COMMISSION REGULATION (EEC) No 1058/77

of 18 May 1977

on the characteristics of olive oil and of certain products containing olive oil and amending the Common Customs Tariff nomenclature as regards olive oil

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic 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 (EEC) No 1707/73 (2), and in particular Articles 13 (4) and 18 (3) thereof,

Having regard to Council Regulation No 162/66/EEC of 27 October 1966 on trade in oils and fats between the Community and Greece (3), and in particular Articles 3 (4) and 9 thereof,

Having regard to Council Regulation (EEC) No 443/72 of 29 February 1972 on the levies on refined olive oil and on certain products containing olive oil (4), and in particular Article 8 thereof,

Whereas at present all virgin olive oils fall within subheading 15.07 A II a) of the Common Customs Tariff; whereas, therefore, a single levy is fixed for those products; whereas, so that the import levy may achieve its objective, it should be fixed according to the various types of virgin oil and for that purpose the types of olive oil should be distinguished in accordance with their physical and chemical characteristics; whereas, consequently, the nomenclature of the Common Customs Tariff should be amended;

Whereas the tariff nomenclature resulting from Regulation No 136/66/EEC is incorporated in the Common Customs Tariff annexed to Regulation (EEC) No 950/68 (5), as last amended by Regulation (EEC) No 874/77 (6);

Whereas to ensure the proper functioning of the system of levies applicable to imports to olive-oil cakes, a single method should be laid down for determining their oil content;

Whereas Commission Regulation (EEC) No 618/72 of 29 March 1972 on the characteristics of olive oil and of certain products containing olive oil (7) has been amended on several occasions, in particularly by Regulation (EEC) No 3366/75 (8); whereas in view of the new amendments to be made thereto, it should be repealed and all its provisions incorporated in a new Regulation;

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

1. For the purposes of subheading 15.07 A of the Common Customs Tariff, 'olive oil' means oil obtained solely from the processing of olives, excluding re-esterified olive oil and mixtures of olive oil with other types of oil.

Tests for the presence of re-esterified olive oil or of other types of oil shall be carried out by means of the methods set out in Annexes VII and VIII respectively.

2. Oils which have the characteristics described in paragraphs 1, 2 and 3 of Annex I shall be classified under subheadings 15.07 A I a), 15.07 A I b) and 15.07 A I c) of the Common Customs Tariff. The said characteristics shall be determined in accordance with the methods laid down in Annexes IV, V and VI.

3. Oils which have the characteristics described in paragraph 4 of Annex I shall be classified under subheading 15.07 A II a) of the Common Customs Tariff.

4. Products falling within heading No 15.17 which have the characteristics described in Annex II shall not be classified under subheading 15.17 A of the Common Customs Tariff.

Article 2

1. The olive oil content of olive-oil cake and other residues resulting from the extraction of olive oil falling within subheading 23.04 A shall be determined in accordance with the method set out in Annex IX.

(1) OJ No 172, 30. 9. 1966, p. 3025/66.
(2) OJ No L 175, 29. 6. 1973, p. 5.
(3) OJ No 197, 29. 10. 1966, p. 3393/66.
(4) OJ No L 54, 3. 3. 1972, p. 3.
(5) OJ No L 172, 22. 7. 1968, p. 1.
(6) OJ No L 106, 29. 4. 1977, p. 20.
(7) OJ No L 78, 31. 3. 1972, p. 5.
(8) OJ No L 333, 30. 12. 1975, p. 13.

2. The olive oil content referred to in paragraph 1 shall be expressed in terms of its weight as a percentage of dry matter.

Article 3

The Common Customs Tariff annexed to Regulation (EEC) No 950/68 is hereby amended as follows :

1. In Additional Note 1 to Chapter 15, '15.07' is replaced by 'subheading 15.07 D'.

2. Additional Notes 2, 3 and 4 to Chapter 15 are replaced by Additional Notes 2, 3 and 4 in Annex III to this Regulation.

3. Subheading 15.07 A is amended to read as follows :

Heading No	Description	Rate of duty	
		Autonomous % or levy (L)	Conventional %
1	2	3	4
15.07	A. Olive oil :		
	I. Untreated :		
	a) Virgin olive oil	20 (L)	
	b) Virgin lampante olive oil	20 (L)	
	c) Other	20 (L)	
	II. Other :		
	a) Obtained by processing oils falling within subheading 15.07 A I a) or 15.07 A I b), whether or not blended with virgin olive oil	20 (L)	
	b) Other	20 (L)	

Article 4

Any reference in a Community instrument to subheadings 15.07 A II or 15.07 A I a) and b) of the Common Customs Tariff shall be construed, according to the characteristics of the product concerned, as a reference to subheadings 15.07 A I a), b) and c) or 15.07 A II a) and b) respectively of the Common Customs Tariff.

Article 5

Commission Regulation (EEC) No 618/72 is hereby repealed.

Article 6

This Regulation shall enter into force on the 43rd day following its publication in the *Official Journal of the European Communities.*

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

Done at Brussels, 18 May 1977.

For the Commission

Finn GUNDELACH

Vice-President

ANNEXES

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ANNEX I

CHARACTERISTICS OF OLIVE OILS

1. For the purposes of subheading 15.07 A I a) of the Common Customs Tariff, 'virgin olive oil' means natural olive oil obtained exclusively by mechanical processes, including pressure, but does not include mixtures with olive oil obtained otherwise, having the following characteristics :

   (a) a free fatty acid content, expressed as oleic acid, not greater than 3 % ;

   (b) a K_{270} extinction coefficient (absorption under a thickness of 1 cm of solution of 1 g of oil per 100 ml in iso-octane (2,2,4-trimethylpentane) at a wavelength of 270 nm) not higher than 0·25 and, after treatment of the sample of oil with activated alumina, not higher than 0·11 ;

   (c) an extinction coefficient variation, in the 270 nm region, not higher than 0·01.

   This variation is defined by :

   $$\Delta K = K_m - 0{\cdot}5\ (K_{m-4} + K_{m+4})$$

   where K_m is the 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} are the extinction coefficients at wavelengths 4 nm lower and higher, respectively than that of K_m ;

   (d) negative Bellier and modified Vizern reactions, determined by the methods specified in Annex V, Sections A and B ;

   (e) negative soap test carried out according to the method described in Annex VI.

2. For the purposes of subheading 15.07 A I b) of the Common Customs Tariff, 'virgin lampante oil', whatever its acidity, means olive oil having the following characteristics :

   (a) a K_{270} extinction coefficient higher than 0·25 and, after treatment of the sample with activated alumina, not higher than 0·11.

   Some oils having a free fatty acid content, expressed as oleic acid, of more than 3 % may have, after passage through activated alumina, a K_{270} extinction coefficient higher than 0·11. If so, after neutralization and decolorization in the laboratory by the method specified in Annex IV, they must have the following characteristics :

   — a K_{270} extinction coefficient not higher than 1·10,

   — an extinction coefficient variation, in the 270 nm region, higher than 0·01 but not higher than 0·16 ;

   (b) negative Bellier and modified Vizern reactions determined by the methods specified in Annex V, Sections A and B ;

   (c) a negative soap test carried out according to the method described in Annex VI.

3. Subheading 15.07 A I c) of the Common Customs Tariff covers oils, especially oils from 'olive residues', having the following characteristics :

   (a) a free fatty acid content, expressed as oleic acid, higher than 3 % ;

   (b) positive Bellier and modified Vizern reactions, determined by the methods described in Annex V, Sections A and B ;

   (c) a negative soap test, carried out by the method described in Annex VI.

4. Subheading 15.07 A II a) of the Common Customs Tariff covers olive oil obtained by the treatment of olive oils falling within subheading 15.07 A I a) or 15.07 A I b), whether or not blended with virgin olive oil, having the following characteristics :

   (a) a free fatty acid content, expressed as oleic acid, not exceeding 3 % ;

   (b) a positive soap test carried out by the method described in Annex VI,

or :

— a K_{270} extinction coefficient higher than 0·25 but not higher than 1·10 and, after treatment of the sample of oil with activated alumina, higher than 0·11,

and :

— an extinction coefficient variation, in the 270 nm region, higher than 0·01 but not higher than 0·16.

This variation is defined by :

$$\Delta K = K_m - 0{\cdot}5\ (K_{m-4} + K_{m+4})$$

where K_m is the extinction coefficient at the wavelength of maximum absorption on the absorption curve in the 270 nm region, and

K_{m-4} and K_{m+4} are the extinction coefficients at wavelengths 4 nm lower and higher, respectively than that of K_m ;

(c) negative Bellier and modified Vizern reactions, determined by the methods specified in Annex V, Sections A and B.

ANNEX II

PRODUCTS FALLING WITHIN SUBHEADING 15.17 A

Subheading 15.17 A does not cover :

(a) residues resulting from the treatment of fatty substances containing oil having an iodine index, determined by the Wijs method without catalyst, lower than 70 or higher than 100 ;

(b) residues resulting from the treatment of fatty substances containing oil having an iodine index not lower than 70 or not higher than 100, of which the peak area representing the retention volume of β-sitosterol, determined in accordance with the provisions of Annex VIII, is less than 93 % of the total sterol peak areas.

ANNEX III

ADDITIONAL NOTES 2, 3 AND 4 TO CHAPTER 15 OF THE COMMON CUSTOMS TARIFF

2. A. For the purposes of subheading 15.07 A, 'olive oil' means oil derived solely from the treatment of olives, excluding re-esterified olive oil and mixtures of olive oil with other oils.

B. 'Untreated olive oil' means oil with characteristics as defined in Sections I, II and III below.

I. For the purposes of subheading 15.07 A I a), 'virgin olive oil' means natural olive oil obtained exclusively by mechanical processes, including pressure, but does not include mixtures with olive oil obtained otherwise, having the following characteristics :

(a) a free fatty acid content, expressed as oleic acid, not greater than 3 % ;

(b) a K_{270} extinction coefficient (absorption under a thickness of 1 cm of solution of 1 g of oil per 100 ml in iso-octane (2,2,4-trimethylpentane) at a wavelength of 270 nm) not higher than 0·25 and, after treatment of the sample of oil with activated alumina, not higher than 0·11 ;

(c) an extinction coefficient variation, in the 270 nm region, not higher than 0·01.

This variation is defined by :

$$\Delta K = K_m - 0{\cdot}5\ (K_{m-4} + K_{m+4})$$

where K_m is the 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} are the extinction coefficients at wavelengths 4 nm lower and higher, respectively than that of K_m ;

(d) negative Bellier and modified Vizern reactions ;

(e) a negative soap test.

II. For the purposes of subheading 15.07 A I b), 'virgin lampante oil', whatever its acidity, means olive oil having the following characteristics :

(a) a K_{270} extinction coefficient higher than 0·25 and, after treatment of the sample with activated alumina, not higher than 0·11. Some oils having a free fatty acid content, expressed as oleic acid, of more than 3 % may have, after passage through activated alumina, a K_{270} extinction coefficient higher than 0·11. If so, after neutralization and decolorization in the laboratory, they must have the following characteristics :

— a K_{270} extinction coefficient not higher than 1·10 ;

— an extinction coefficient variation, in the 270 nm region, higher than 0·01 but not higher than 0·16 ;

(b) negative Bellier and modified Vizern reactions ;

(c) a negative soap test.

III. Subheading 15.07 A I c) covers oils, especially oils from 'olive residues', having the following characteristics :

(a) a free fatty acid content, expressed as oleic acid, higher than 3 % ;

(b) positive Bellier and/or modified Vizern reactions ;

(c) a negative soap test.

C. Subheading 15.07 A II a) covers olive oil obtained by the treatment of olive oils falling within subheading 15.07 A I a) or 15.07 A I b), whether or not blended with virgin olive oil, having the following characteristics :

(a) a free fatty acid content, expressed as oleic acid, not exceeding 3 % ;

(b) — a positive soap test, or a K_{270} extinction coefficient higher than 0·25 but not higher than 1·10 and, after treatment of the sample of oil with activated alumina, higher than 0·11, and

— an extinction coefficient variation, in the 270 nm region, higher than 0·01 but not higher than 0·16.

This variation is defined by :

$$\Delta K = K_m - 0{\cdot}5\ (K_{m-4} + K_{m+4})$$

where K_m is the extinction coefficient at the wavelength of maximum absorption curve in the 270 nm region and

K_{m-4} and K_{m+4} are the extinction coefficients at wavelengths 4 nm lower and higher, respectively than that of K_m ;

(c) negative Bellier and modified Vizern reactions.

3. Subheading 15.17 A does not cover :

(a) residues resulting from the treatment of fatty substances containing oil having an iodine index, determined by the Wijs method, without catalyst, lower than 70 or higher than 100 ;

(b) residues resulting from the treatment of fatty substances containing oil having an iodine index not lower than 70 or higher than 100, of which the peak area representing the retention volume of β-sitosterol, determined in accordance with the provisions in Annex VIII to the Regulation mentioned in Additional Note 4 below, is less than 93 % 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 1058/77.

ANNEX IV

I. TREATMENT OF THE SAMPLE WITH ACTIVATED ALUMINA

1. Into a chromatography column approximately 35 mm in diameter and 450 mm in length, fitted with an outlet tube approximately 10 mm in diameter, place 30 g of basic alumina prepared in accordance with the process described in Section 2.

Tamp down the alumina mechanically by allowing the column, held in the vertical plane, to fall gently on a wooden surface, repeating the process as often as necessary. Into the column prepared in this way place 100 ml of a 10 % solution of the oil in hexane.

Collect the eluate and evaporate off the solvent under vacuum at a temperature not exceeding 25 °C.

Determination of the extinction coefficient at 270 nm must be carried out immediately on the oil thus obtained.

2. Basic alumina of Brockmann activity I (0 % moisture) is prepared by heating basic alumina (chromatography grade) of particle size in the range 30 to 130 µm (average 80 µm) for three hours at 380 to 400 °C. Add 5 ml of distilled water to 100 g of this product to obtain basic alumina with Brockmann activity II-III. Shake frequently and allow to stand overnight in a hermetically sealed container.

Alumina activity test

Into a chromatography column approximately 35 mm in diameter and 450 mm in length place 30 g of basic alumina (prepared in the manner described above). Pass a mixture of 95 % of olive oil having a K_{270} extinction coefficient not exceeding 0·18 and 5 % of peanut oil treated with bleaching clay during its refining and having a K_{270} extinction coefficient not exceeding four through this column under the conditions specified by the method. If the mixture has an extinction coefficient exceeding 0·11, the alumina is acceptable. If the conjugated trienes remain uneluted by the alumina, it is necessary to use an alumina with a higher degree of hydration, after ascertaining whether it complies with the requirements of the foregoing test.

II. NEUTRALIZATION AND DECOLORIZATION OF THE OLIVE OIL IN THE LABORATORY

A. NEUTRALIZATION OF THE OIL

1. **Apparatus**

— beaker, 300 ml, tall,
— laboratory centrifuge with 100 ml tubes,
— beaker, 250 ml,
— round-bottomed flasks, 100 ml,
— separating funnel, 1 litre.

2. **Reagents**

— aqueous solution of 12 % sodium hydroxide,
— ethyl alcohol solution of 1 % phenolphtalein,
— pure hexane, AR,
— pure propan-2-ol of AR.

3. **Procedure**

(a) *Oils with a free fatty acid content, expressed as oleic acid, of less than 30 %.*

Place 50 g of crude oil in a tall 300 ml beaker and heat to 65 °C in a water bath. Add a quantity of 12 % solution of sodium hydroxide corresponding to the free acid of the oil, with an excess of 5 %, stirring gently all the time. Continue to stir for five minutes, keeping the temperature at 65 °C.

Transfer the mixture into 100 ml centrifuge tubes and separate the soapy paste by centrifugation. Pour the decanted oil into a 250 ml beaker and wash with 50 to 60 ml of boiling distilled water, removing the water by means of a siphon. Repeat the washings until all traces of residual soap are removed (disapperance of the pink colouring in the phenolphtalein).

Centrifuge the oil to eliminate any small quantities of residual water.

(b) *Oils with a free fatty acid content expressed as oleic acid exceeding 30 %.*

In a 1 litre separating funnel place 50 g of crude oil, 200 ml of hexane, 100 ml of propan-2-ol and a quantity of 12 % solution of sodium hydroxide corresponding to the free acid of the oil, with an excess of 0·3 %.

Stir vigorously for one minute. Add 100 ml of distilled water, stir again and allow to stand.

After separation of the layers, allow the lower layer containing soaps to drain off. Between the two layers (oily on top and aqueous underneath) an intermediary layer often forms made up of mucilages and insoluble substances which must also be eliminated.

B. DECOLORIZATION OF NEUTRALIZED OIL

1. **Apparatus**

— round-bottomed flask, 250 ml, with three ground glass necks for the insertion of:
  (a) a thermometer graduated in degrees and allowing readings to be taken at 90 °C;
  (b) a mechanical stirrer operating at 250 to 300 revolutions per minute, equipped to operate in a vacuum;
  (c) a vacuum pump connection,
— vacuum pump, with a manometer, capable of giving residual pressure of 15 to 30 millibars.

2. **Procedure**

Weigh about 100 g of neutralized oil in the three-necked flask. Insert the thermometer and the stirrer, connect the vacuum pump and heat to 90 °C, stirring all the time. Maintain that temperature, continuing to stir, until the oil to be analyzed is entirely free from water (about 30 minutes). Then break the vacuum and add 2 to 3 g of activated earth. Re-establish the vacuum until a residual pressure of 15 to 30 millibars is obtained and, maintaining a temperature of 90 °C, stir for 30 minutes at about 250 revolutions per minute.

Filter while still hot in a thermostatic oven (50 to 60 °C).

ANNEX V

TEST FOR THE PRESENCE OF OIL FROM OLIVE RESIDUES IN OLIVE OILS

A. 'BELLIER' METHOD

1. Apparatus

— round-bottomed flask, 100 ml, fitted with reflux condenser,

— pipette, 5 ml, graduated in tenths,

— heating system with which it is possible to attain a temperature of about 80 °C,

— thermometer graduated from 15 to 60 °C.

2. Reagents

— aqueous alcoholic potassium hydroxide solution (42·5 g of KOH dissolved in 72 ml of distilled water, the volume then being brought up to 500 ml with 95 ° ethyl alcohol),

— ethyl alcohol solution of 70 ° titre,

— solution of acetic acid in water, 1 + 2 (by volume), adjusted to strength so that 1·5 ml exactly neutralize 5 ml of the aqueous alcoholic potassium hydroxide solution in the presence of phenolphtalein.

3. Sample preparation

Remove any moisture from the oil by decantation and filtration through paper, both operations being carried out at a temperature slightly higher than the melting point of any solid constituents which might have separated from the liquid.

4. Procedure

Into the flask place about 1 ml of oil prepared as shown in Section 3. Add 5 ml of aqueous alcoholic potassium hydroxide solution. Fit the reflux condenser and bring to the boil for 10 minutes with occasional stirring. Allow to cool down to room temperature. Add 1·5 ml of diluted acetic acid and 50 ml of ethyl alcohol solution previously warmed to 50 °C. Mix by stirring, insert the thermometer and allow to cool, observing the appearance of the solution as soon as it has dropped to 45 °C. If a flocculent precipitate is formed at a temperature higher than 40 °C, the reaction is positive. If the characterizing flocculent precipitate does not materialize, keep the liquid at room temperature which must not be below 20 °C or above 22 °C, for at least 24 hours or, if necessary, for 48 hours. Observe the solution once again : the formation of a flocculent precipitate in suspension in the liquid also proves the reaction to be positive.

Reporting of results

The test for the presence of oil from olive residues (Bellier method) shall be reported as a positive or negative test.

B. 'MODIFIED VIZERN' METHOD

The unsaponifiable matter of the oil to be analyzed is isolated and its behaviour in 85 ° alcohol studied under specified conditions.

1. Apparatus

— round-bottomed alkali-resistant glass flask, 300 ml, fitted with reflux condenser,

— separating funnels, 500 or 1 000 ml,

— beakers, 300 and 250 ml,

— glass test-tube.

2. **Reagents**

— alcoholic potassium hydroxide solution, 2N,

— petroleum ether,

— 50 % ethyl alcohol,

— 96 ° ethyl alcohol, checked by alcoholmeter,

— Hydrogen peroxide, 10 volume,

— 85° ethyl alcohol, checked by alcoholometer.

3. **Procedure**

Into a 300 ml round-bottomed flask weigh approximately 5 g of the sample to be analyzed. Add 50 ml of 2N alcoholic potash solution. Fit the reflux condenser and bring to the boil gently. After an hour of heating, shake and cool to 30 to 35 °C; transfer to a separating funnel, using 50 ml of distilled water.

Rinse out the flask carefully with 50 ml of petroleum ether and repeat this operation several times. Transfer the petroleum ether into a separating funnel. Shake the contents vigorously for slightly more than a minute. After decantation, remove the aqueous layer by transferring it into a second separating funnel. Add another 50 ml of petroleum ether, shake the contents vigorously for just over a minute and allow to separate. Transfer the aqueous layer to a third separating funnel, adding another 50 ml of petroleum ether. Shake vigorously and allow to separate.

In a separating funnel, collect the ethereal extracts from the various extractions of unsaponifiable matter and wash at least three times with 50 % alcohol (50 ml each time) until the washing liquid is no longer alkaline to phenolphtalein.

Filter the unsaponifiable matter solution into a 300 ml round-bottomed flask, wash the filter with petroleum ether and remove the solvent by distillation. Add 10 ml of 96 ° alcohol, warm moderately (to about 40 °C) and filter into a 100 ml beaker. Wash the 300 ml flask with 10 ml of 96 ° alcohol and then filter into the beaker.

To the 100 ml beaker containing the alcoholic unsaponifiable matter extracts, add 5 ml of 10 volume hydrogen peroxide and heat on a water bath until completely evaporated. Add another 20 ml of 96 ° alcohol and 5 ml of hydrogen peroxide and re-evaporate completely.

Withdraw the beaker from the water bath and add 20 ml of 85 ° alcohol. Warm gently on the water bath, being very careful not to lower the concentration by causing loss of alcohol. This is a very important factor which must not be overlooked.

Filter through paper, allow to cool and examine the behaviour of the solution at the end of an hour, and again at the end of four hours. If the solution is quite transparent at the end of an hour, the test is negative, i.e., there is no oil from olive residues. If the solution is cloudy at the end of an hour, repeat the observation four hours after that.

If the solution is still cloudy but contains no flocculence at the end of four hours, the test is still negative. On the other hand, if flocculence is observed, the test is positive and oil from olive residues is present.

Reporting of results

The test for the presence of oil from olive residues (modified Vizern method) shall be reported as a positive or a negative test.

Remarks

Olive oils give a transparent or, at the most, a milky solution throughout the test. Pure or blended oils from olive residues give rise to a characteristic flocculence which remains in suspension like small clouds and which finally precipitate after being allowed to stand for several hours.

ANNEX VI

SOAP TEST FOR DETECTION OF ALKALINITY

The principle of the method is the detection of alkali soaps through their action on bromophenol blue.

Reagents

— 0·1 % solution of bromophenol blue in 96 % (v/v) ethanol,

— freshly distilled acetone with a 2 % (v/v) water content.

This acetone with a 2 % water content must give a yellow or greenish yellow coloration in the presence of a few drops of the bromophenol blue solution.

Apparatus

— stoppered test-tube, 150 × 15 mm.

Procedure

Into the stoppered test-tube pour 10 ml of acetone and one drop of bromophenol blue solution ; the solution should turn yellow. If it does not, rinse the tube and its stopper with acetone until the blue coloration disappears. Pour 10 g of the oil into the tube, close it by means of its own stopper, shake and allow to settle. The upper acetone layer turning blue means the presence of soap.

Reporting of results

These shall be reported as positive or negative results.

ANNEX VII

TEST FOR THE PRESENCE OF RE-ESTERIFIED OILS

The purpose of the method is to determine the composition of the fraction of fatty acids which are esterified at the 2-position (β- or internal position) of the glycerol in the oils or fats, as there is more palmitic acid in the β-position with re-esterified olive oils than with those which have not been re-esterified.

Principle

This method is based on the partial and specific hydrolysis of the triglycerides by pancreatic lipase with a preferential formation of 2-monoglycerides. This hydrolysis gives rise to a mixture containing diglycerides, 2-monoglycerides and some free fatty acids, in addition to unhydrolyzed triglycerides. This mixture is fractionated and the monoglycerides isolated by thin-layer chromatography. These monoglycerides are converted with methanol into methyl esters, which are analyzed by gas chromatography.

If the percentage of palmitic acid found at the 2-position of the triglycerides is higher than 2 %, the product analyzed is considered as containing added re-esterified oil.

1. **Apparatus**

— separating funnel, 500 ml,

— chromatography column of glass, 13 mm in diameter and 400 mm long, fitted with a sintered glass disc and a stopcock,

— wide-necked round-bottomed flask, 250 ml,

— round-bottomed flask, 100 ml,

— centrifuge tube with ground stopper, 10 ml,

— hypodermic syringe fitted with a fine needle, 1 ml,

— round-bottomed flask, 25 ml, with ground-jointed air-cooled condenser 1 m long,
— beaker, 50 ml,
— burette, 5 ml, graduated in 1/20 ml,
— thin-layer chromatography spreader with glass plates, 20 × 20 cm,
— microsyringe delivering 3 to 4 µl drops,
— thin-layer chromatography developing tank,
— rotary evaporator,
— oven controllable at 103 ± 2 °C,
— thermostat controllable between 30 and 45 °C to within ± 0·5 °C,
— vibrating electric shaker permitting vigorous shaking of the centrifuge tube,
— thin-layer chromatography spray,
— UV lamp for examining the chromatography plates,
— laboratory shaker of suitable design for the dispersion or mixing of heterogeneous substances,
— pH meter,
— paddle stirrer,
— stop-clock.

2. **Reagents**

— aqueous sodium hydroxide solution, 12 % (m/v),
— solution of phenolphalein, 1 % (m/v), in ethanol, 95 % (v/v),
— peroxide-free diethyl ether,
— isopropyl or ethyl alcohol, analytical grade, 95 % (v/v),
— neutral activated alumina, chromatography grade, of Brockmann I activity, activated recently for two hours at 206 °C, and stored in a dessicator,
— hexane, or if not available, petroleum ether (boiling range 30 to 50 °C), chromatography grade,
— formic acid, not less than 98 % (m/m),
— pancreatic lipase of suitable activity (Notes 1 and 2),
— buffer solution consisting of a 1M solution of tris-hydroxymethylaminomethane in water adjusted to pH 8 with 6N hydrochloric acid (potentiometric standard),
— aqueaus sodium cholate (enzyme grade) solution, 0·1 % (m/v),
— hydrogen chloride solution, 6N,
— developing solvent consisting of hexane (or, failing this, petroleum ether) mixed with diethyl ether and formic acid in the proportions of 70 : 30 : 1 (v/v/v),
— aqueous gum arabic solution, 10 % (m/v),
— aqueous calcium chloride ($CaCl_2$) ^{2} solution, 22 % (m/v),
— alcoholic solution of 2',7'-dichlorofluorescein, 0·2 % (m/v), rendered slightly alkaline by the addition of 1N sodium hydroxide solution at the rate of one drop per 100 ml,
— powder silica with binder, thin-layer chromatography grade,
— aqueous sodium cholate solution, 20 % (m/v),
— aqueous sodium hydroxide solution, 0·1N,
— neutralized vegetable oil.

3. **Sample preparation**

If the acidity of the sample is less than 3 %, direct neutralization with alumina as in Section 3.2.

If the acidity of the sample is higher than 3 %, alkali neutralization in the presence of solvent as in Section 3.1 followed by passage through alumina as in Section 3.2.

3.1. *Alkali neutralization in the presence of solvent*

Into a 500 ml separating funnel pour about 10 g of raw oil, 100 ml of hexane or, failing this, petroleum ether, 50 ml of 95 % isopropyl or ethyl alcohol, a few drops of phenolphthalein solution and a sufficient quantity of 12 % sodium hydroxide to take up the free acidity of the oil and give 0·3 % in excess. Shake vigorously for one minute, add 50 ml of distilled water, shake once more and allow to settle.

After separation, remove the lower layer containing the soaps. Discard any intermediate layers (mucilages or insoluble substances), wash the hexane solution of the neutralized oil with successive 25 or 30 ml portions of a 1 : 1 (v/v) solution of isopropyl or ethyl alcohol and distilled water until the pink phenolphtalein coloration disappears. Remove most of the hexane by vacuum distillation and take the oil to dryness at 30 to 40 °C under vacuum in a stream of pure nitrogen until solvent removal is complete.

3.2. *Passage through alumina*

Prepare a suspension of 15 g of activated alumina in 50 ml of hexane, or petroleum ether, and pour it into the chromatography column of glass while stirring. Ensure that the alumina is evenly spread and allow the solvent to run down to 1 to 2 mm above the top of the absorbent. Into the column carefully pour a solution prepared about 15 minutes earlier by dissolving 5 g of oil in 25 ml of hexane, or petroleum ether, and collect in a 100 ml round-bottomed flask all the liquid issuing from the column.

Remove most of the solvent by vacuum distillation and then take the oil to dryness at 30 to 40 °C under vacuum in a stream of pure nitrogen until solvent removal is complete.

4. **Preparation of chromatography plates**

Into wide-necked 250 ml round-bottomed flask place 30 g of prowdered silica with 60 ml of distilled water and stir until a fully homogeneous slurry is obtained. De-gas by keeping it under the vacuum of a water injector pump for one minute.

Spread the slurry in the usual way over the plates by means of the spreader, adjusting the layer thickness to 0·25 mm.

This quantity of slurry is sufficient for the preparation of five 20 × 20 cm plates.

Allow the plates to air-dry for about 15 minutes and then dry them in the oven at 103 ± 2 °C for two hours.

Store the plates so prepared in a dessicator.

5. **Procedure**

5.1. *Pancreatic lipase hydrolysis*

Into a 10 ml centrifuge tube weigh about 0·1 g of prepared sample.

Add 20 mg of lipase and 2 ml of buffer solution. Stir carefully and with appropriate precautions then add 0·5 ml of 0·1 % sodium cholate solution and 0·2 ml of calcium chloride solution. Close the tube with its ground stopper, shake cautiously (avoid wetting the stopper) and place the tube immediately in a thermostat adjusted to 40 ± 0·5 °C shaking for exactly one minute.

Remove the tube from the thermostat and shake it vigorously for exactly two minutes.

Cool immediately under running water and add 1 ml of 6N hydrogen chloride solution and 1 ml of diethyl ether. Stopper and shake vigorously. Allow to settle and sample the upper organic layer with a syringe.

5.2. *Thin-layer chromatography separation of the monoglycerides*

On a chromatography plate, about 1·5 cm from its bottom edge, deposit the extract in a continuous uniform line with the object of obtaining as fine a base line as possible.

Insert the plate in a well-saturated developing tank and develop with the developing solvent up to about 1 cm from the top edge. The development of the plate must take place at a temperature of about 20 °C.

Air-dry the plate at the tank temperature and spray it with the 2', 7',-dichlorofluorescein solution. Identify the monoglycerides band (R_f = about 0·035) under UV light ; remove them with a metal spatula (avoid removing any compounds remaining on the base line) and place the silica in the 25 ml round-bottomed methylation flask.

Convert the monoglycerides into their methyl esters by direct treatment of the previously collected silicy in accordance with the universal method for the preparation of the methyl esters of fatty acids mentioned in Section 7.3, then perform the gas chromatography of the esters in accordance with the method indicated in Section 7.4.

On the same sample determine the composition of the total fatty acids, comparison of which with that of the fatty acids at the 2-position is useful for the interpretation of the results obtained.

6. **Reporting of results**

Calculate the composition of the 2-position fatty acids as a percentage to one decimal place (Note 3).

7. **Notes**

7.1. *Lipase activity test*

In a suitable mixer prepare an oil emulsion by stirring a mixture of 165 ml of 10 % gum arabic solution, 15 g of crushed ice and 20 ml of an already neutralized oil for about 10 minutes.

Into a 50 ml beaker place 10 ml of this emulsion, 0·3 ml of 20 % sodium cholate solution and 20 ml of distilled water in succession.

Place the beaker in a thermostat controlled to 37 ± 0·5 °C and insert into the beaker the electrodes of a pH p*H* meter and a paddle stirrer.

From a 5 ml burette add some 0·1N sodium hydroxide solution in drops until a pH of 8·5 is reached.

Add an appropriate volume of aqueous lipase powder suspension (see above). As soon as the pH meter indicates a pH of 8·3, start the stop-clock and add 0·1N sodium hydroxide solution at the necessary rate to maintain the pH at the value of 8·3. Note the volume of alkali solution consumed each minute.

Plot the data obtained in a system of coordinate axes, plotting times as abscissae and ml of alkali solution consumed in keeping the pH constant as ordinates. The resultant graph must be a straight line.

The lipase suspension referred to in the previous section is a 1 % suspension by weight in water. For each test take the necessary quantity of this suspension to ensure that about 1 ml of alkali solution is consumed in four to five minutes. This result is usually obtained with 1 to 5 mg of powder.

A lipase unit is defined as the quantity which liberates 10 μ -equivalents of acid per minute.

If A is the activity of the powder used, measured in lipase units per mg, then :

$$A = \frac{V \times 10}{m}$$

where : V = number of ml of 0·1N sodium hydroxide solution per minute, calculated from the graph and

m = mass of the test portion of powder, in mg.

The lipase used must have an activity of not less than 0·8 and not more than two units per mg.

7.2. *Lipase preparation*

Lipases with a satisfactory lipase activity are available from trade sources. It is also possible to prepare it in the laboratory as follows :

Chill 5 kg of pig pancreas down to 0 °C, remove all surrounding fat and connective tissues and triturate in a mill with cutting blades until a fluid paste is obtained. Stir this paste in the cold state for four to six hours with 2·5 litres of anhydrous acetone, then centrifuge. Extract the residue a further three times with the same volume of acetone, twice with a 1 : 1 (v/v) mixture of acetone and diethyl ether and twice with diethyl ether.

Dry the residue for 48 hours under a vacuum to obtain a stable powder which must be stored in the refrigerator.

7.3. *General method of preparing the methyl esters fatty acid*

In accordance with the method set out in Section II of Annex VI to Commission Regulation (EEC) No 72/77 of 13 January 1977 amending Regulation (EEC) No 1470/68 on the drawing and reduction of samples and the determination of oil content, impurities and moisture in oil seeds [1].

7.4. *Gas chromatography of the methyl esters of the fatty acids*

In accordance with the method set out in Section III of Annex VI to Commission Regulation (EEC) No 72/77.

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[1] OJ No L 12, 15. 1. 1977, p. 11.

ANNEX VIII

TEST FOR THE PRESENCE OF OTHER OILS IN OLIVE OILS: ANALYSIS OF THE STEROL FRACTION OF THE OIL OR FAT

Principle

Gas chromatography of sterols prepared by means of thin-layer chromatography from unsaponifiable matter carefully dried.

Apparatus

1. Thin-layer chromatography apparatus, including in particular, four glass plates of size 20 × 20 × 0·4 cm, two of size 20 × 5 × 0·4 cm and one 0·1 ml microsyringe.
2. 50 ml beaker.
3. Porous filters, porosity 3, diameter 15 mm.
4. 100 ml round-bottomed flask.
5. 10 ml conical-bottomed centrifuge tube, fitted with ground glass stopper.
6. 1 ml graduated pipettes.
7. Gas chromatography apparatus equipped with a flame-ionization detector and a silver or glass injector or a system of direct injection into the column and coupled to a recorder.
8. A U-shaped or spiral glass or stainless steel gas chromatography column, 1 to 2 m long and 3 to 4 mm in internal diameter, with a stationary phase of silicone rubber (Methyl type) (1), stable up to at least 300 °C, impregnating a calcined diatomaceous earth to the extent of not less than 2 % and not more than 4 %, acid washed and silanized and of a particle size analysis of 80 to 100 mesh or 100 to 120 mesh.

   Note: Glass is recommended, as some types of stainless steel can give rise to erroneous results by deteriorating the sterols.
9. 5 or 10 µl microsyringe.

Reagents

1. Chloroform, chromatography grade.
2. Benzene, chromatography grade.
3. Heptane.
4. Silica gel (e.g., Kieselgel G).
5. Thin-layer chromatography reference solution composed of 5 % cholesterol in chloroform.
6. Acetone, chromatography grade.
7. 0·1 % absolute alcohol solution of 2',7'-dichlorofluorescein sodium salt.
8. Pyridine.
9. Hexamethyldisilazane.
10. Trimethylchlorosilane.
11. Sensitivity test solution composed of 1 mg of cholesterol per ml of n-pentane.
12. Peak-resolution test solution composed of 0·9 mg of colza oil phytosterols and 0·1 mg of cholesterol per ml of n-pentane. The sterols must be freshly prepared in accordance with the procedure described in Section B of the Procedure.
13. Reference test solution composed of 1 mg of sunflower seed oil phytosterols, freshly prepared as described in Section B of the Procedure, per ml of n-pentane.

Preparation of the chromatography plates

On the spreader place one plate 20 × 5 × 0·4 cm, four plates 20 × 20 × 0·4 cm and one plate 20 × 5 × 0·4 cm, in that order.

(1) E.g., SE 30.

In a wide-necked 500 ml round-bottomed flask place 40 g of silica gel and 80 ml of water. Stir with a glass rod or, if available, a mechanical glass stirrer until a homogeneous suspension results. Remove any gas by creating a vacuum, using a water injector pump ; for at least one minute. Then transfer the suspension to the spreader, adjust the thickness to 0·5 mm and coat the plates uniformly. Allow the plates to air-dry for about 15 minutes and then dry off in an oven at 105 °C for two hours. Store the plates so prepared in an evacuated dessicator.

PROCEDURE

A. **Preparation of the unsaponifiable matter**

Foreword

The unsaponifiable matter is defined as the substances soluble in the fat which are insoluble in water after saponification but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, alcohols and hydrocarbons as well as any foreign organic matter which may be present that is not volatile at 100 °C (mineral oils). Light petroleum or diethyl ether is used as a solvent but in most cases the results will differ according to the solvent selected and, generally, the use of diethyl ether will give a higher result. In the case of olive oil, petroleum ether (light petroleum) has been adopted as the solvent to be used in view of the temperature conditions in which the majority of laboratory analyses are carried out.

Light petroleum method

Apparatus

— 150 ml flask fitted with a reflux condenser,

— 500 ml separating funnels,

— oven regulated at 103 °C (± 2 °C).

Reagents

Approximately 2N KOH solution in ethanol (dissolve 120 g potassium hydroxide in 95 % v/v ethanol and make up to 1 litre). The reagent must not be darker in colour than straw yellow.

Light petroleum (B.p. 40 to 60 °, bromine value 1), free from residue.

Procedure

Weigh about 5 g of fat to within 0·01 g into the flask.

Add 50 ml of approximately 2N ethanolic KOH solution. Attach the condenser. Boil gently for an hour.

Stop heating. Add 50 ml of distilled water through the top of the condenser and shake.

After cooling, transfer to a separating funnel and rinse the flask several times using 50 ml of light petroleum in all.

Shake vigorously for a minute.

Let it stand until there is complete separation of the two phases, and draw off the soap solution into a second separating funnel. If an emulsion should form, break it by adding small quantities of ethanol or concentrated potassium hydroxide solution.

Extract the soap solution twice more, using 50 ml light petroleum each time.

Combine the three ethereal extracts in one separating funnel, wash three times with 50 ml portions of 50 % (v/v) ethanol.

Pour off the petroleum extract quantitatively, if necessary in instalments through the top of the funnel into a tared 250 ml flask. Rinse the funnel with small quantities of light petroleum.

Remove the solvent by careful heating under vacuum and dry the residue under vacuum at a temperature not exceeding 50 °C in order to avoid undesirable oxidative changes.

B. Separation of the sterol fraction by means of thin-layer chromatography

Into the development tank pour some of the 85 : 15 (v/v) mixture of n-heptane and acetone or 95 : 5 (v/v) mixture of benzene and acetone to a depth of about 1 cm ; close with the lid and allow to stand for at least three hours so that liquid and vapour can reach equilibrium. It is also recommended that strips of filter paper dipping into the eluant be attached to the inside surface of the tank. The advantage this precaution offers is that it reduces by about a third the time taken for the front of the liquid to migrate and causes the compounds to elute more uniformly.

Meanwhile, prepare a 5 % solution in chloroform of the unsaponifiable matter extracted with petroleum ether. Take about 0·3 ml of this solution and deposit it, using a 0·1 ml microsyringe, in a continuous and uniform stripe on the chromatography plate at about 1·5 cm from the bottom edge in such a way as to give as thin a base line as possible. In accordance with standard practice, deposit a few µl of the reference solution containing cholesterol at one end of the plate in order to ascertain the R_f value of the sterol fraction.

Place the plate in the development tank prepared as described above. The room temperature must be about 20 °C. Close the tank with the lid and develop until the solvent front has reached a level about 1 cm from the top edge of the plate. Withdraw the plate from the tank and allow the solvent to evaporate in a stream of warm nitrogen.

Develop by spraying the plate uniformly and carefully with the alcoholic 2',7'-dichlorofluorescein sodium salt solution. By examination of the plate under ultraviolet light, the position of the sterols is determined by means of alignment on the spot of cholesterol derived from the reference solution. Collect the sterol band by scraping it away with a metal spatula. Place the separated silica gel in a 50 ml beaker with 15 ml of hot chloroform, shake and transfer the whole of the silica gel to a porous filter and filter.

Wash the filter three times, each time with a 15 ml portion of hot chloroform, collecting the filtrage in a 100 ml round-bottomed flask.

Evaporate the chloroform solution down to 4 to 5 ml and pour it into a previously tared centrifuge tube fitted with a ground stopper. Bring to dryness by evaporating the solvent off with gentle heating in a stream of nitrogen and weigh the resultant sterol fraction.

C. Gas chromatography analysis of the sterols

1. *Preparation of the trimethylsilylethers (TMS)*

Into the centrifuge tube add for each mg of sterol 0·02 ml of silanization reagent composed of a 9 : 3 : 1 (v/v/v) mixture of pryridine, hexamethyldisilazine and trimethylchlorosilane, being careful to exclude every trace of moisture. Place the tube in a dessicator for about 30 minutes, insert the stopper and centrifuge for a few minutes. Sample the remaining solution for subsequent analysis.

2. *Conditions for a gas chromatography analysis*

Column temperature : 220 to 250 °C.

If heated separately, the injection system must be maintained at a temperature 20 to 40 °C above the column temperature. Nitrogen flowrate : 30 to 60 ml/min. Disconnect the detector and equilibrate any new column under these conditions for 16 to 24 hours. Reconnect the detector, light the flame and adjust the hydrogen, oxygen or air flowrates to give a suitable flame height and sensitivity. Switch on the recording apparatus and see that the paper unwinds at the right speed ; adjust the zero and sensitivity control. When the base line is stable, the apparatus is ready for use.

3. *Sensitivity test*

Take 5 ml of sensitivity test solution, evaporate off the solvent and treat it as shown in Section 1 ; inject 0·1 to 0·2 µl of the TMS solution so prepared. The cholesterol peak must appear alone on the chromatogram.

Adjust the sensitivity control so as to use approximately the full scale of the recorder.

4. *Peak resolution test*

Take 5 ml of so prepared solution (TMS). Inject 0·1 to 2 ml of resolution-test. Evaporate off the solvent and treat as described in Section 1.

Inject 0·1 to 0·2 µl of the TMS solution so prepared.

The cholesterol, brassiscasterol, campesterol and β-sitosterol peaks will appear on the chromatogram. Measure the retention distances (distances from the point of injection to the points of maximum peak height), d_{CH} in respect of cholesterol, d_B in respect of brassiscasterol, d_C in respect of campesterol and d_S in respect of β-sitosterol, and the widths of the peaks at their base (retention lengths between the points at which the tangents to the points of inflection located on the front side and rear side of the peak intersect the base line), ω_{CH} in respect of cholesterol and ω_B in respect of brassicasterol. Peak resolution, expressed by the formula:

$$PR = 2\frac{(d_B - d_{CH})}{\omega_B + \omega_{CH}}$$

must equal at least 1.

Calculate the relative retention times (cholesterol = 1·00) for brassicasterol, campesterol and β-sitosterol.

5. *Reference test*

Take 5 ml of reference test solution, evaporate off the solvent, treat as described in Section 1 and inject 0·1 to 0·2 µl of the TMS solution so prepared. The campesterol, stigmasterol, β-sitosterol and Δ7-stigmastenol peaks will appear on the chromatogram.

Measure the peak retention distances, d_C in respect of campesterol, d_{ST} in respect of stigmasterol, d_S in respect of β-sitosterol and $d_{ST\text{-}7}$ in respect of Δ7-stigmastenol.

Calculate the relative retention times which are approximately:

cholesterol	1·0
brassicasterol	1·1
campesterol	1·3
stigmasterol	1·4
β-sitosterol	1·6 (1)
Δ7-stigmastenol	1·8 (2)

6. *Analysis*

Inject 0·1 to 0·2 µl of the sterol TMS solution to be analyzed and record the chromatogram.

D. Reporting of results

In interpreting the composition of the sterol fraction analyzed, ignore any peaks having different retention times from those determined experimentally for the six sterols mentioned above.

The percentage β-sitosterol content is given by the formula:

$$\frac{\text{Area of the β-sitosterol peak}}{\text{Sum of the areas of the six sterol peaks}} \times 100$$

The β-sitosterol content must not be less than 93 % of the total sterol percentage composition.

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(1) If other sterols, e.g., Δ5-avenasterol, have the same retention volume under these conditions as β-sitosterol, they shall be counted as though they were β-sitosterol.

(2) If other sterols have the same retention volume under these conditions as Δ7-stigmastenol; they shall be counted as though they were Δ7-stigmastenol.

ANNEX IX

OIL CONTENT OF OLIVE RESIDUE

Apparatus

— suitable extraction apparatus fitted with a 200 to 250 ml round-bottomed flask,
— electrically heated bath (e.g., sand bath, water bath) or hotplate,
— analytical balance,
— oven regulated to a maximum of 80 °C,
— electrically heated oven fitted with a thermostatic device regulated to 103 ± 2 °C and one that can be swept with a stream of air or operated at reduced pressure,
— mechanical mill, easy to clean, and one that allows the olive residues to be ground without a rise in their temperature or any appreciable alteration in their content of moisture, volatile matter or substances extractable with hexane,
— extraction thimble and cotton wool or filter paper from which substances extractable with hexane have already been removed,
— dessicator,
— sieve with 1 mm diameter apertures,
— small particles of previously dried pumice stone.

Reagent

Normal hexane, technical grade, which must leave a residue of less than 0·002 g per 100 ml, on complete evaporation.

PROCEDURE

Preparation of the test sample

If necessary, use the mechanical mill, which has previously been properly cleaned, to grind the laboratory sample in order to reduce it to particles that can pass completely through the sieve.

Use about one twentieth of the sample to complete the process of cleaning the mill, discard the ground material, grind the remainder and collect, mix carefully and analyze without delay.

Test portion

As soon as the grinding operation has been completed, weigh out about 10 g of the sample to the nearest 0·01 g for testing.

Preparation of the extraction thimble

Place the test portion in the thimble and plug with cotton wool. If a filter paper is used, envelope the test portion in it.

Preliminary drying

If the olive residues are very moist (i.e., moisture and volatile matter content more than 10 %), carry out preliminary drying by placing the loaded thimble (or filter paper) in the oven heated for an appropriate time at not more than 80 °C in order to reduce the moisture and volatile matter content to less than 10 %.

Preparation of the round-bottomed flask

Weigh to the nearest 1 mg the flask containing one or two particles of pumice stone, previously dried in the stove at 103 ± 2 °C and then cooled in a dessicator for not less than one hour.

Initial extraction

Into the extraction apparatus insert the thimble (or filter paper) containing the test portion. Pour into the flask the requisite quantity of hexane. Fit the flask to the extraction apparatus and place the whole on the electrically heated bath. Adjust the rate of heating in such a way that the reflux rate is not less than three drops per second (moderate, not violent boiling). After four hours extraction, allow to cool. Remove the thimble from the extraction apparatus and place it in a stream of air in order to drive off most of the impregnating solvent.

Second extraction

Tip the contents of the thimble into the micro-grinder and grind as finely as possible. Return the ground mixture to the thimble without loss and place it back in the extraction apparatus.

Continue the extraction for a further two hours, using the same round-bottomed flask containing the initial extract.

The resultant solution in the extraction flask must be clear. If not, filter it through a filter paper and wash the original flask and the filter paper several times with hexane. Collect the filtrate and the washing solvent in a second round-bottomed flask which has been dried and tared to the nearest 1 mg.

Removal of solvent and weighing of extract

Remove the greater part of the solvent by distillation on an electrically heated bath. Remove the last traces of solvent by heating the flask in the oven at 103 ± 2 °C for 20 minutes. Assist the elimination process either by blowing in air, or preferably an inert gas, at intervals or by using reduced pressure.

Leave the flask in a dessicator to cool for at least one hour and weigh to the nearest 1 mg.

Heat again for 10 minutes under the same conditions, cool in a dessicator and reweigh.

The difference between the two weighings shall not exceed 10 mg. If it does, heat again for periods of 10 minutes followed by cooling and weighing until the weight difference is 10 mg or less. Note the last weight of the flask.

Carry out duplicate determinations on the test sample.

EXPRESSION OF RESULTS

Method of calculation and formula

(a) The extract expressed as a percentage by mass of the product as received is equal to :

$$S = m_1 \times \frac{100}{m_0}$$

where : S is the percentage by mass of extract of the product as received,

m_0 is the mass, in grams, of the test portion,

m_1 is the mass, in grams, of the extract after drying.

Take as the result the arithmetic mean of the duplicate determinations, providing the repeatability conditions are satisfied.

Express the result to the first decimal place.

(b) The extract is expressed on a dry matter basis by using the formula :

$$S \times \frac{100}{100 - U} = \text{oil percentage of extract on a dry basis}$$

where : S is the percentage of extract by means of the product as received (see (a)),

U is its moisture and volatile matter content.

Repeatability

The difference between the duplicate determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0·2 g of hexane extract per 100 g of sample.

If this condition is not satisfied, repeat the analysis on two other test portions. If in this case too the difference exceeds 0·2 g, take as the result the arithmetic mean of the four determinations.