COMMISSION DIRECTIVE

of 25 July 1980

relating to the Community method of analysis for determining the erucic acid content in oils and fats intended to be used as such for human consumption and foodstuffs containing added oils or fats

(80/891/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 76/621/EEC of 20 July 1976 relating to the fixing of the maximum level of erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils or fats (1), and in particular Article 3 thereof,

Whereas Article 2 of Directive 76/621/EEC provides that, as from 1 July 1979, the erucic acid content of the products referred to in Article 1 of that Directive, calculated on the total level of fatty acids in the fat component, may not be greater than 5 % ;

Whereas Article 3 of Directive 76/621/EEC provides that the erucic acid content shall be determined by a Community method of analysis ;

Whereas Regulation (EEC) No 1470/68 of 23 September 1968 on the drawing and reduction of samples and the determination of the oil content, impurities and moisture in oil seeds (2), lays down in Annex VI, as introduced by Regulation (EEC) No 72/77 (3), a method of analysis determining the erucic acid content of colza and rape seeds ; whereas this method should be used as a screening method ;

Whereas it is not possible, when the constituent fatty acids of oils and fats are analyzed by gas-liquid chromatography under normal conditions, to distinguish erucic acid from other isomers of docosenoic acid such as cetoleic acid ;

Whereas it is necessary to determine the level of erucic acid in oils and fats, as well as in foodstuffs to which oils or fats have been added, which may contain cetoleic acid and other isomers of docosenoic acid ;

Whereas the level of erucic acid need not be determined in oils and fats and in foodstuffs to which oils or fats have been added when, after using the screening analysis methods, they have been found not to contain more than 5 % of total docosenoic acids or of cis-docosenoic acids ;

Whereas, pending the introduction of an improved method of analysis for the determination of erucic acid, this method of analysis is considered to be the most suitable at present ;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Foodstuffs,

HAS ADOPTED THIS DIRECTIVE :

Article 1

Member States shall require that the analysis necessary for the determination of the erucic acid content of the products referred to in Article 1 of Directive 76/621/EEC be carried out as laid down in Article 2.

Article 2

1. For screening purposes either of the following shall be determined :

(a) the total docosenoic acid content of products referred to in Article 1 using the method set out in Annex VI to Regulation (EEC) No 1470/68 ; or

(b) the total cis-docosenoic acid content of products referred to in Article 1, by the method set out in Annex VI to Regulation (EEC) No 1470/68 using gas-liquid chromatography in conditions whereby the cis- and trans-isomers of docosenoic acids are separated ; stationary phases suitable for this purpose are, for example, the cyanopropylpolysiloxanes or liquid crystals.

2. If the total content of either :

(a) docosenoic acids, determined according to paragraph 1 (a), or

(b) cis-docosenoic acids, determined according to paragraph 1 (b),

(1) OJ No L 202, 28. 7. 1976, p. 35.
(2) OJ No L 239, 28. 9. 1968, p. 2.
(3) OJ No L 12, 15. 1. 1977, p. 11.

of the products referred to in Article 1, calculated on their total fatty acid content in the fat component, does not exceed 5 %, no further determination shall be required. Otherwise, the erucic acid content shall be determined by the method set out in the Annex hereto.

Article 3

Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive not later than 1 February 1982. They shall forthwith inform the Commission thereof.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 25 July 1980.

For the Commission

Étienne DAVIGNON

Member of the Commission

ANNEX

THE DETERMINATION OF THE ERUCIC ACID CONTENT IN OILS AND FATS INTENDED TO BE USED AS SUCH FOR HUMAN CONSUMPTION AND IN THE FAT OR OIL COMPONENT OF FOODSTUFFS TO WHICH OILS OR FATS HAVE BEEN ADDED

I. INTRODUCTION

1. SAMPLE PREPARATION

1.1. General

The mass of the sample presented to the laboratory for analysis shall normally be 50 g unless a larger quantity is required.

1.2. Preparation of the sample for analysis in the laboratory

The sample must be homogenized before it is analyzed.

1.3. Containers

A sample so prepared shall be stored in an air-tight and moisture-tight container.

2. REAGENTS

2.1. Water

2.1.1. Where water is required as a solvent, diluent or for washing, distilled water or demineralized water of at least equivalent purity shall be used.

2.1.2. Where 'solution' or 'dilution' is mentioned without any other reagent being specified, an aqueous solution or dilution is meant.

2.2. Chemicals

All chemicals used, shall be of recognized analytical quality except where otherwise specified.

3. APPARATUS

3.1. List of apparatus

This list contains only those items with a specialized use and with a specification.

3.2. Analytical balance

'Analytical balance' means a balance with a sensitivity of 0·1 mg or better.

4. EXPRESSION OF THE RESULTS

4.1. Results

The result referred to in the official analysis report shall be the mean value obtained from not less than two determinations, the repeatability of which is satisfactory.

4.2. Calculation of the percentage

Unless otherwise stated the results shall be expressed as percentages (m/m) of the total fatty acids in the sample as received by the laboratory.

4.3. Number of significant figures

The number of significant figures in the result so expressed shall be governed by the precision of the method.

II. DETERMINATION OF ERUCIC ACID

1. SCOPE AND FIELD OF APPLICATION

The method determines the erucic acid content of:

(i) oils and fats containing cetoleic acid (a particular cis-isomer of docosenoic acid which occurs in fish oils), and

(ii) hydrogenated oils and fats containing trans and cis-isomers of docosenoic acid.

2. DEFINITION

Erucic acid content: the content of erucic acid as determined by the method specified.

3. PRINCIPLE

The methyl esters of the component fatty acids of the oil or fat are separated by low temperature argentation thin-layer chromatography and quantitatively determinated by gas-liquid chromatography.

4. REAGENTS

4.1. Diethyl ether peroxide-free freshly distilled.

4.2. n-hexane.

4.3. Silica gel G, for thin-layer chromatography.

4.4. Silica gel, for column chromatography.

4.5. Silver nitrate solution, 200 g/litre. Dissolve 24 g silver nitrate in water and make up to 120 ml with water.

4.6. Methyl erucate solution 5 mg/ml. Dissolve 50 mg methyl erucate in a few ml of n-hexane and dilute to 10 ml with n-hexane.

4.7. Methyl tetracosanoate, internal standard solution, 0·25 mg/ml. Dissolve 25 mg methyl tetracosanoate in a few ml of n-hexane (as 4·6) and dilute to 100 ml with n-hexane.

4.8. Development solvent. Toluene : n-hexane 90 : 10 (v/v).

4.9. 2,7 Dichlorofluorescein solution 0·5 g/litre. Dissolve by warming and stirring 50 mg of 2,7 dichlorofluorescein in 100 ml of 50 % aqueous methanol.

5. APPARATUS

5.1. Apparatus for thin-layer chromatography to include, in particular:

5.1.1. Deep-freeze unit, capable of maintaining developing tank and contents at a temperature of minus 20 to minus 25 °C.

5.1.2. Glass plates, 200 × 200 mm.

5.1.3. Ultra-violet lamp.

5.1.4. Glass columns, length about 200 mm, internal diameter about 10 mm with filter of glass wool or sintered glass. Alternatively, small funnels with sintered glass filters.

5.1.5. Applicator, for depositing solutions in the form of a narrow band or streak on TLC plates.

5.2. Gas-liquid chromatograph, together with an electronic integrator, as described in Section III of Annex VI to Commission Regulation (EEC) No 72/77.

6. PROCEDURE

6.1. **Preparation of fatty-acid methyl esters**

Take about 400 mg of the oil or fat component of the sample for analysis and prepare a solution containing about 20 to 50 mg/ml of the fatty acid methyl esters in n-hexane by the method described in Section II.3 of Annex VI to Commission Regulation (EEC) No 72/77.

6.2. **Thin-layer chromatography**

6.2.1. *Preparation of plates*

Place 60 g silica gel (4.3) in a 500 ml round-bottomed flask, add 120 ml of silver nitrate solution (4.5) and shake for one minute to obtain a fully homogeneous slurry. Spread the slurry in the usual manner over the plates ; the thickness of the layer should be approximately 0·5 mm. This quantity of slurry is sufficient for the preparation of five 200 × 200 mm plates.

Allow the plates to partially air-dry (preferably by leaving them in the dark for about 30 minutes). Fully dry and activate the plates by placing them in an oven, maintained at 100 °C, for two hours 30 minutes. Use the plates as soon as possible after activation or carefully store in a dark cabinet and then reactivate before use. (Note : activation at 110 °C for one hour may be found satisfactory provided the plates are not darkened as a result). Score lines through the coating 10 mm from the sides and the top of each plate before use to reduce edge effects during the development.

6.2.2. *Application of methyl esters*

Using the applicator (5.1.5) deposit 50 µl of the solution of methyl esters (6.1) prepared from the sample in a narrow streak about 50 mm long, at least 40 mm from the side of the plate and 10 mm from the bottom. Apply in a similar way 100 µl of a solution containing equal volumes of the prepared solution of methyl esters (6.1) and the methyl erucate solution (4.6). Take particular care during the application of solutions because of the fragile nature of the coating. (Note : if desired, 50 µl of the methyl erucate solution (4.6) may be applied to the plate to assist in identifying the methyl erucate band after development : see figure). After the application of the methyl esters stand the bottom edge of the plate in diethyl ether until the ether ascends to about 5 mm above the area of sample application. This concentrates the methyl esters in a narrow band.

6.2.3. *Development of the plates*

Pour the development solvent (4.8) into the tank to a depth of about 5 mm and place the tank, complete with lid, in a deep freeze cabinet (5.1.1) held at minus 25 °C, or as near to this temperature as possible. (In some cases it may be advantageous to line the tank). After two hours, place the plate carefully in the tank and allow the solvent to ascend to about one half to two thirds of the height of the plate. Remove the plate and gently evaporate the solvent from it in a nitrogen stream. Replace the plate in the tank and allow the solvent to ascend to the top of the plate. Remove the plate and as previously dry in a nitrogen stream and then spray carefully with 2,7 dichlorofluorescein solution (4.9).

View the plate under ultra-violet light and locate the band containing methyl erucate in the sample by reference to the intensified band in the sample to which methyl erucate has been added (see figure).

6.2.4. *Separation of the methyl ester fractions*

Scrape off the methyl erucate band derived from the sample into a 50 ml beaker taking care to avoid losses. Similarly transfer the silica gel located above and below the methyl erucate band into another 50 ml beaker. This band will contain all the other fatty-acid methyl ester fractions. Add 1·0 ml of the methyl tetracosanoate standard solution (4.7) and 10 ml of diethyl ether (4.1) to each beaker. Stir, and transfer the contents of the beakers to separate columns or funnels (5.1.4) each containing about 1 g silica gel (4.4) ; elute the methyl esters using three or four 10 ml portions of diethyl ether. Collect the filtrates in small flasks. Evaporate each filtrate to a small volume using a gentle nitrogen stream and transfer the methyl esters to small pointed-bottom glass tubes. Remove all the solvent by evaporation with a nitrogen stream in such a way that the methyl esters concentrate at the bottom of the tubes. Dissolve the methyl esters in about 25 to 50 µl of n-hexane (4.2).

6.3. **Gas-liquid chromatography**

6.3.1. Carry out the procedure described in Section III of Annex VI to Commission Regulation 72/77/EEC and analyze 1 to 2 µl of the methyl ester solutions obtained from (i) the fraction containing methyl erucate, and (ii) the fractions containing the remainder of the methylated fatty acids.

6.3.2. Obtain from the electronic integrator the following peak areas :

(i) from the chromatogram of the fraction containing the methyl erucate :

(a) methyl erucate [E]

(b) internal standard [L_1]

(c) total methyl ester peak areas excluding the internal standard [EF]

(ii) from the chromatogram of the fractions containing the remainder of te fatty acid methyl esters

(a) total methyl ester peak areas excluding the internal standard [RF]

(b) internal standard [L_2]

7. EXPRESSION OF RESULTS

7.1. **Method of calculation and formula**

7.1.1. The erucic acid content of the sample, expressed in terms of its methyl ester as a percentage of the total fatty acid methyl esters prepared from the sample, is given by :

$$L_1 \frac{E}{\left(\frac{EF}{L_1} + \frac{RF}{L_2} \right)} \times 100$$

where

E, EF, RF, L_1 and L_2 are the peak areas referred to in 6.3.2, corrected as necessary by the use of calibration factors.

For practical purposes the value for methyl erucate given by the above formula is equivalent to the level of erucic acid expressed as a percentage of the total level of fatty acids in the sample.

7.1.2. If peak areas are obtained in percentages the values for EF and RF may be calculated as follows :

$$EF = 100 - L_1$$

$$RF = 100 - L_2$$

7.1.3. The method of calculation (7.1.1) assumes that the level of tetracosanoic acid in the sample is negligible. If significant amounts of this acid are shown to be present the value for tetracosanoic acid (L_2) obtained from the chromatogram of the fractions containing the remainder of the fatty acid methyl esters must be reduced to :

$$L_2 - T_2$$

where

$$T_2 = \frac{T_0 P_2}{P_0}$$

and

T_2 = peak area of methyl tetracosanoate derived from the sample and which forms part of the peak area attributed to the internal standard in the chromatogram of the remaining fraction of fatty acid methyl esters

P_2 = peak area of methyl palmitate obtained from the chromatogram of the remaining fraction

T_0 = peak area of methyl tetracosanoate obtained from the chromatogram of the methyl esters of the total fatty acids as determined by the analysis referred to in Article 2 of this Directive

P_0 = peak area of methyl palmitate obtained from the chromatogram of the methyl esters of the total fatty acids as as determined by the analysis referred to in Article 2 of this Directive.

7.1.4. *Derivation of formula*

The proportion of fatty acids in the fraction containing the methyl erucate, expressed as a percentage of the total fatty acids in the sample, is given by :

$$\frac{\frac{EF}{L_1}}{\frac{EF}{L_1} + \frac{RF}{L_2}} \times 100 \quad \text{or} \quad \frac{EF}{L_1\left(\frac{EF}{L_1} + \frac{RF}{L_2}\right)} \times 100$$

The proportion of erucic acid in the fraction containing the methyl erucate, is given by :

$$\frac{E}{EF}$$

Hence the erucic acid content of the sample, expressed as a percentage of the total fatty acids, is given by :

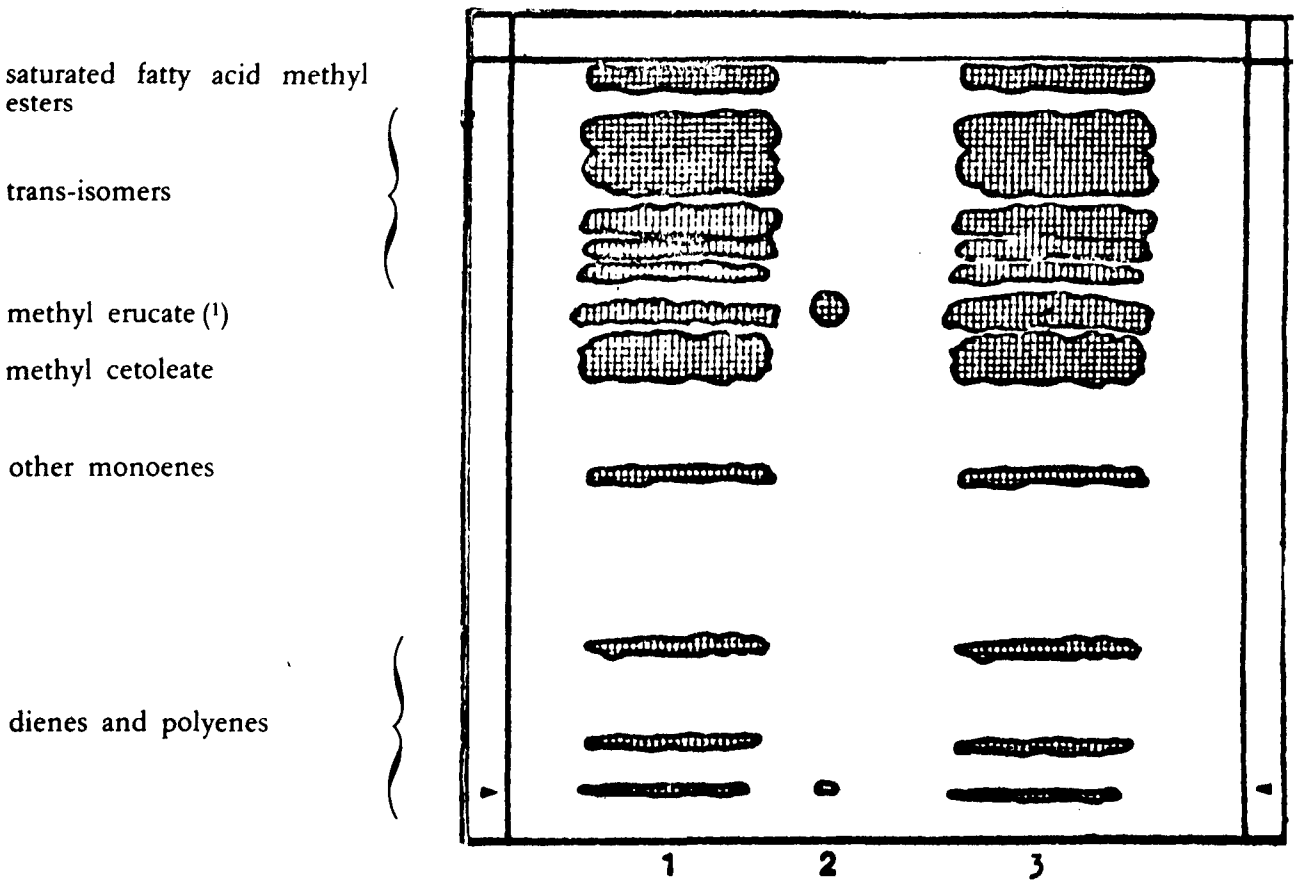
$$\frac{EF}{L_1\left(\frac{EF}{L_1} + \frac{RF}{L_2}\right)} \times \frac{E}{EF} \times 100 \quad \text{or} \quad \frac{E}{L_1\left(\frac{EF}{L_1} + \frac{RF}{L_2}\right)} \times 100$$

7.1.5. *Repeatability*

The difference between the values of two deteriminations when carried out simultaneously or in rapid succession on the same sample, by the same analyst under the same conditions, shall not exceed 10 % of the result or 0·5 g per 100 g of sample, taking the greater value.

FIGURE

Typical thin-layer chromatogram showing the separation of the methyl esters of erucic acid, cetoleic acid and trans-isomers of docosenoic acid



1 sample ;
2 methyl erucate ;
3 sample + methyl erucate

(1) The fraction designated methyl erucate will usually contain methyl esters of other monoenoic acids but should be free of methyl cetoleate.