1513/2001 [2], and in particular Article 35a thereof,

Having regard to Council Regulation (EEC) No 2658/87 of 23 July 1987 on the tariff and statistical nomenclature and on the Common Customs Tariff [3], as last amended by Commission Regulation (EC) No 578/2002 [4], and in particular Article 9 thereof,

Whereas:

(1) Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis [5], as last amended by Regulation (EC) No 2042/2001 [6], defines the physical, chemical and organoleptic characteristics of olive and olive-pomace oils and stipulates methods of assessing these characteristics. From 1 November 2001, the definition of olive-pomace oil given in point 4 of the Annex to Regulation No 136/66/EEC stipulates that certain olive-pomace oils correspond to lampante olive oils with the exception of certain specific characteristics.

(2) In order to distinguish between oils obtained by centrifuging olive pomace and lampante olive oils in the absence of an analytical parameter, limit values for wax, erythrodiol and uvaol composition or total aliphatic alcohol composition should be set for differentiating them independently of production method. To this end, a method for determining total aliphatic alcohol content should be specified.

(3) Introduction of these new limits requires an amendment to Additional Note 2 to Chapter 15 of the Combined Nomenclature in Annex I to Regulation (EEC) No 2658/87. This opportunity should also be taken to delete Article 5 and Annex XIV to Regulation (EEC) No 2568/91 and to correct certain errors in the text of that Regulation.

(4) In order to harmonise methods for preparing methyl esters of fatty acids to be used for analysing the fatty-acid composition of oils, technical developments in analysis methods have made it possible to use the free acidity of oils, so that the number of methods included in Annex X B can be reduced to two.

(5) Drawing on experience, the International Olive Oil Council has devised a new method of organoleptic assessment of virgin oils that is simpler and more reliable than that given in Annex XII to Regulation (EEC) No 2568/91. The method provided for in Annex XII should therefore be replaced by the new method of organoleptic assessment of virgin oils.

(6) Use of the new method requires that a new arbitration procedure be available for dealing with cases of discrepancy between the category declared and that assigned by the assessing panel.

(7) In order to ensure that analyses are carried out in the correct conditions and in view of the distances between regions, different time limits should be set for sending samples to the laboratory after sampling, taking account of the weather conditions in each season. For the purpose of grading oils, the results of analyses should be compared with the limits laid down in Regulation (EEC) No 2568/91, which already include the repeatability and reproducibility limits for the analysis methods used.

(8) In order both to give time for adjustment to the new standards and assembly of the means of applying them and to avoid disturbance to commercial transactions, the amendments to this Regulation should not apply until 1 September 2002, and olive and olive-pomace oil packaged for retail sale before that date should be exempt.

(9) The measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Oils and Fats and the Customs Code Committee regarding the issues coming within their respective areas of responsibility,

[1] OJ 172, 30.9.1966, p. 3025/66.
[2] OJ L 201, 26.7.2001, p. 4.
[3] OJ L 256, 7.9.1987, p. 1.
[4] OJ L 97, 13.4.2002, p. 1.
[5] OJ L 248, 5.9.1991, p. 1.
[6] OJ L 276, 19.10.2001, p. 8.

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EEC) No 2568/91 is amended as follows:

1. In Article 2(1):

   (a) the third indent is replaced by the following:

   '— for determination of the wax content, the method given in Annex IV,';

   (b) the following indent is added:

   '— for determination of the aliphatic alcohol content, the method given in Annex XIX,'.

2. Article 2(2) is replaced by the following:

   '2. Verification by national authorities or their representatives of the organoleptic characteristics of virgin oils shall be effected by tasting panels approved by the Member States.

   The organoleptic characteristics of an oil as referred to in the first subparagraph shall be deemed consonant with the category declared if a panel approved by the Member State confirms the grading.

   Should the panel not confirm the category declared as regards the organoleptic characteristics, at the interested party's request the national authorities or their representatives shall have two counter-assessments carried out by other approved panels, at least one by a panel approved by the producer Member State concerned. The characteristics concerned shall be deemed consonant with the characteristics declared if at least two of the counter-assessments confirm the declared grade. If that is not the case, the interested party shall be responsible for the cost of the counter-assessments.'

3. The second subparagraph of Article 2(3) is replaced by the following:

   'Without prejudice to standard EN ISO 5555 and Chapter 6 of standard EN ISO 661, the samples taken shall be put in a dark place away from strong heat as quickly as possible and sent to the laboratory for analysis no later than:

   — the tenth working day after they are taken, during the period from October to May, and

   — the fifth working day after they are taken, during the period from June to September.'

4. The following paragraph is added at the end of Article 2:

   '5. For the purpose of determining the characteristics of olive oils by the methods provided for in paragraph 1, the analysis results shall be directly compared with the limits laid down in this Regulation.'

5. Articles 3 and 3a are deleted.

6. Article 3b becomes Article 3.

7. Article 4(1) is replaced by the following:

   '1. The Member States may approve assessment panels so that national authorities or their representatives can assess and verify organoleptic characteristics.

   The terms of approval shall be set by Member States and ensure that:

   — the requirements of Annex XII.4 are met,

   — the panel head is given training recognised for this purpose by the Member State,

   — continued approval depends on performance in annual checks arranged by the Member State.

   Member States shall notify to the Commission a list of approved panels and the action taken under this paragraph.'

8. Article 5 is deleted.

9. The Annexes are amended in accordance with the Annex to this Regulation.

Article 2

Additional Note 2 to Chapter 15 of the Combined Nomenclature in Annex I to Regulation (EEC) No 2658/87 is amended as follows:

1. Point B.I(a) is replaced by the following:

   '(a) a wax content not exceeding 300 mg/kg;'.

2. Point B.I(g)(4) is replaced by the following:

   '4. organoleptic characteristics showing a median of defects above 6,0 in accordance with Annex XII to Regulation (EEC) No 2568/91;'.

3. Point B.II(g) is replaced by the following:

   '(g) organoleptic characteristics showing a median of defects not higher than 6,0 in accordance with Annex XII to Regulation (EEC) No 2568/91;'.

4. Point D(b) is replaced by the following:

   '(b) an erythrodiol and uvaol content higher than 4,5 %;'.

Article 3

This Regulation shall enter into force on the seventh day following its publication in the *Official Journal of the European Communities*.

It shall apply from 1 September 2002 to olive and olive-pomace oil packaged for retail sale.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 6 May 2002.

For the Commission
Franz FISCHLER
Member of the Commission

ANNEX

1. In the summary of Annexes to Regulation (EEC) No 2568/91:
   (a) Annex XIV: Additional Notes 2, 3 and 4 to Chapter 15 of the Combined Nomenclature is deleted.
   (b) the following is added: 'Annex XIX: Method for determining aliphatic alcohol content.'
2. Annex I is replaced by the following:

'ANNEX I

CHARACTERISTICS OF OLIVE OIL

Category	Acidity (%) (*)	Peroxide value mEq 02/kg (*)	Halogenated solvents mg/kg (*) (1)	Waxes mg/kg (**)	Saturated fatty acids in position 2 triglyceride (%)	Stigmasta-dienes mg/kg (2)	Difference between HPLC and theoretical calculation of ECN42	K_{232} (*)	K_{270} (*)	K_{270} after alumina (3)	Delta-K (*)	Organoleptic assessment Median of defects (Md) (*)	Organoleptic assessment Median for "fruity" (Mf) (*)
1. Extra virgin olive oil	≤ 1,0	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,50	≤ 0,20	≤ 0,10	≤ 0,01	Md = 0	Mf > 0
2. Virgin olive oil	≤ 2,0	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,60	≤ 0,25	≤ 0,10	≤ 0,01	Md ≤ 2,5	Mf > 0
3. Ordinary virgin olive oil	≤ 3,3	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,60	≤ 0,25	≤ 0,10	≤ 0,01	Md ≤ 6,0 (4)	—
4. Virgin lampante olive oil	> 3,3	> 20	> 0,20	≤ 300 (5)	≤ 1,3	≤ 0,50	≤ 0,3	≤ 3,70	> 0,25	≤ 0,11	—	Md > 6	—
5. Refined olive oil	≤ 0,5	≤ 5	≤ 0,20	≤ 350	≤ 1,5	—	≤ 0,3	≤ 3,40	≤ 1,20	—	≤ 0,16	—	—
6. Olive oil	≤ 1,5	≤ 15	≤ 0,20	≤ 350	≤ 1,5	—	≤ 0,3	≤ 3,30	≤ 1,00	—	≤ 0,13	—	—
7. Crude olive-pomace oil	> 0,5 (**)	—	—	>350 (6)	≤ 1,8	—	≤ 0,6	—	—	—	—	—	—
8. Refined olive-pomace oil	≤ 0,5	≤ 5	≤ 0,20	> 350	≤ 2,0	—	≤ 0,5	≤ 5,50	≤ 2,50	—	≤ 0,25	—	—
9. Olive-pomace oil	≤ 1,5	≤ 15	≤ 0,20	> 350	≤ 2,0	—	≤ 0,5	≤ 5,30	≤ 2,00	—	≤ 0,20	—	—

(1) Overal upper limit for compounds detected by electron capture detector.
For compounds detected individually the upper limit is 0,10 mg/kg.
(2) Sum of isomers that could (or could not) be separated by capillary column.
(3) For the purpose of determining the presence of refined oil, where the K_{270} exceeds the limit for the category concerned, it must be determined again after passage over alumina.
(4) Where the median for "fruity" is 0, the median of the defects must be not more than 2,5.
(5) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if total aliphatic alcohol is less than or equal to 350 mg/kg or if the percentage of erythrodiol and uvaol is less than or equal to 3,5.
(6) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if total aliphatic alcohol is greater than 350 mg/kg and the percentage of erythrodiol and uvaol is greater than 3,5.

Category	Acid content						Sum of the translino-leic isomers (%)	Sum of the translino-leic and translino-lenic isomers (%)	Cholesterol (%)	Brassica-sterol (%)	Campe-sterol (%)	Stigma-sterol (%)	Betasito-sterol (%) (1)	Delta-7-Stigma-stenol (%)	Total sterols (mg/kg)	Erythrodiol and uvaol (%) (**)
	Myristic (%)	Linolenic (%)	Arachidic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)										
1. Extra virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
2. Virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
3. Ordinary virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
4. Virgin lampante olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,10	≤ 0,10	≤ 0,5	≤ 0,1	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5 (2)
5. Refined olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
6. Olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
7. Crude olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,20	≤ 0,10	≤ 0,5	≤ 0,2	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 2 500	> 4,5 (3)
8. Refined olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 800	> 4,5
9. Olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 600	> 4,5

(1) Delta-5,23-Stigmastadienol + Chlerosterol + Beta-Sitosterol + Sitostanol + Delta-5-Avenasterol + Delta-5,24-Stigmastadienol.
(2) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if total aliphatic alcohol is less than or equal to 350 mg/kg or if the percentage of erythrodiol and uvaol is less than or equal to 3,5.
(3) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if total aliphatic alcohol is greater than 350 mg/kg and the percentage of erythrodiol and uvaol is greater than 3,5.

Notes:

(a) The results of the tests must be expressed to the same number of significant digits as that specified for each characteristic.
The last significant digit must be rounded up to the next digit if the non-significant digit that follows it is greater than 4.
(b) An oil is to be placed in a different category or declared not in conformity in terms of purity if any one of the characteristics lies outside the limit laid down.
(c) The characteristics marked with an asterisk (*), relating to the quality of the oil, mean that:
— for virgin lampante olive oil, these limits (with the exception of K_{232}) do not have to be respected simultaneously,
— in the case of other virgin olive oils, non-compliance with at least one of these limits shall involve a change in category, while remaining classed in one of the categories foe virgin olive oils.
(d) The characteristics marked with two asterisk (**) mean that, for all olive-pomace oils concerned, these limits do not have to be respected simultaneouisly.'

3. Annex X.B is replaced by the following:

'ANNEX X B

PREPARATION OF THE FATTY ACID METHYL ESTERS FROM OLIVE OIL AND OLIVE-POMACE OIL

The following two methods are recommended for preparing the fatty acid methyl esters from olive oils and olive-pomace oils:

Method A: Trans-esterification with cold methanolic solution of potassium hydroxide

Method B: Methylation by heating with sodium methylate in methanol followed by esterification in acid medium.

Each method will be applied according to the analytical parameter to be determined and the oil category as indicated below:

(a) determination of difference between actual and theoretical content of triglycerides with ECN42 (ΔECN42):
- method A will be applied to samples of all the oil categories after purification of the oil by passing it through a silica gel column;

(b) determination of the fatty acid composition:
- method A will be applied directly to samples of the following oil categories:
  - virgin olive oils with an acidity of less than 3,3 %,
  - refined olive oil,
  - olive oil (blend of virgin olive oils and refined olive oil),
  - refined olive-pomace oil,
  - olive-pomace oil (blend of virgin olive oils and refined olive-pomace oil);
- method B will be applied directly to samples of the following oil categories:
  - virgin olive oil with an acidity of more than 3,3 %,
  - crude olive-pomace oil;

(c) determination of trans-isomers of fatty acids:
- method A will be applied directly to samples of the following oil categories:
  - virgin olive oils with an acidity of less than 3,3 %,
  - refined olive oil,
  - olive oil (blend of virgin olive oils and refined olive oil),
  - refined olive-pomace oil,
  - olive-pomace oil (blend of virgin olive oils and refined olive-pomace oil);
- method A will be applied to the following categories of oils after purification of the oil by passing it through a silica gel column:
  - virgin olive oil with an acidity of more than 3,3 %,
  - crude olive-pomace oil.

PURIFICATION OF OIL SAMPLES

When necessary, the samples will be purified by passing the oil through a silica gel column, eluting with hexane/diethyl ether (87:13, v/v) as described in IUPAC method 2.507.

Alternatively, solid-phase extraction on silica gel phase cartridges can be used. A silica gel cartridge (1 g, 6 ml) is placed in a vacuum elution apparatus and washed with 6 ml of hexane. The vacuum is released to prevent the column from becoming dry and then a solution of the oil (0,12 g approximately) in 0,5 ml of hexane is loaded into the column and vacuum is applied. The solution is pulled down and then eluted with 10 ml of hexane/diethyl ether (87:13 v/v) under vacuum. The combined eluates are homogenised and divided in two similar volumes. An aliquot is evaporated to dryness in a rotary evaporator under reduced pressure at room temperature. The pomace is dissolved in 1 ml of heptane and the solution is ready for fatty acid analysis by GC. The second aliquot is evaporated and the pomace is dissolved in 1 ml of acetone for triglyceride analysis by HPLC, if necessary.

METHODS FOR PREPARING THE FATTY ACID METHYL ESTERS

1. ***Method A: Trans-esterification with cold methanolic solution of potassium hydroxide***

1.1. **Purpose**

This rapid method is applicable to olive oils and olive-pomace oils with a free fatty acid content of less than 3,3 %. Free fatty acids are not esterified by potassium hydroxide. Fatty acid ethyl esters are trans-esterified at a lower rate than glyceridic esters and may be only partially methylated.

1.2. **Principle**

Methyl esters are formed by trans-esterification with methanolic potassium hydroxide as an intermediate stage before saponification takes place (title 5 in ISO-5509:2000, title 5 in IUPAC method 2.301).

1.3. **Reagents**

Methanol containing not more than 0,5 % (m/m) water.

Heptane, chromatographic quality.

Potassium hydroxide, approximately 2 N methanolic solution: dissolve 11,2 g of potassium hydroxide in 100 ml of methanol.

1.4. **Apparatus**

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

Graduated or automatic pipettes, 2 ml and 0,2 ml

1.5. **Procedure**

In a 5 ml screw-top test tube weigh approximately 0,1 g of the oil sample. Add 2 ml of heptane, and shake. Add 0,2 ml of 2 N methanolic potassium hydroxide solution, put on the cap fitted with a PTFE joint, tighten the cap, and shake vigorously for 30 seconds. Leave to stratify until the upper solution becomes clear. Decant the upper layer containing the methyl esters. The heptane solution is suitable for injection into the gas chromatograph. It is advisable to keep the solution in the refrigerator until gas chromatographic analysis. Storage of the solution for more than 12 hours is not recommended.

2. ***Method B: Methylation by heating with sodium methylate in methanol followed by esterification in acid medium***

2.1. **Purpose**

This method is applicable to olive oils and olive-pomace oils with a free fatty acid content of more than 3,3 %.

2.2. **Principle**

Neutralisation of the free fatty acids and alkaline methanolysis of the glycerides, followed by esterification of the fatty acids in acid medium (title 4.2. in IUPAC method 2.301).

2.3. **Reagents**

— heptane, chromatographic quality,
— methanol containing not more than 0,05 % (m/m) water,
— sodium methylate, 0,2 N methanolic solution: dissolve 5 g of sodium in 1 000 ml of methanol (this may be prepared from commercial solutions),
— phenolphthalein, 0,2 % methanolic solution,
— sulphuric acid, 1 N in methanolic solution: add 3 ml of 96 % sulphuric acid to 100 ml of methanol,
— saturated solution of sodium chloride in water.

2.4. **Apparatus**

— 50 ml flat-bottomed volumetric flask with long, narrow, ground neck,
— reflux condenser: air condenser (1 m long) with ground joint appropriate to the neck of the flask,
— boiling chips,
— glass funnel.

2.5. **Procedure**

Transfer about 0,25 g of the oil sample into a 50 ml ground-necked volumetric flask. With the aid of a funnel, add 10 ml of 0,2 N sodium methylate in methanol and the boiling chips. Fit a reflux condenser, shake, and bring to the boil. The solution should become clear, which usually occurs in about 10 minutes. The reaction is complete after 15 minutes. Remove the flask from the source of heat, wait until the reflux stops, remove the condenser, and add two drops of phenolphthalein solution. Add a few ml of 1 N sulphuric acid in methanol solution until the solution becomes colourless and then add 1 ml in excess. Fit the condenser and boil again for 20 minutes. Withdraw from the source of heat and cool the flask under running water. Remove the condenser, add 20 ml of saturated sodium chloride solution, and shake. Add 5 ml of heptane, plug the flask, and shake vigorously for 15 seconds.

Leave to settle until the two phases have separated. Add saturated sodium chloride solution again until the aqueous layer reaches the lower end of the flask neck. The upper layer containing the methyl esters fills the flask neck. This solution is ready to be injected in the GC.

Caution: Methylation by method B must be done under a hood.

2.6. **Alternatives to methylation Method B**

2.6.1. *Method C*

2.6.1.1. Principle

The fatty matter undergoing analysis is treated with methanol-hydrochloric acid, in a sealed vial, at 100 °C.

2.6.1.2. Apparatus

— Strong glass vial of a capacity of about 5 ml (height 40 to 45 mm, diameter 14 to 16 mm).
— 1 and 2 ml graduated pipettes.

2.6.1.3. Reagents

Solution of hydrochloric acid in 2 % methanol. This is prepared from gaseous hydrochloric acid and anhydrous methanol (Note 1).

Hexane, chromatographic quality.

Note 1: Commercial solutions of hydrogen chloride in methanol can be used. Small amounts of gaseous hydrochloric acid can easily be prepared in the laboratory by simple displacement from the commercial solution (p = 1,18) by dripping concentrated sulphuric acid. Since hydrochloric acid is very rapidly absorbed by methanol, it is advisable to take the usual precautions when dissolving it, e.g. introduce the gas through a small inverted funnel with the rim just touching the surface of the liquid. Large quantities of methanolic hydrochloric acid solution can be prepared in advance, as it keeps perfectly in glass-stoppered bottles stored in the dark. Alternatively, this reagent can be prepared by dissolution of acetyl chloride in anhydrous methanol.

2.6.1.4. Procedure

— Place in the glass vial 0,2 g of the fatty matter, which has previously been dried out on sodium sulphate and filtered, and 2 ml of hydrochloric acid-methanol solution. Heat seal the vial.
— Immerse the vial at 100 °C for 40 minutes.
— Cool the vial under running water, open, add 2 ml of distilled water and 1 ml of hexane.
— Centrifuge and remove the hexane phase, which is ready for use.

2.6.2. *Method D*

2.6.2.1. Principle

The fatty matter undergoing analysis is heated under reflux with methanol-hexane-sulphuric acid. The methyl esters obtained are extracted with petroleum ether.

2.6.2.2. Apparatus

— Test tube of a capacity of about 20 ml, fitted with an air reflux condenser approximately 1 m in length, with ground glass joints.
— 5 ml graduated pipette.
— 50 ml separating funnel.
— 10 ml and 25 ml measuring beakers.
— 15 ml test tube with conical base.

2.6.2.3. Reagents

— Methylation reagent: anhydrous methanol-hexane-concentrated sulphuric acid (p = 1,84) in the ratio 75:25:1 (V/V/V).

— 40 to 60 °C petroleum ether.

— Anhydrous sodium sulphate.

2.6.2.4. Procedure

Place 0,1 g of oil in the 20 ml test tube and add 5 ml of methylation reagent.

Fit the reflux condenser and heat for 30 minutes in a boiling water bath (Note 2).

Transfer quantitatively the mixture into a 50 ml separating funnel, with the aid of 10 ml distilled water and 10 ml petroleum ether. Shake vigorously, and allow the phases to separate, remove the aqueous phase and wash the ether layer twice with 20 ml distilled water. Add to the separating funnel a small quantity of anhydrous sodium sulphate, shake, allow to settle for a few minutes and filter, collecting the filtrate in a 15 ml test tube with a conical base.

Evaporate the solvent over a water bath in a current of nitrogen.

Note 2: To control boiling, insert a glass rod into the test tube and limit the temperature of the water bath to 90 °C.

3. ***Precision parameters***

The statistical evaluation of the precision of methods A and B was published by the International Olive Oil Council in its method COI/T.20/CO. No 24.

RECOMMENDATIONS FOR GAS CHROMATOGRAPHIC ANALYSIS OF THE FATTY ACID ESTERS FROM OLIVE OIL AND OLIVE-POMACE OIL

1. **Procedure**

The gas chromatographic analysis of solutions of fatty esters in heptane is to be carried out according to standard ISO-5508 using a capillary column (50 m length x 0,25 or 0,32 mm i.d.) impregnated with cyanopropylsilicone phase as indicated for the determination of fatty acid trans-isomers (COI/T.20/Doc. no. 17).

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

2. **Calculations**

2.1. For the calculation of the fatty acid composition and ΔECN42, all the following fatty acids will be taken into account:

Myristic (C14:0).

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

Palmitoleic (C16:1). Sum of the areas of the peaks corresponding to the ω9 and ω7 isomers of the methyl ester.

Margaric (C17:0).

Margaroleic (C17:1).

Stearic (C18:0).

Oleic (C18:1). Sum of the areas of the peaks corresponding to the ω9 and ω7 isomers of the methyl ester, ethyl ester, and trans-isomers of the methyl ester.

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

Arachidic (C20:0).

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

Eicosenoic (C20:1).

Behenic (C22:0).

Lignoceric (C24:0).

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

2.2. For the calculation of the percentage of trans-C18:1 the peak corresponding to the methyl esters of this fatty acid is to be used. For the sum [trans-C18:2 + trans-C18:3], all the peaks corresponding to the trans-isomers of these two fatty acids are to be added together. For the calculation of the total area, all the peaks mentioned in 2.1. are to be taken into account (see COI/T.20/Doc. No. 17).

The calculation of the percentage of each fatty acid will be carried out according to the formula:

% X = (Area X x 100)/(total area)

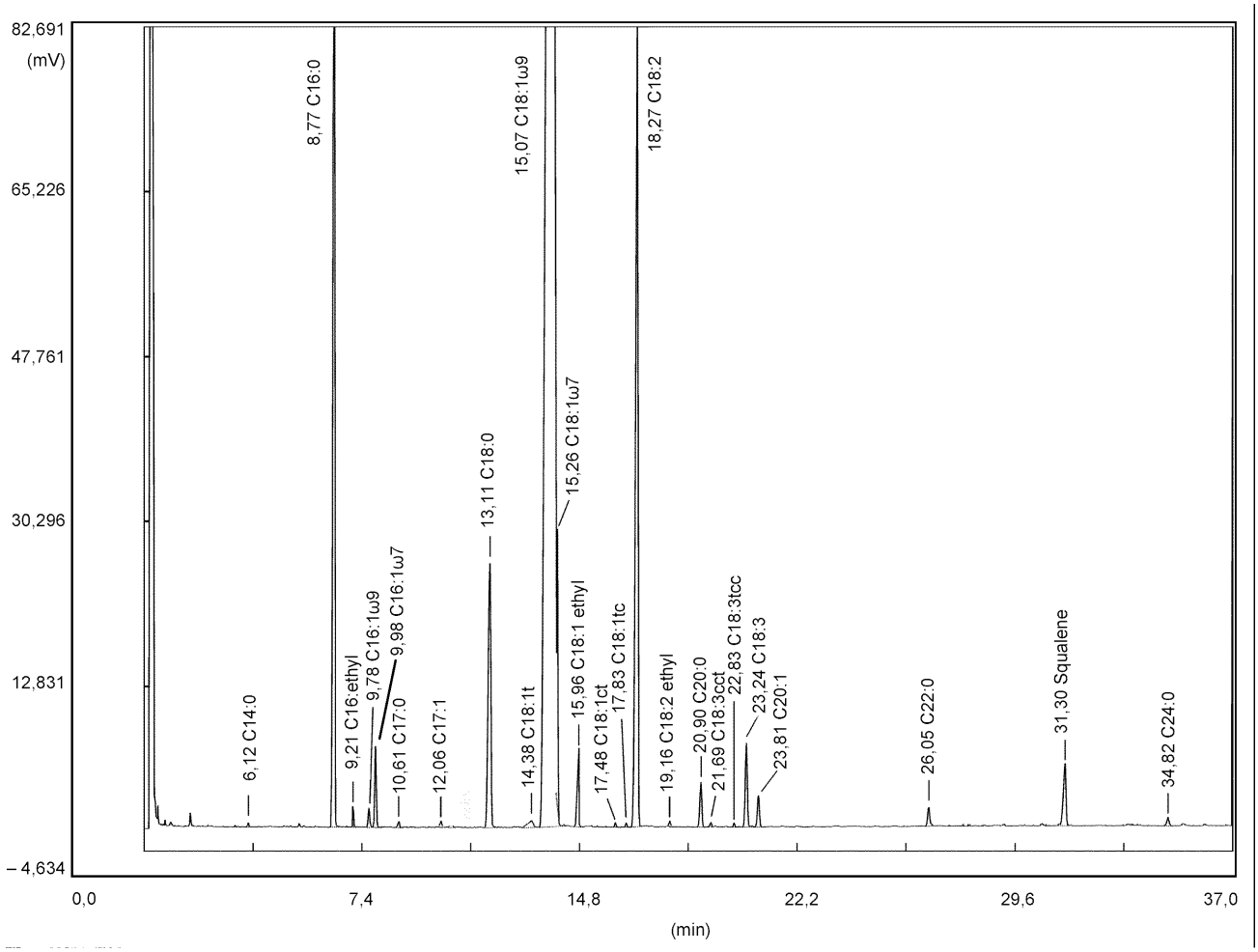


Figure 1: Gas chromatographic profile obtained by the cold methylation method from olive-pomace oil. The chromatographic peaks correspond to the methyl and ethyl esters except where otherwise indicated.'

4. Annex XII is replaced by the following:

'ANNEX XII

ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OILS

1. PURPOSE AND SCOPE

This Annex sets the criteria required for organoleptic assessment of the virgin oils defined at 1 in the Annex to Regulation No 136/66/EEC and describes the method of grading them with reference to characteristics.

This method can be used only for grading virgin oils on the basis of fruitiness and intensity of defects by a group of selected trained tasters operating as a panel in line with section 4.

2. GENERAL

For the general basic vocabulary, the tasting room, the general methodology and the tasting glass compliance with the stipulations of the International Olive Oil Council is recommended.

3. SPECIFIC VOCABULARY

3.1. **Positive attributes**

Fruity: range of smells (dependent on variety) characteristic of oil from healthy fresh fruit, green or white, perceived directly or retronasally.

Bitter: characteristic taste of oil from green olives or olives turning colour.

Pungent: tingling sensation characteristic of oil made at the beginning of the season mainly from olives that are still green.

3.2. **Negative attributes**

"*Atrojado*" (*fusty*): characteristic flavour of oil from piled olives in advanced anaerobic fermentation.

Mustiness/humidity: characteristic flavour of oil from olives in which large numbers of fungi and yeasts had developed as a result of storage for several days in humid conditions.

Muddy sediment: characteristic flavour of oil that has remained in contact with sediment in vats and tanks.

Winey/vinegary: characteristic flavour of certain oils reminiscent of wine or vinegar, due basically to formation of acetic acid, ethyl acetate and ethanol by fermentation of the olives.

Metallic: flavour reminiscent of metal, characteristic of oil that has been in prolonged contact with metal surfaces during crushing, mixing, pressing or storage.

Rancid: flavour of oil that has become oxidised.

Heated or burnt: characteristic flavour caused by excessive and/or prolonged heating during production, particularly by thermo-mixing of the paste in unsuitable conditions.

Hay/wood: characteristic flavour of certain oils from dry olives.

Rough: thick and pasty mouthfeel produced by some oils.

Greasy: flavour reminiscent of diesel, grease or mineral oil.

Vegetable water: flavour acquired by oil through prolonged contact with the vegetable water.

Brine: flavour of oil from olives preserved in salt solution.

Esparto: characteristic flavour of oil from olives pressed in new esparto mats. It can vary according to whether the mats are of green or dried esparto.

Earthy: flavour of oil from olives collected with earth or mud on them and not washed.

Grubby: flavour of oil from olives heavily attacked by grubs of the olive fly (Bactrocera oleae).

Cucumber: characteristic flavour of oil kept too long in hermetically sealed containers, notably in tins, attributed to formation of 2,6-nonadienal.

4. PANEL

The panel is appointed by the Member State and consists of a panel head and from eight to twelve tasters. However, for the 2001/02 marketing year, the panel may consist of fewer than eight tasters.

The panel head must be a soundly trained expert in the various types of oil. He or she is responsible for the panel and its organisation and operation, including preparation, coding and presentation of the samples to the tasters and collection and processing of the data.

He or she selects the testers, sees to their training and checks that their performance remains of adequate standard.

The testers must be selected and trained on account of their skill in distinguishing between similar samples. The International Olive Oil Council's manual on the selection, training and monitoring of qualified virgin oil tasters must be followed.

Panels must undertake to participate in national, Community and international organoleptic assessments organised for the purposes of periodic monitoring and harmonisation of perception criteria. They must also provide the Member State concerned with full information each year on the composition of the panel and the number of assessments made in their capacity as an approved panel.

5. PROCEDURE FOR ORGANOLEPTIC ASSESSMENT AND GRADING

5.1. **Use of profile sheet by taster**

The profile sheet to be used by the taster is reproduced as Appendix A.

Tasters must each smell and then taste [1] the oil submitted for examination contained in the tasting glass, analysing their olfactory, gustatory, tactile and kinaesthetic perceptions and mark on the sheet the intensity of their perception of each negative and positive attribute.

If negative attributes not listed on the profile sheet are perceived these must be noted under "Other" using those of the terms defined in 3.2 above that best describe them.

5.2. **Processing of data by panel head**

The panel head collects the profile sheets completed by the tasters and scrutinises the intensities assigned. In the event of an anomaly he or she will ask tasters to re-examine their sheet and if necessary repeat the test.

The panel head may feed each tester's data into a computer programme for calculating the median (Appendix B). Input of each sample shall be made with the help of a grid of 10 vertical columns for the 10 sensory attributes and one line for each panel member.

If a negative attribute is mentioned under "Other" by 50 % of the panel the head must calculate the median for this attribute and grade accordingly.

In cases of assessment in connection with monitoring of conformity to standards and of counter-assessment the panel head shall arrange for the assessment to be repeated twice at intervals of at least one day. The attribute medians shall be calculated using the data from the profile sheets of the three assessments.

5.3. **Grading of oils**

The oil is graded as follows in line with the median of the defects and the median for "fruity". By this is understood the median of the negative attribute perceived with greatest intensity. The value of the robust variation coefficient for this negative attribute must be no greater than 20 %.

(a) *extra virgin olive oil*: the median of the defects is 0 and the median for "fruity" is above 0;

(b) *virgin olive oil*: the median of the defects is above 0 but not above 2,5 and the median for "fruity" is above 0;

(c) *ordinary virgin olive oil*: the median of the defects is above 2,5 but not above 6,0; or the median of the defects is not above 2,5 and the median for "fruity" is 0;

(d) *lampante virgin olive oil*: the median of the defects is above 6,0.

From 1 November 2003 categories c) and d) are replaced by:

(c) *lampante olive oil*: the median of the defects is above 2,5; or the median of the defects is not above 2,5 and the median for "fruity" is 0.

5.4. **Special case**

If the median of a positive attribute other than "fruity" is above 5,0 the panel head must note this on the analysis certificate.

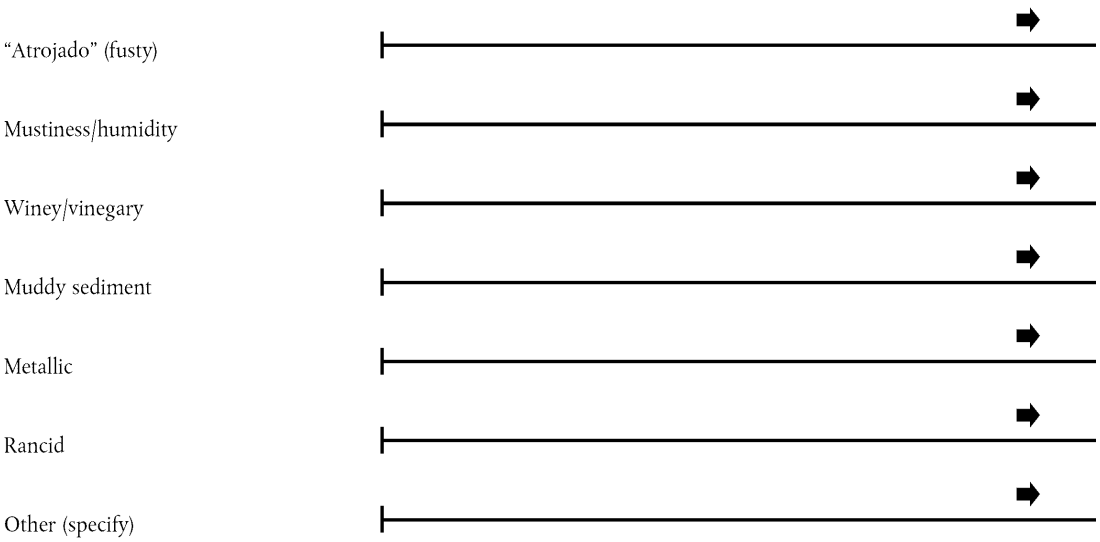
[1] But may refrain from tasting if they note some extremely intense negative attributes; they will note this exceptional circumstance on the profile sheet.

APPENDIX A

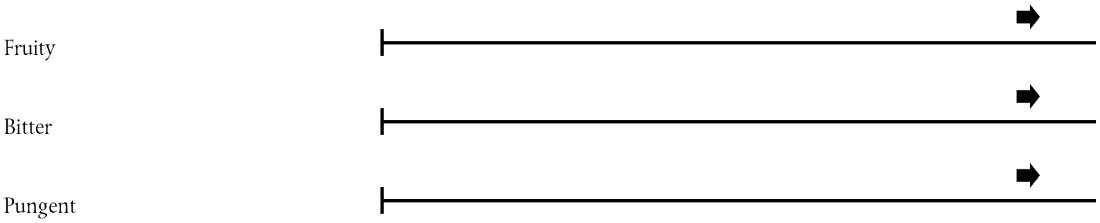
Profile sheet

(for use by taster)

DEFECTS PERCEIVED — INTENSITY



POSITIVE ATTRIBUTES PERCEIVED



Name of taster **Sample code**

Date

APPENDIX B

METHOD OF CALCULATING MEDIAN AND CONFIDENCE INTERVALS

Median

$$Me = [P\,(X < Xm) \leq 1/2 \wedge P\,(X \leq Xm) \geq 1/2]$$

The median is the real number Xm characterised by the fact that the probability (P) that the values of the distribution (X) are below that number (Xm) is not more than 0,5 and that simultaneously the probability (P) that the values of the distribution (X) are not above Xm is not less than 0,5. Another definition considers the median to be the 50th percentile of a distribution of numbers ranked in order of increase. In other terms the median represents the central value of an ordered series of uneven numbers or the average of the two central values of an ordered series of even numbers.

Robust standard deviation

$$S = \frac{1{,}25\ IQR}{1{,}35\ \sqrt{N}}$$

To obtain a reliable estimate of the variability that arises around the median recourse is required to the Stuart and Kendall method of estimating the robust standard deviation. The formula for the asymptotic standard deviation S involves N and IQR. N is the number of observations and IQR the interquartile range, i.e. the robust estimate of the variability of the data under consideration (the interquartile range covers exactly 50 % of the cases of any probability distribution). The interquartile range is given by calculating the size of the deviation between the 75th and the 25th percentiles.

$$IQR = 75th\ \text{percentile} - 25th\ \text{percentile}$$

The percentile is the value Xpc characterised by the fact that the probability (P) that the valves of the distribution are below Xpc is not more than a determined hundredth and that simultaneously the probability (P) that the valves of the distribution are not above Xpc is not less than the said hundredth. The hundredth indicates the distribution fraction used. In the case of the median this is 50/100.

$$\text{Percentile} = [P\,(X < Xpc) \leq \frac{n}{100} \wedge P\,(X \leq Xpc) \geq \frac{n}{100}]$$

In other words the percentile is the distribution value corresponding to a determined area plotted from the distribution or density curve. For example, the 25th percentile represents the distribution value corresponding to an area equal to 0,25 or 25/100.

Robust variation coefficient %

$$CVR = \frac{S}{Me}\ 100$$

The RVC represents a pure number, i.e. without dimension, that indicates the percentage of variability of the series of numbers analysed against the Av value of the median. For that reason it is very useful for verifying the reliability of the panel members.

Confidence intervals at 95 % on the median

The confidence intervals (C.I.) at 95 % (value of the error of first kind equal to 0,05 or 5 %) represent the range in which the value of the median would be able to vary should it be possible to repeat the experiment an infinite number of times. In practice this interval indicates the range of variability of the test under the operating conditions selected should it be possible to repeat the test several times. The interval helps evaluate, as in the case of the RVC, the reliability of the test.

$$\text{Upper C.I.} = Av + (c.S)$$

$$\text{Lower C.I} = Av - (c.S)$$

where c in the case of a confidence interval of 0,95 is equal to 1,96.

Grading is effected by comparing the median values with the reference ranges set in section 5.3. The software package permits a visualised grading on a table of the statistics or on a graph.'

5. Annex XIV is deleted.

6. The following Annex XIX is added.

'ANNEX XIX

DETERMINATION OF ALIPHATIC ALCOHOLS CONTENT BY CAPILLARY GAS CHROMATOGRAPHY

1. OBJECT

The procedure describes a method for the determination of aliphatic alcohols content in oils and fats.

2. PRINCIPLE OF THE METHOD

The fatty substance, with 1-eicosanol added as internal standard, is saponified with ethanolic potassium hydroxide and then the unsaponifiable matter extracted with ethyl ether. The alcoholic fraction is separated from the unsaponifiable matter by chromatography on a basic silica gel plate; the alcohols recovered from the silica gel are transformed into trimethylsilyl ethers and analysed by capillary gas chromatography.

3. EQUIPMENT

3.1. 250 ml round-bottomed flask fitted with a reflux condenser having ground-glass joints.

3.2. 500 ml separating funnel.

3.3. 250 ml round-bottomed flasks.

3.4. Chromatographic tank for thin-layer chromatographic analysis, for glass plates of dimensions 20 x 20 cm.

3.5. Ultraviolet lamp having a wavelength of 366 or 254 nm.

3.6. 100 µl and 500 µl microsyringes.

3.7. A cylindrical filter funnel with a G3 porous septum (porosity 15 to 40 µm) of diameter approximately 2 cm and a depth of some 5 cm, with an attachment suitable for filtration under vacuum and a 12/21 male ground glass joint.

3.8. 50 ml vacuum conical flask with a 12/21 ground-glass female joint which can be fitted to the filter funnel (3.7).

3.9. A 10 ml test tube with a tapering bottom and a sealing stopper.

3.10. Gas chromatograph for use with a capillary column, and provided with a splitting system composed of:

3.10.1. Thermostatic chamber for columns (column oven) to hold the temperature desired with a precision of ± 1 °C.

3.10.2. A temperature-adjustable injection unit with a persilanised glass vaporising element.

3.10.3. A flame ionisation detector and converter-amplifier.

3.10.4. Recorder-integrator for operation with the converter-amplifier (3.10.3), with response time not exceeding one second and with variable paper-speed.

3.11. Glass or fused silica capillary column, of length 20 to 30 m, internal diameter 0,25 to 0,32 mm, with SE-52 or SE-54 liquid phase or equivalent, with a film thickness between 0,10 and 0,30 µm.

3.12. Microsyringe for gas chromatography, of 10 µl capacity with hardened needle.

3.13. Analytical balance sensitive to 1 mg (with 0,1 mg display).

4. REAGENTS

4.1. Potassium hydroxide, approximately 2 N ethanolic solution: 130 g potassium hydroxide (minimum concentration 85 %) is dissolved, with cooling, in 200 ml distilled water and then made up to one litre with ethanol. The solution should be stored in a well-stoppered opaque glass bottle.

4.2. Ethyl ether, pure for analysis.

4.3. Anhydrous sodium sulphate, analytical purity.

4.4. Glass plates coated with silica gel, without fluorescence indicator, thickness 0,25 mm (commercially available ready for use).

4.5. Potassium hydroxide, approximately 0,2 N ethanolic solution; 13 g of potassium hydroxide are dissolved in 20 ml of distilled water and made up to one litre with ethanol.

4.6. Benzene, for chromatography (see 5.2.2).

4.7. Acetone, for chromatography (See 5.2.2).

4.8. Hexane, for chromatography (see 5.2.2).

4.9. Ethyl ether, for chromatography (see 5.2.2).

4.10. Chloroform, for chromatography.

4.11. Reference solution for thin-layer chromatography: cholesterol or phytosterols, 5 % solution in chloroform.

4.12. 0,2 % solution of 2', 7'-dichlorofluorescein in ethanol. Make slightly basic by adding a few drops of 2 N alcoholic potassium hydroxide solution.

4.13. Anhydrous pyridine, for chromatography.

4.14. Hexamethyl disilazane.

4.15. Trimethylchlorosilane.

4.16. Standard solutions of trimethylsilyl ethers of aliphatic alcohols from C_{20} to C_{28}. They may be prepared from mixtures of pure alcohols at the time they are required for use.

4.17. A 0,1 % (m/v) solution of 1-eicosanol in chloroform (internal standard).

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

4.19. Auxiliary gas: nitrogen, gas-chromatographic purity.

5. PROCEDURE

5.1. **Preparation of the unsaponifiables**

5.1.1. Using a 500 µl microsyringe place, into a 250 ml round-bottom flask, a volume of 0,1 % 1-eicosanol solution in chloroform (4.17) containing a quantity of 1-eicosanol approximately equal to 10 % of the aliphatic alcohols content in that portion of sample to be taken for analysis. For example, to 5 g of sample add 250 µl of the 0,1 % 1-eicosanol solution if olive oil and 1 500 µl if olive pomace oil.

Evaporate to dryness in current of nitrogen and then weigh accurately 5 g of the dry filtered sample into the same flask.

5.1.2. Add 50 ml of 2 N potassium hydroxide ethanolic solution, fit the reflux condenser and heat the apparatus to slight boiling on a steam bath, stirring continuously throughout the heating process until saponification has taken place (the solution becomes clear). Continue heating for a further 20 minutes and then add 50 ml of distilled water through the condenser. The condenser is then disconnected and the flask cooled to approximately 30 °C.

5.1.3. The contents of the flask are quantitatively transferred to a separating funnel of 500 ml capacity by adding distilled water several times, using a total of around 50 ml distilled water. Add approximately 80 ml of ethyl ether, shake vigorously for approximately 30 seconds and allow to settle (Note 1).

Separate off the lower aqueous phase collecting it in a second separating funnel. Two further extractions are effected on the aqueous phase, in the same manner, using each time 60 to 70 ml ethyl ether.

Note 1: Emulsions may be eliminated by adding, using as a spray, small quantities of ethyl alcohol or methyl alcohol.

5.1.4. The ethyl ether extracts are combined in a separating funnel and washed with distilled water (50 ml at a time) until the washing water gives a neutral reaction.

Discard the washing water, dry with anhydrous sodium sulphate and filter, into a flask of 250 ml capacity which has been weighed beforehand, the funnel and filter being washed with small quantities of ethyl ether which are added to the total.

5.1.5. Distil the ether down to a few ml, then bring to dryness under a slight vacuum or in a current of nitrogen, completing drying in an oven at 100 °C for approximately a quarter of an hour, and then weigh after cooling in a desiccator.

5.2. **Separation of alcoholic fractions**

5.2.1. Preparation of basic TLC plates: the silica gel plates (4.4) are immersed completely, in 0,2 N potassium hydroxide solution (4.5) for 10 seconds, and then left to dry for two hours under an extractor hood and finally placed in an oven at 100 °C for one hour.

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

Note 2: When basic silica gel plates are used to separate the alcoholic fraction there is no need to treat the unsaponifiables with alumina. It follows that all acid compounds (fatty acids and others) are retained at the origin thereby obtaining both aliphatic alcohol and terpenic alcohol bands which are both separated distinctly from the sterol band.

5.2.2. Place a 65/35 by volume hexane/ethyl ether mixture in the plate-developing chamber to a depth of approximately 1 cm (*).

Close the chamber using an appropriate cover and leave for half an hour to allow equilibration between vapour and liquid. Strips of filter paper dipping into the eluent may be affixed to the inside surfaces of the tank to reduce the development time by approximately one third and obtain more uniform, regular elution of the components.

Note 3: The developing solution must be replaced for each analysis in order to obtain reproducible developing conditions.

5.2.3. An approximately 5 % solution of unsaponifiable matter (5.1.5) in chloroform is prepared and 0,3 ml of the solution is streaked as a uniform strip of minimum thickness, using the 100 µl microsyringe, on a TLC plate at approximately 2 cm from the bottom of the TLC plate. Aligned with the origin, 2 to 3 µl of the aliphatic alcohols reference solution (4.11) are spotted for the identification of the aliphatic alcohols band after development has been completed.

5.2.4. Place the plate inside the development tank as stated in 5.2.2. The ambient temperature should be maintained between 15 and 20 °C. Immediately close the chamber with the cover and allow to elute until the solvent front reaches approximately 1 cm from the upper edge of the plate.

The plate is then removed from the development chamber and the solvent evaporated under a hot air current or the plate is left for a while under the extractor hood.

5.2.5. The plate is sprayed lightly and evenly with the solution of 2', 7'-dichlorofluorescein when the plate is observed under ultra violet light. The aliphatic alcohols band can be identified through being aligned with the stain obtained from the reference solution: mark the limits of the band with a black pencil; outlining the band of aliphatic alcohols and the band immediately above that, which is the terpenic alcohols band, together.

Note 4: The aliphatic alcohols band and the terpenic alcohols band are to be grouped together in view of the possible migration of some aliphatic alcohols into the triterpenic alcohols band.

5.2.6. Using a metal spatula scrape off the silica gel in the marked area. Place the finely comminuted material removed into the filter funnel (3.7). Add 10 ml of hot chloroform, mix carefully with the metal spatula and filter under vacuum, collecting the filtrate in the conical flask (3.8) attached to the filter funnel.

Wash the pomace in the flask three times with ethyl ether (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 (3.9), evaporate to dryness by mild heating in a gentle flow of nitrogen, make up again using a few drops of acetone, evaporate again to dryness, place in an oven at 105 °C for approximately 10 minutes and then allow to cool in a desiccator and weigh.

The pomace inside the test tube is composed of the alcoholic fraction.

5.3. **Preparation of the trimethylsilyl ethers**

5.3.1. The reagent for silylation, consisting of a mixture of 9:3:1 by volume (Note 5) of pyridine-hexamethyldisilazane-trimethylchlorosilane in the proportion of 50 µl for each milligram of aliphatic alcohols, is added to the test tube containing the alcoholic fraction, avoiding all absorption of moisture (Note 6).

Note 5: Solutions which are ready for use are available commercially. Other silanising reagents such as, for example, bis-trimethylsilyl, trifluor acetamide + 1 % trimethyl chlorosilane, which has to be diluted with an equal volume of anhydrous pyridine, are also available.

Note 6: The slight opalescence which may form is normal and does not cause any interference. The formation of a white floc or the appearance of a pink colour are indicative of the presence of moisture or deterioration of the reagent. If these occur the test must be repeated.

5.3.2. Stopper the test tube, shake carefully (without overturning) until the aliphatic alcohols are completely dissolved. 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.

(*) In these cases in particular, a 95/5 by volume benzene/acetone eluent mixture must be used to obtain distinct band separation.

5.4. **Gas chromatography analysis**

5.4.1. Preliminary operations, column packing

5.4.1.1. Fit the column in the gas chromatograph, attaching the inlet end to the injector connected to the splitting system and the outlet end to the detector. Carry out a general check of the gas chromatography assembly (tightness of gas fittings, efficiency of the detector, efficiency of the splitting system and of the recording system, etc.).

5.4.1.2. If the column is being used for the first time it is recommended that it should be subjected to conditioning. A little carrier gas is passed through the capillary column and then the gas chromatography assembly is switched on and gradually heated until a temperature not less than 20 °C above the operating temperature (see Note 7) is attained. That temperature is held for not less than two hours and then the assembly is brought to the operating conditions (regulation of gas flow, split flame ignition, connection to the electronic recorder, adjustment of the temperature of the capillary column oven, the detector and the injector, etc.) and the signal is adjusted to a sensitivity not less than twice the highest level contemplated for the execution of the analysis. The course of the base line must be linear, without peaks of any kind, and must not drift. A negative straight-line drift indicates leakage from the column connections; a positive drift indicates inadequate conditioning of the column.

Note 7: The conditioning temperature shall be at least 20 °C less than the maximum temperature contemplated for the liquid phase employed.

5.4.2. Choice of operating conditions

5.4.2.1. The guideline operating conditions are as follows:

— column temperature: the initial isotherm is set at 180 °C for eight minutes and then programmed at 5 °C/minute to 260 °C and a further 15 minutes at 260 °C,

— temperature of evaporator: 280 °C,

— temperature of detector: 290 °C,

— linear velocity of carrier gas: helium 20 to 35 cm/s, hydrogen 30 to 50 cm/s,

— splitting ratio: 1:50 to 1:100,

— sensitivity of instrument: 4 to 16 times the minimum attenuation,

— sensitivity of recording: 1 to 2 mV fs,

— paper speed: 30 to 60 cm/h,

— quantity of substance injected: 0,5 to 1 µl of TMSE solution.

The above conditions may be modified according to the characteristics of the column and of the gas chromatograph to obtain chromatograms satisfying the following conditions:

— alcohol C_{26} retention time shall be 18 ± 5 minutes,

— the alcohol C_{22} peak shall be 80 ± 20 % of the full scale value for olive oil and 40 ± 20 % of the full scale value for seed oil.

5.4.2.2. The above requirements are checked by repeated injection of the standard TMSE mixture of alcohols and the operating conditions are adjusted to yield the best possible results.

5.4.2.3. The parameters for the integration of peaks shall be set so that a correct appraisal of the areas of the peaks considered is obtained.

5.4.3. Analytical procedure

5.4.3.1. Using the microsyringe of 10 µl capacity draw in 1 µl of hexane followed by 0,5 µl of air and subsequently 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 injection unit and after one to two seconds inject rapidly, then slowly remove the needle after some five seconds.

5.4.3.2. Recording is effected until the TMSE of the aliphatic alcohols present have been eluted completely. The base line shall always correspond to the requirements of 5.4.1.2.

5.4.4. Peak identification

The identification of individual peaks is effected according to the retention times and by comparison with the standard TMSE mixture, analysed under the same conditions.

A chromatogram of the alcoholic fraction of a virgin olive oil is shown in Figure 1.

5.4.5. Quantitative evaluation

5.4.5.1. The peak areas of 1-eicosanol and of the aliphatic alcohols C_{22}, C_{24}, C_{26} and C_{28} are calculated by electronic integration.

5.4.5.2. The contents of each aliphatic alcohol, expressed in mg/1 000 g fatty substance, are calculated as follows:

$$\text{alcohol x} = \frac{A_x \cdot m_s \cdot 1\,000}{A_s \cdot m}$$

where:

Ax = area of the alcohol peak x

A_s = area of 1-eicosanol

m_s = mass of 1-eicosanol in milligrams

m = mass of sample drawn for determination, in grams.

6. EXPRESSION OF THE RESULTS

The contents of the individual aliphatic alcohols in mg/1 000 g of fatty substance and the sum of the "total aliphatic alcohols" are reported.

APPENDIX

Determination of the linear velocity of the gas

1 to 3 µl of methane or propane are injected into the gas chromatograph set at normal operating conditions and the time taken for the methane or propane to flow through the column from the instant of injection to the instant the peak elutes (tM) is measured using a stop clock.

The linear velocity in cm/s is given by L/tM, where L is the length of the column in centimetres and tM is the measured time in seconds.

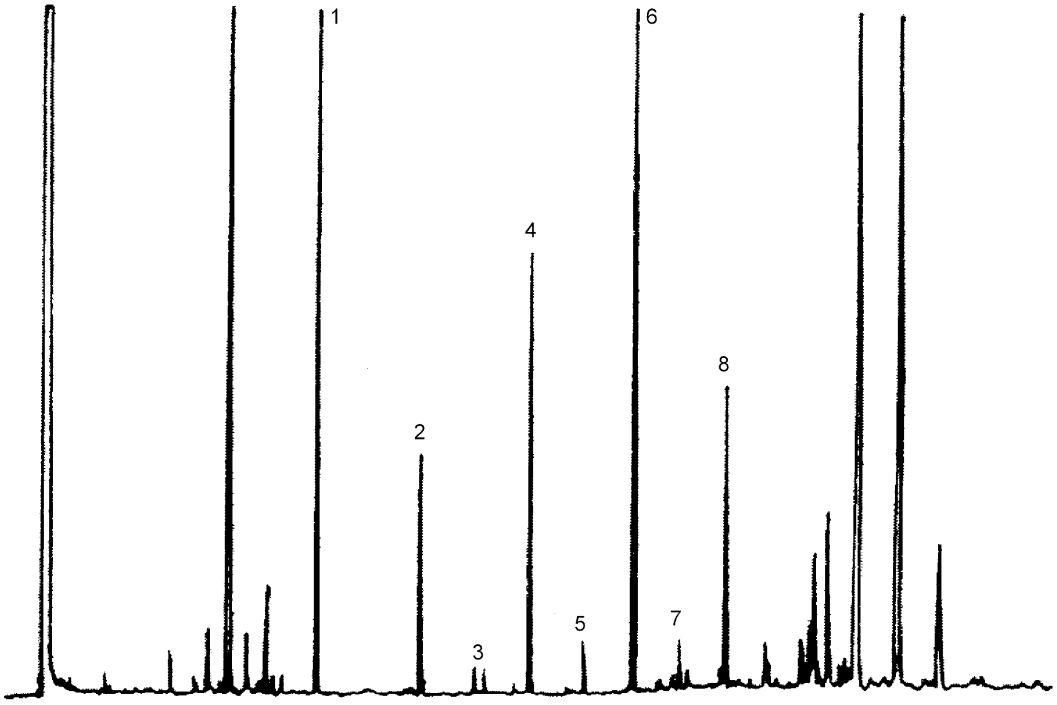


Figure 1 — Chromatogram of the alcoholic fraction of virgin oil

1 =	Eicosanol	5 =	Pentacosanol
2 =	Decosanol	6 =	Hexacosanol
3 =	Tricosanol	7 =	Heptacosanol
4 =	Tetracosanol	8 =	Octacosanol'