COMMISSION REGULATION (EC) No 2472/97

of 11 December 1997

amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis and 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 organization of the market in oils and fats (¹), as last amended by Regulation (EC) No 1581/96 (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 Regulation (EC) No 2308/97 (⁴), and in particular Article 9 thereof,

Whereas Commission Regulation (EEC) No 2568/91 (⁵), as last amended by Regulation (EC) No 2527/95 (⁶), defines the characteristics of olive oil and olive-residue oil and the relevant methods of analysis; whereas Regulation (EEC) No 2568/91 also amends Additional Notes 2, 3 and 4 to Chapter 15 of the combined nomenclature contained in Annex I to Regulation (EEC) No 2658/87;

Whereas, because of developments in research, the characteristics of olive oil as defined in Regulation (EEC) No 2568/91 should be amended so as better to ensure the purity of the products marketed and the relevant method of analysis should be defined;

Whereas in order to take into account developments in extraction techniques, in particular two-phase extraction, and continue the process of harmonization with the international standards laid down by the International Olive Oil Council, certain limits concerning the characteristics of olive oil and olive-residue oil should be adjusted;

Whereas the changes to the characteristics of olive oil referred to above require the amendment of Additional Notes 2, 3 and 4 to Chapter 15 of the combined nomenclature;

Whereas, to allow a period of adjustment to the new standards and the introduction of the means needed to

apply them and to avoid disturbance to trade, the entry into force of this Regulation should be deferred for approximately two months and provision should be made for oil packaged prior to its entry into force to be disposed of during a limited period;

Whereas, therefore, Regulations (EEC) No 2658/87 and (EEC) No 2568/91 should be amended;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Oils and Fats,

HAS ADOPTED THIS REGULATION:

Article 1

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

1. in Article 2, the following indent is added:

'— for determining the content of triglycerides with ECN42, the method set out in Annex XVIII.';

2. the Annexes are amended in accordance with Annex I hereto.

Article 2

Additional Notes 2, 3 and 4 to Chapter 15 of the combined nomenclature contained in Annex I to Regulation (EEC) No 2658/87 are replaced by the text set out in Annex II hereto.

Article 3

This Regulation shall enter into force on the 60th day following its publication in the *Official Journal of the European Communities.*

It shall not apply to olive oil and olive-residue oil packaged prior to the date of its entry into force and marketed up to the end of the 10th month following the said entry into force.

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

Done at Brussels, 11 December 1997.

For the Commission

Franz FISCHLER

Member of the Commission

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ANNEX I

1. The following title is added to the summary of the Annexes to Regulation (EEC) No 2568/91:

'Annex XVIII: Method for determining the content of triglycerides with ECN42'.

2. Annex I is replaced by the following tables and text:

'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 (%)	Stigmastadienes mg/kg (²)	Difference between HPLC and theoretical calculation of ECN42	K_{232} (*)	K_{270} (*)	K_{270} after alumina (³)	Delta-K (*)	Panel test (*)
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	≥6,5
2. Virgin olive oil	≤2,0	≤20	≤0,20	≤250	≤1,3	≤0,15	≤0,2	≤2,60	≤0,25	≤0,10	≤0,01	≥5,5
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	≥3,5
4. Virgin lampante olive oil	>3,3	>20	>0,20	≤350	≤1,3	≤0,50	≤0,3	≤3,70	>0,25	≤0,11	—	<3,5
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-residue oil	>0,5	—	—	—	≤1,8	—	≤0,6	—	—	—	—	—
8. Refined olive-residue oil	≤0,5	≤5	≤0,20	—	≤2,0	—	≤0,5	≤5,50	≤2,50	—	≤0,25	—
9. Olive-residue oil	≤1,5	≤15	≤0,20	>350	≤2,0	—	≤0,5	≤5,30	≤2,00	—	≤0,20	—

(¹) Overall 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) To check the presence of refined oil, if the K_{270} exceeds the limit for the category concerned, it shall be determined again after passage over alumina.

Notes

The results of the tests must be expressed to the same number of decimals as that specified for each characteristic.
The last digit shall be increased by one unit if the following digit is greater than 4.

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.
An asterisk (*) after the characteristic signifies, with regard to the quality of the oil, that:
— in the case of virgin lampante olive oil, the limits laid down (with the exception of that for K_{232}) do not all have to be complied with simultaneously,
— in the case of other virgin olive oils, failure to comply with one or more of the limits is to entail a change of category within the virgin olive oil group.

| Category | Acidic composition | | | | | | Sum of the transoleic isomers (%) | Sum of the translinoleic and translinolenic isomers (%) | Cholesterol (%) | Brassicasterol (%) | Campesterol (%) | Stigmasterol (%) | Betasitosterol [1] (%) | Delta-7-Stigmasterol (%) | 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	≥1000	≤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	≥1000	≤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	≥1000	≤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	≥1000	≤4,5
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	≥1000	≤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	≥1000	≤4,5
7. Crude olive-residue 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	≥2500	≥12
8. Refined olive-residue 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	≥1800	≥12
9. Olive-residue 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	≥1600	>4,5

[1] Delta-5,23-Stigmastadienol + Clerosterol + Sitosterol + Sitostanol + Delta-5-Avenasterol + Delta-5-Avenasterol + Delta-5,24-Stigmastadienol.

Note:

The results of the tests must be expressed to the same number of decimals as that specified for each characteristic.

The last digit shall be increased by one unit if the following digit is greater than 4.

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.'

3. The following Annex XVIII is added:

'ANNEX XVIII

DETERMINATION OF TRIACYGLYCEROLS WITH ECN 42 (DIFFERENCE BETWEEN HPLC DATA AND THEORETICAL CONTENT)

1. **Scope**

   Determination of the composition of triacylglycerols (TAGs) in olive oils, in terms of their equivalent carbon number by differences between the analytical results obtained by high performance liquid chromatography (HPLC) and the theoretical content, calculated starting from the fatty acid composition.

2. **Field application**

   The standard is applicable to olive oils. The method is applicable to the detection of the presence of small amounts of seed oils (rich in linoleic acid) in every class of olive oils.

3. **Principle**

   The content of triacylglycerols with ECN42 determined by HPLC analysis and the theoretical content of triacylglycerols with ECN42 (calculated on the basis of GLC determination of fatty acid composition) correspond within a certain limit for pure oils. A difference larger than the values stated in the Regulation for each type of oil points out that the oil contains seed oils.

4. **Method**

   The method for calculation of theoretical content of triacylglycerols with ECN42 and of the difference between the HPLC data and this one essentially is made by the coordination of analytical data obtained by means of other methods: it is possible to distinguish three phases: determination of fatty acid composition by capillary gas chromatography, calculation of theoretical composition of triacylglycerols with ECN42, HPLC determination of ECN42 triacylglycerols

4.1. **Apparatus**

4.1.1. Round bottom flasks, 250 and 500 ml.

4.1.2. Beakers 100 ml.

4.1.3. Glass chromatographic column, 21 mm internal diameter, 450 mm length, with cock and normalized cone (female) at the top.

4.1.4. Separator funnels, 250 ml, with normalized cone (male) at the bottom, suitable to be connected with the top of the column.

4.1.5. Glass rod, 600 mm length.

4.1.6. Glass funnel, 80 mm diameter.

4.1.7. Volumetric flasks, 50 ml.

4.1.8. Volumetic flasks, 20 ml.

4.1.9. Rotative evaporator.

4.1.10. High performance liquid chromatography, allowing thermostatic control of column temperature.

4.1.11. Injection units for 10 µl delivery.

4.1.12. Detector: differential refractometer. The full scale sensitivity should be at least 10^{-4} units of refractive index.

4.1.13. Column: stainless steel tube 250 mm length and 4,5 mm internal diameter packed with 5 µm diameter particles of slica with 22 to 23 % carbon in the form of octadecylsilane (note 2).

4.1.14. Recorder and/or integrator.

4.2. **Reagents**

   The reagents should be of analytical purity. Elution solvents should be de-gassed, and may be recycled several times without effect on the separations.

4.2.1. Petroleum ether 40 to 60 °C chromatographic grade.

4.2.2. Ethil ether, peroxides free, freshly distilled.

4.2.3.　Glass chromatographic elution solvent: mixture petroleum ether/ethil ether 87/13 (v/v).

4.2.4.　Silicagel, 70-230 mesh, type Merck 7734, with water content standardized at 5 % (w/w).

4.2.5.　Glass wool.

4.2.6.　Acetone.

4.2.7.　Acetonitrile.

4.2.8.　HPLC elution solvent: acetonitrile + acetone (proportions to be adjusted to obtain the desired separation; begin with 50:50 mixture).

4.2.9.　Solubilization solvent: acetone.

4.2.10.　Reference triglycerides commercial triglycerides tripalmitin, triolein, etc.) may be used and the retention times thence plotted in accordance with the equivalent carbon number, or alternatively reference chromatograms obtained from soya oil, mixture 30:70 soya oil/olive oil and pure olive oil (see notes 3 and 4 and figure 1, 2, 3, 4).

4.3.　**Sample preparation**

As a number of interfering substances can rise false positive results, the sample must always be purified according to IUPAC method 2.507, used for determination of polar substances in oxidised oils.

4.3.1.　**Chromatographic column preparation**

Fill the column (4.1.3) with about 30 ml of elution solvent (4.2.3), then introduce inside the column some glass wool (4.2.5) pushing it to the bottom of the column by means of the glass rod (4.1.5).

In a 100 ml beaker, suspend 25 g of silicagel (4.2.4) in 80 ml of elution mixture (4.2.3), then transfer it inside the column, by means of a glass funnel (4.1.6).

To ensure the complete transfer of silicagel inside the column, wash the beaker with the elution mixture and transfer the washing portions inside the column, too.

Open the cock and let solvent elute from the column until its level is about 1 cm over the silicagel.

4.3.2.　**Column chromatography**

Weigh with the accuracy of 0,001 g, 2,5 ± 0,1 g of oil, previously filtered, homogenized and anhydrified, if necessary, in a 50 ml volumetric flask (4.1.7). Solve it in about 20 ml of elution solvent (4.2.3), if necessary, slightly heat it to make the dissolution easily. Cool at room temperature and adjust the volume with elution solvent.

By means of a volumetric pipette, introduce 20 ml of solution inside the column prepared according to 4.3.1, open the cock and let solvent elute to the silicagel layer level.

Then elute with 150 ml of elution solvent (4.2.3), adjusting the solvent rate at about 2 ml/min (150 ml will take about 60 to 70 minutes to pass through the column).

The eluated is recovered in a 250 ml round bottom flask (4.1.1) previously tared in an oven and exactly weighted. Eliminate solvent at reduce pressure (Rotavapor) and weigh the residue that will be used to prepare the solution for HPLC analysis and for methyl ester preparation.

The sample recovery from the column must be 90 % at least for extra virgin, virgin, ordinary refined and olive oil categories, and a minimum of 80 % for lampante and residue olive oils.

4.4.　**HPLC analysis**

4.4.1.　**Preparation of the samples for chromatographic analysis**

A 5 % solution of the sample to be analysed is prepared by weighing 0,5 ± 0,001 g of the sample into a 10 ml graduated flask and making up to 10 ml with the solubilization solvent (4.2.9).

4.4.2.　**Procedure**

Set up the chromatographic system. Pump elution solvent (4.2.8) at a rate of 1,5 ml/min to purge the entire system. Wait until a stable base line is obtained. Inject 10 µl of the sample prepared as in 4.3.

4.4.3. Calculation and expression of results

Use the area normalization method, i.e. assume that the sum of the areas of the peaks corresponding to TAGs from ECN42 up to ECN52 is equal to 100 %. Calculate the relative percentage of each triglyceride using the formula:

% triglyceride = area of peak × 100/ sum of peak areas.

The results are to be given to within at least two decimal places.

Note 1: The elution order can be determined by calculating the equivalent carbon numbers, often defined by the relation $ECN = CN-2n$, where CN is the carbon number and n is the number of double bounds, it can be calculated more precisely by taking into account the origin of the double bond. If n_o, n_l and n_{ln} are the numbers of double bonds attributed to oleic, linoleic and linolenic acids respectively, the equivalent carbon number can be calculated by means of the relation of the formula:

$$ECN = CN - d_o n_o - d_l n_l - d_{ln} n_{ln},$$

where the coefficient d_o, d_l and d_{ln} can be calculated by means of the reference triglycerides. Under the conditions specified in this method, the relation obtained will be close in:

$$ECN = CN - (2{,}60\ n_o) - (2{,}35\ n_l) - (2{,}17\ n_{ln})$$

Note 2: Examples: Lichrosorb (Merck) RP 18 Art 50333

Lichrosphere or equivalent (Merck) 100 CH18 Art 50377.

Note 3: With several reference triglycerides, it is also possible to calculate the resolution with respect to triolein:

$$\alpha = RT' / RT \text{ triolein}$$

by use of the reduced retention time $RT' = RT - RT$ solvent.

The graph of $\log \alpha$ against f (number of double bonds) enables the retention values to be determined for all the triglycerides of fatty acids contained in the reference triglycerides — see figure 2.

Note 4: The efficiency of the column should permit clear separation of the peak of trilinoein from the peaks of the triglycerides with an adjacent RT. The elution is carried out up to ECN52 peak.

Note 5: A correct measure of the areas of all peaks of interest for the present determination is ensured if the second peak corresponding to ECN50 is 50 % of full scale of the recorder.

4.5. **Calculation of triacylglycerols composition**

4.5.1. Determination of fatty acid composition

Fatty acid composition is carried out by means of the EEC gas chromatographic method reported in Annex X A of Regulation (EEC) No 2568/91, by means of a capillary column. The methyl esters preparation is carried out according to Annex X B (sodium methylate alcohol solution).

4.5.2. Fatty acids for calculation

Glycerides are grouped by their equivalent carbon number (ECN), taking into account the following equivalencies between ECN and fatty acids. Only fatty acids with 16 and 18 carbon atoms were taken in consideration, because only these are important for olive oil.

Fatty acid (FA)	Abbreviation	Molecular weight (MW)	ECN
Palmatic acid	P	256,4	16
Palmatoleic acid	Po	254,4	14
Stearic acid	S	284,5	18
Oleic acid	O	282,5	16
Linoleic acid	L	280,4	14
Linolenic acid	Ln	278,4	12

4.5.3. Conversion of area % into moles for all fatty acids

$$\text{moles } P = \frac{\text{area \% P}}{\text{MW P}} \qquad \text{moles } S = \frac{\text{area \% S}}{\text{MW S}} \qquad \text{moles } Po = \frac{\text{area \% Po}}{\text{MW Po}}$$

$$\text{moles } O = \frac{\text{area \% O}}{\text{MW O}} \qquad \text{moles } L = \frac{\text{area \% L}}{\text{MW L}} \qquad \text{moles } Ln = \frac{\text{area \% Ln}}{\text{MW Ln}}$$

(1)

4.5.4. Normalization of fatty acids to 100 %

$$\text{moles \% P } (1,2,3) = \frac{\text{moles P} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

$$\text{moles \% S } (1,2,3) = \frac{\text{moles S} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

$$\text{moles \% Po } (1,2,3) = \frac{\text{moles Po} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

$$\text{moles \% O } (1,2,3) = \frac{\text{moles O} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

$$\text{moles \% L } (1,2,3) = \frac{\text{moles L} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

$$\text{moles \% Ln } (1,2,3) = \frac{\text{moles Ln} * 100}{\text{moles } (P + S + Po + O + L + Ln)}$$

(2)

The result gives the percentage of each fatty acid in moles % in the overall (1,2,3-) position of the TAGs.

Then the sum of the saturated fatty acids P and S (SFA) and the unsaturated fatty acids Po, O, L and Ln (UFA) are calculated:

moles % SFA = moles % P + moles % S

moles UFA = 100 − moles % SFA

(3)

4.5.5. Calculation of the fatty acid composition in 2- and 1,3- positions of TAGs

The fatty acids are distributed to three pools as follows: two identical for 1- and 3- positions and one for 2- position, with different coefficients for the saturated (P and S) and unsaturated acids (Po, O, L and Ln).

4.5.5.1. Saturated fatty acids in 2- position [P(2) and S(2)]

moles % P(2) = moles % P (1,2,3) * 0,06

moles % S(2) = moles % S (1,2,3) * 0,06

(4)

4.5.5.2. Unsaturated fatty acids in 2- position [Po(2), O(2), L(2) and Ln(2)]:

$$\text{moles \% Po(2)} = \frac{\text{moles \% Po(1,2,3)}}{\text{moles \% UFA}} * [100 - \text{moles \% P(2)} - \text{moles \% S(2)}]$$

$$\text{moles \% O(2)} = \frac{\text{moles \% O(1,2,3)}}{\text{moles \% UFA}} * [100 - \text{moles \% P(2)} - \text{moles \% S(2)}]$$

$$\text{moles \% L(2)} = \frac{\text{moles \% L(1,2,3)}}{\text{moles \% UFA}} * [100 - \text{moles \% P(2)} - \text{moles \% S(2)}]$$

$$\text{moles \% Ln(2)} = \frac{\text{moles \% Ln(1,2,3)}}{\text{moles \% UFA}} * [100 - \text{moles \% P(2)} - \text{moles \% S(2)}]$$

(5)

4.5.5.3. Fatty acids in 1,3-positions [P(1,3), S(1,3), Po(1,3) O(1,3), L(1,3) and Ln(1,3)]:

$$\text{moles \% P(1,3)} = \frac{\text{moles \% P(1,2,3)} - \text{moles \% P(2)}}{2} + \text{moles \% P(1,2,3)}$$

$$\text{moles \% S(1,3)} = \frac{\text{moles \% S(1,2,3)} - \text{moles \% S(2)}}{2} + \text{moles \% S(1,2,3)}$$

$$\text{moles \% Po(1,3)} = \frac{\text{moles \% Po(1,2,3)} - \text{moles \% Po(2)}}{2} + \text{moles \% Po(1,2,3)}$$

$$\text{moles \% O(1,3)} = \frac{\text{moles \% O(1,2,3)} - \text{moles \% O(2)}}{2} + \text{moles \% O(1,2,3)}$$

$$\text{moles \% L(1,3)} = \frac{\text{moles \% L(1,2,3)} - \text{moles \% L(2)}}{2} + \text{moles \% L(1,2,3)}$$

$$\text{moles \% Ln(1,3)} = \frac{\text{moles \% Ln(1,2,3)} - \text{moles \% Ln(2)}}{2} + \text{moles \% Ln(1,2,3)}$$

(6)

4.5.6. Calculation of triacylglycerols

4.5.6.1. TAGs with one fatty acid (AAA, here LLL, PoPoPo)

$$\text{moles \% AAA} = \frac{\text{moles \% A(1,3)}^* \text{moles \% A(2)}^* \text{moles \% A(1,3)}}{10\ 000}$$

(7)

4.5.6.2. TAGs with two fatty acids (AAB, here PoPoL, PoLL)

$$\text{moles \% AAB} = \frac{\text{moles \% A(1,3)}^* \text{moles \% A(2)}^* \text{moles \% B(1,3)}^* 2}{10\ 000}$$

$$\text{moles \% ABA} = \frac{\text{moles \% A(1,3)}^* \text{moles \% B(2)}^* \text{moles \% A(1,3)}}{10\ 000}$$

(8)

4.5.6.3. TAGs with three different fatty acids (ABC, here OLLn, PLLn, PoOLn, PPoLn)

$$\text{moles \% ABC} = \frac{\text{moles \% A(1,3)}^* \text{moles \% B(2)}^* \text{moles \% C(1,3)}^* 2}{10\ 000}$$

$$\text{moles \% BCA} = \frac{\text{moles \% B(1,3)}^* \text{moles \% C(2)}^* \text{moles \% A(1,3)}^* 2}{10\ 000}$$

$$\text{moles \% CAB} = \frac{\text{moles \% C(1,3)}^* \text{moles \% A(2)}^* \text{moles \% B(1,3)}^* 2}{10\ 000}$$

(9)

4.5.6.4. Triacylglycerides with ECN42

The following triglycerides with ECN42 are calculated according equation 7, 8 and 9 in order of expected elution in HPLC (normally only three peaks).

LLL

PoLL and the positional isomer LPoL

OLLn and the positional isomers OLnL and LnOL

PoPoL and the positional isomer PoLPo

PoOLn and the positional isomers OPoLn and OLnPo

PLLn and the positional isomers LLnP and LnPL

PoPoPo

SLnLn and the positional isomer LnSLn

PPoLn and the positional isomers PLnPo and PoPLn

The triacylglycerides with ECN42 are given by the sum of the nine triacylglycerols including their positional isomers. The results to be given with at least two decimal places.

5. Evaluation of the results

The calculated theoretical content and the content determined by the HPLC analysis are compared. If the difference between HLPC data minus theoretical data is greater than the values states for the appropriate oil category in the Regulation, the sample contains seed oil.

Note: Results are given to within one decimal figure.

6. **Example** (The numbers refer to the sections in the text of the method)

4.5.1. Calculation of moles % fatty acids from GLC data (area %)

The following data are obtained for the fatty acid composition by GLC:

FA MW	P 256,4	S 284,5	Po 254,4	O 282,5	L 280,4	Ln 278,4
area %	10,0	3,0	1,0	75,0	10,0	1,0

4.5.3. Conversion of area % into moles for all fatty acids

$$\text{moles P} = \frac{10}{256,4} = 0,03900 \text{ moles P} \qquad \text{See formula (1)}$$

$$\text{moles S} = \frac{3}{284,5} = 0,01054 \text{ moles S} \qquad \text{See formula (1)}$$

$$\text{moles Po} = \frac{1}{254,4} = 0,00393 \text{ moles Po} \qquad \text{See formula (1)}$$

$$\text{moles O} = \frac{75}{282,5} = 0,26549 \text{ moles O} \qquad \text{See formula (1)}$$

$$\text{moles L} = \frac{10}{280,4} = 0,03566 \text{ moles L} \qquad \text{See formula (1)}$$

$$\text{moles Ln} = \frac{1}{278,4} = 0,003594 \text{ moles Ln} \qquad \text{See formula (1)}$$

Total $\qquad = 0,35822$ moles TAGs

4.5.4. Normalization of fatty acids to 100 %

$$\text{moles \% P(1,2,3)} = \frac{0,03900 \text{ moles P} * 100}{0,35822 \text{ moles}} = 10,888 \% \qquad \text{See formula (2)}$$

$$\text{moles \% S(1,2,3)} = \frac{0,01054 \text{ moles S} * 100}{0,35822 \text{ moles}} = 2,944 \% \qquad \text{See formula (2)}$$

$$\text{moles \% Po(1,2,3)} = \frac{0,00393 \text{ moles Po} * 100}{0,35822 \text{ moles}} = 1,097 \% \qquad \text{See formula (2)}$$

$$\text{moles \% O(1,2,3)} = \frac{0,26549 \text{ moles O} * 100}{0,35822 \text{ moles}} = 74,113 \% \qquad \text{See formula (2)}$$

$$\text{moles \% L(1,2,3)} = \frac{0,03566 \text{ moles L} * 100}{0,35822 \text{ moles}} = 9,956 \% \qquad \text{See formula (2)}$$

$$\text{moles \% Ln(1,2,3)} = \frac{0,00359 \text{ moles Ln} * 100}{0,35822 \text{ moles}} = 1,003 \% \qquad \text{See formula (2)}$$

Total moles % $\qquad = 100,0 \ \%$

Sum of the saturated and unsaturated fatty acids in the 1,2,3- position of TAGs

moles % SFA = 10,888 % + 2,944 % = 13,831 % $\qquad$ See formula (3)

moles % UFA = 100,000 % − 13,831 % = 86,169 % $\qquad$ See formula (3)

4.5.5. Calculation of the fatty acid composition in 2- and 1,3- positions of the TAGs

4.5.5.1. Saturated fatty acids in 2- position [P(2) and S(2)]

moles % P(2) = 10,888 % * 0,06 = 0,653 % $\qquad$ See formula (4)

moles % S(2) = 2,944 % * 0,06 = 0,177 % $\qquad$ See formula (4)

4.5.5.2. Unsaturated fatty acids in 1,3-position [Po(1,3), O(1,3), L(1,3) and Ln(1,3)]

$$\text{moles \% Po(2)} = \frac{1{,}097\ \%}{86{,}169\ \%} \cdot (100 - - 0{,}659 - 0{,}177) = 1{,}263 \text{ moles \%} \quad \text{See formula (5)}$$

$$\text{moles \% O(2)} = \frac{74{,}113\ \%}{86{,}169\ \%} \cdot (100 - - 0{,}659 - 0{,}177) = 85{,}295 \text{ moles \%} \quad \text{See formula (5)}$$

$$\text{moles \% L(2)} = \frac{9{,}956\ \%}{86{,}169\ \%} \cdot (100 - - 0{,}659 - 0{,}177) = 11{,}458 \text{ moles \%} \quad \text{See formula (5)}$$

$$\text{moles \% Ln(2)} = \frac{1{,}003\ \%}{86{,}169\ \%} \cdot (100 - - 0{,}659 - 0{,}177) = 1{,}154 \text{ moles \%} \quad \text{See formula (5)}$$

4.5.5.3. Fatty acids in 1,3-positions [P(1,3), S(1,3), Po(1,3), O(1,3), L(1,3) and Ln(1,3)]

$$\text{moles \% P(1,3)} = \frac{10{,}888 - 0{,}659}{2} \quad 10{,}888 = 16{,}005 \text{ moles \%} \quad \text{See formula (6)}$$

$$\text{moles \% S(1,3)} = \frac{2{,}944 - 0{,}177}{2} \quad 2{,}944 = 4{,}327 \text{ moles \%} \quad \text{See formula (6)}$$

$$\text{moles \% Po(1,3)} = \frac{1{,}097 - 1{,}263}{2} \quad 1{,}097 = 1{,}015 \text{ moles \%} \quad \text{See formula (6)}$$

$$\text{moles \% O(1,3)} = \frac{74{,}113 - 85{,}295}{2} \quad 74{,}113 = 68{,}522 \text{ moles \%} \quad \text{See formula (6)}$$

$$\text{moles \% L(1,3)} = \frac{9{,}956 - 11{,}458}{2} \quad 9{,}956 = 9{,}205 \text{ moles \%} \quad \text{See formula (6)}$$

$$\text{moles \% Ln(1,3)} = \frac{1{,}003 - 1{,}154}{2} \quad 1{,}003 = 0{,}927 \text{ moles \%} \quad \text{See formula (6)}$$

4.5.6. Calculation of triacylglycerols

From the calculated fatty acid composition in sn-2- and sn-1,3- positions (see above):

FA in	1,3-pos.	2-pos.
P	16,005 %	0,653 %
S	4,327 %	0,177 %
Po	1,015 %	1,263 %
O	68,522 %	85,295 %
L	9,205 %	11,458 %
Ln	0,927 %	1,154 %
Sum	100,0 %	100,0 %

the following triacylglycerols are calculated:

LLL
PoPoPo

PoLL   with 1 positional isomer
SLnLn   with 1 positional isomer
PoPoL   with 1 positional isomer

PPoLn   with 2 positional isomers
OLLn   with 2 positional isomers
PLLn   with 2 positional isomers
PoOLn   with 2 positional isomers.

4.5.6.1. TAGs with one fatty acid (LLL, PoPoPo)   See formula (7)

$$\text{mol \% LLL} = \frac{9{,}205\ \% \cdot 11{,}458\ \% \cdot 9{,}205\ \%}{10\ 000} = 0{,}09708 \text{ mol LLL}$$

$$\text{mol \% PoPoPo} = \frac{1{,}015\ \% \cdot 1{,}263\ \% \cdot 1{,}015\ \%}{10\ 000} = 0{,}00013 \text{ mol PoPoPo}$$

4.5.6.2. TAGs with two fatty acids (PoLL, SLnLn, PoPoL)   See formula (8)

$$\text{mol \% PoLL + LLPo} = \frac{1,015\ \% * 11,458\ \% * 9,205\ \% * 2}{10\ 000} = 0,02141$$

$$\text{mol \% LPoL} = \frac{9,205\ \% * 1,263\ \% * 9,205\ \%}{10\ 000} = 0,01070$$

0,03211 mol PoLL

$$\text{mol \% SLnLn + LnLnS} = \frac{4,327\ \% * 1,154\ \% * 0,927\ \% * 2}{10\ 000} = 0,00093$$

$$\text{mol \% LnSLn} = \frac{0,927\ \% * 0,177\ \% * 0,927\ \%}{10\ 000} = 0,00002$$

0,00095 mol SLnLn

$$\text{mol \% PoPoL + LPoPo} = \frac{1,015\ \% * 1,263\ \% * 9,205\ \% * 2}{10\ 000} = 0,00236$$

$$\text{mol \% PoLPo} = \frac{1,015\ \% * 11,458\ \% * 1,015\ \%}{10\ 000} = 0,00118$$

0,00354 mol PoPoL

4.5.6.3. TAGs with three different fatty acids (PoPLn, OLLn, PLLn, PoOLn)   See formula (9)

$$\text{mol \% PPoLn} = \frac{16,005\ \% * 1,263\ \% * 0,927\ \% * 2}{10\ 000} = 0,00375$$

$$\text{mol \% LnPPo} = \frac{0,927\ \% * 0,653\ \% * 1,015\ \% * 2}{10\ 000} = 0,00012$$

$$\text{mol \% PoLnP} = \frac{1,015\ \% * 1,154\ \% * 16,005\ \% * 2}{10\ 000} = 0,00375$$

0,00762 mol PPoLn

$$\text{mol \% OLLn} = \frac{68,522\ \% * 11,458\ \% * 0,927\ \% * 2}{10\ 000} = 0,14577$$

$$\text{mol \% LnOL} = \frac{0,927\ \% * 85,295\ \% * 9,205\ \% * 2}{10\ 000} = 0,14577$$

$$\text{mol \% LLnO} = \frac{9,205\ \% * 1,154\ \% * 68,522\ \% * 2}{10\ 000} = 0,14577$$

0,43671 mol OLLn

$$\text{mol \% PLLn} = \frac{16,005\ \% * 11,458\ \% * 0,927\ \% * 2}{10\ 000} = 0,03400$$

$$\text{mol \% LnPL} = \frac{0,927\ \% * 0,653\ \% * 9,205\ \% * 2}{10\ 000} = 0,00111$$

$$\text{mol \% LLnP} = \frac{9,205\ \% * 1,154\ \% * 16,005\ \% * 2}{10\ 000} = 0,03400$$

0,06911 mol PLLn

$$\text{mol \% PoOLn} = \frac{1,015\ \% * 85,295\ \% * 0,927\ \% * 2}{10\ 000} = 0,01605$$

$$\text{mol \% LnPoO} = \frac{0,927\ \% * 1,263\ \% * 68,522\ \% * 2}{10\ 000} = 0,01605$$

$$\text{mol \% OLnPo} = \frac{68,522\ \% * 1,154\ \% * 1,015\ \% * 2}{10\ 000} = 0,01605$$

0,04815 mol PoOLn

ECN42 = 0,69540 mol TAGs'

ANNEX II

'2. A. Heading Nos 1509 and 1510 cover only oils derived solely from the treatment of olives the analytical characteristics of whose fatty acid content — determined using the methods set out in Annexes V, X-A and X-B to Regulation (EEC) No 2568/91 — and sterol content are as follows:

Table I

Fatty acid content as percentage of total fatty acids

Fatty acid	Percentage
Myristic acid	≤ 0,05
Linolenic acid	≤ 0,9
Arachidic acid	≤ 0,6
Eicosenoic acid	≤ 0,4
Behenic acid (¹)	≤ 0,3
Lignoceric acid	≤ 0,2

(¹) ≤ 0,2 for the oils of heading No 1509.

Table II

Sterol content as percentage of total sterols

Sterol	Percentage
Cholesterol	≤ 0,5
Brassicasterol (¹)	≤ 0,1
Campesterol	≤ 4,0
Stigmasterol (²)	< Campesterol
Betasitosterol (³)	≥ 93,0
Delta-7-Stigmasterol	≤ 0,5

(¹) ≤ 0,2 for the oils of heading No 1509.

(²) Requirement not valid for virgin lampante oil (subheading 1509 10 10) or for crude olive-residue oil (subheading 1510 00 10).

(³) Delta-5,23-stigmastadienol + clerosterol + betasitosterol + sitostanol + delta-5-avenasterol + delta-5,24-stigmastadienol.

Heading Nos 1509 and 1510 do not cover chemically altered olive oil (in particular re-esterified olive oil) and mixtures of olive oil with other oils. The presence of re-esterified olive oil or other oils is ascertained using the methods set out in Annex VII to Regulation (EEC) No 2568/91.

B. Subheading 1509 10 covers only olive oils defined in Sections I and II below obtained solely using mechanical or other physical means under conditions, and particularly thermal conditions, that do not lead to deterioration of the oil, and which have undergone no treatment other than washing, decantation, centrifugation or filtration. Oils derived from olives using solvents fall within heading No 1510.

I. For the purposes of subheading No 1509 10 10, "virgin lampante olive oil", whatever its acidity, means olive oil with:

(a) a wax content not exceeding 350 mg/kg;

(b) an erythrodiol and uvaol content not exceeding 4,5 %;

(c) a content in saturated fatty acids at the 2- position in the triglycerides not exceeding 1,3 %;

(d) a sum of transoleic isomers not exceeding 0,10 % and a sum of translinoleic + transoleic isomers not exceeding 0,10 %;

(e) a stigmastadiene content not exceeding 0,50 mg/kg;

(f) a difference between the HPLC and theoretical content of triglycerides with ECN42 of 0,3 or less; and

(g) one or more of the following characteristics:

1. a peroxide number not lower than 20 meq O_2/kg;

2. a content in volatile halogenated solvents greater than 0,2 mg/kg or greater than 0,1 mg/kg for any one solvent;

3. a K_{270} extinction coefficient greater than 0,25 and, after treatment of the oil with activated alumina, not greater than 0,11. In point of fact some oils having a free fatty acid content, expressed as oleic acid, of more than 3,3 g per 100 g may, after passage through activated alumina in accordance with the method set out in Annex IX to Regulation (EEC) No 2568/91, have a K_{270} extinction coefficient higher than 0,10. If so, after neutralization and decolourization in the laboratory in accordance with the method set out in Annex XIII to the aforementioned Regulation, they must have the following characteristics:

— a K_{270} extinction coefficient not higher than 1,20,

— an extinction coefficient variation (ΔK), in the 270 nm region, higher than 0,01 but not higher than 0,16, i.e.:

ΔK	$= K_m - 0,5 (K_{m-4} + K_{m+4})$
K_m	= extinction coefficient at the wavelength of the maximum of the absorption curve in the 270 nm region, and
K_{m-4} and K_{m+4}	= extinction coefficients at wavelengths 4 nm lower and higher than the K_m wavelength;

4. organoleptic characteristics which include detectable defects exceeding the limits of acceptability and a panel test score lower than 3,5 in accordance with Annex XII to Regulation (EEC) No 2568/91;

II. For the purposes of subheading 1509 10 90, "virgin oil" means olive oil having the following characteristics:

(a) an acid content, expressed as oleic acid, not exceeding 3,3 g per 100 g;

(b) a peroxide number not exceeding 20 meq O_2/kg;

(c) a wax content not exceeding 250 mg/kg;

(d) a content in volatile halogenated solvents not exceeding 0,2 mg/kg overall and not exceeding 0,1 mg/kg for each solvent;

(e) a K_{270} extinction coefficient not higher than 0,25 and, after treatment of the oil with activated alumina, not higher than 0,10;

(f) an extinction coefficient variation (ΔK), in the 270 nm region, not higher than 0,01;

(g) organoleptic characteristics which may include detectable defects within the limits of acceptability and a panel test score not lower than 3,5 in accordance with Annex XII to Regulation (EEC) No 2568/91;

(h) an erythrodiol and uvaol content not exceeding 4,5 %;

(ij) a content in saturated fatty acids at the 2- position in the triglycerides not exceeding 1,3 %;

(k) a sum of transoleic isomers not exceeding 0,05 % and a sum of translinoleic + translinoleic isomers not exceeding 0,05 %;

(l) a content in stigmastadienes not exceeding 0,15 mg/kg;

(m) a difference between the HPLC and theoretical content of triglycerides with ECN42 of 0,2 or less.

C. Subheading 1509 90 covers olive oil obtained by the treatment of olive oils falling within subheading 1509 10 10 and/or 1509 10 90, whether or not blended with virgin olive oil, having the following characteristics:

(a) an acid content, expressed as oleic acid, not exceeding 1,5 g per 100 g;

(b) a wax content not exceeding 350 mg/kg;

(c) a K_{270} extinction coefficient not higher than 1,0;

(d) an extinction coefficient variation (ΔK), in the 270 nm region, not higher than 0,13;

(e) an erythrodiol and uvaol content not exceeding 4,5 %;

(f) a content in saturated fatty acids at the 2- position in the triglycerides not exceeding 1,5 %;

(g) the sum of transoleic isomers not exceeding 0,20 % and the sum of translinoleic + translinolenic isomers not exceeding 0,30 %;

(h) a difference between the HPLC and theoretical content of triglycerides with ECN42 of 0,3 or less.

D. For the purposes of subheading 1510 00 10, "crude oils" means oils, particularly olive-residue oils, with the following characteristics:

(a) an acid content, expressed as oleic acid, greater than 0,5 g per 100 g;

(b) an erythrodiol and uvaol content not lower than 12 %;

(c) a content in saturated fatty acids at the 2- position in the triglycerides not exceeding 1,8 %;

(d) a sum of transoleic isomers not exceeding 0,20 % and a sum of translinoleic + translinolenic isomers not exceeding 0,10 %;

(e) a difference between the HPLC and theoretical content of triglycerides with ECN42 of 0,6 or less.

E. Subheading 1510 00 90 covers oils obtained by the treatment of oils falling within subheading 1510 00 10, whether or not blended with virgin olive oil, and oils not having the characteristics of the oils referred to in additional notes 2 B, 2 C and 2 D. Oils falling within the subheading must have a content in saturated fatty acids at the 2- position in the triglycerides not exceeding 2,0 %, a sum of transoleic isomers lower than 0,40 % and a sum of translinoleic + translinolenic isomers lower than 0,35 % and a difference between the HPLC and theoretical content of triglycerides with ECN42 that does not exceed 0,5.

3. Subheadings 1522 00 31 and 1522 00 39 do not cover:

(a) residues resulting from the treatment of fatty substances containing oil having an iodine index, determined in accordance with the method laid down in Annex XVI to Regulation (EEC) No 2568/91, lower than 70 or higher than 100;

(b) residues resulting from the treatment of fatty substances containing oil having an iodine index higher than 70 or lower than 100, of which the peak area representing the retention volume of betasitosterol [1], determined in accordance with Annex V to Regulation (EEC) No 2568/91, is less than 93,0 % of the total sterol peak areas.

4. The analytical methods for the determination of the characteristics of the products referred to above are those laid down in the Annexes to Regulation (EEC) No 2568/91. Accordingly, account is to be taken also of the footnotes of Annex I to the Regulation.

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[1] Delta-5,23-stigmastadienol + clerosterol + betasitosterol + sitostanol + delta-5-avenasterol + delta-5,24-stigmastadienol.'