BRITISH STANDARD

BS EN ISO
5508 : 1995
BS 684 :
Section 2.35 :
1990

Animal and vegetable fats and oils —

Analysis by gas chromotography of methyl esters of fatty acids

The European Standard EN ISO 5508 : 1995 has the status of a British Standard

BSI
STANDARDS

National foreword

This Section of BS 684 has been prepared under the direction of the Agriculture and Food Standards Policy Committee. It is identical with ISO 5508 : 1990 'Animal and vegetable fats and oils — Analysis by gas chromatography of methyl esters of fatty acids', published by the International Organization for Standardization (ISO), and in the preparation of which the United Kingdom played a full part. It is a revision of BS 684 : Section 2.35 : 1980 which is withdrawn and from which it differs in that a method using capillary columns is included.

In 1995 the European Committee for Standardization (CEN) accepted ISO 5508 : 1990 as European Standard EN ISO 5508 : 1995. As a consequence of implementing the European Standard this British Standard is renumbered as BS EN ISO 5508 and any reference to BS 684 : Section 2.35 should be read as a reference to BS EN ISO 5508.

Cross-reference

International standard	Corresponding British Standard
ISO 5509 : 1978	BS 684 Methods of analysis of fats and fatty oils Section 2.34 : 1980 Preparation of methyl esters of fatty acids (Identical)

Compliance with a British Standard does not of itself confer immunity from legal obligations.

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 5508

April 1995

ICS 67.200.10

Descriptors: agricultural products, fats, animal fats, vegetable fats, oils, animal oils, vegetable oils, fatty acids, methyl ester, gas phase chromotography

English version

Animal and vegetable fats and oils — Analysis by gas chromotography of methyl esters of fatty acids

(ISO 5508 : 1990)

Corps gras d'origines animale et végétale — Analyse par chromotographie en phase gazeuse des esters méthyliques d'acides gras (ISO 5508 : 1990)

Tierische und pflanzliche Fette und Öle — Gaschromatographische Untersuchung der Methylester von Fettsäuren (ISO 5508 : 1990)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Ref. No. EN ISO 5508 : 1995 E

Foreword

The text of the International Standard from ISO/TC 34, Agricultural food products, of the International Organization for Standardization (ISO) has been taken over as a European Standard by the Technical Committee CEN/TC 307, Oilseeds, vegetable and animal fats and oils and their by-products — Methods of sampling and analysis.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 1995, and conflicting national standards shall be withdrawn at the latest by October 1995.

According to the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

Contents

INTERNATIONAL STANDARD ISO 5508:1990(E)

Animal and vegetable fats and oils — Analysis by gas chromatography of methyl esters of fatty acids

1 Scope

This International Standard gives general guidance for the application of gas chromatography, using packed or capillary columns, to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters obtained in accordance with the method specified in ISO 5509.

The method is not applicable to polymerized fatty acids.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5509:1978, *Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids.*

3 Reagents

3.1 Carrier gas

Inert gas (nitrogen, helium, argon, hydrogen, etc.), thorougly dried and with an oxygen content of less than 10 mg/kg.

NOTE 1 Hydrogen, which is used as a carrier gas only with capillary columns, can double the speed of analysis but is hazardous. Safety devices are available.

3.2 Auxiliary gases

3.2.1 Hydrogen (purity ≥ 99,9 %), free from organic impurities.

3.2.2 Air or **oxygen**, free from organic impurities.

3.3 Reference standard

A mixture of methyl esters of pure fatty acids, or the methyl esters of a fat of known composition, preferably similar to that of the fatty matter to be analysed.

Care shall be taken to prevent the oxidation of polyunsaturated fatty acids.

4 Apparatus

The instructions given relate to the usual equipment used for gas chromatography, employing packed and/or capillary columns and a flame-ionization detector. Any apparatus giving the efficiency and resolution specified in 5.1.2 is suitable.

4.1 Gas chromatograph.

The gas chromatograph shall comprise the following elements.

4.1.1 Injection system.

Use an injection system either

a) with packed columns, having the least dead-space possible (in this case the injection system shall be capable of being heated to a temperature 20 °C to 50 °C higher than that of the column), or

b) with capillary columns, in which case the injection system shall be specially designed for use with such columns. It may be of the split type or it may be of the splitless on column injector type.

NOTE 2 In the absence of fatty acids with less than 16 carbon atoms, a moving needle injector may be used.

4.1.2 Oven.

The oven shall be capable of heating the column to a temperature of at least 260 °C and of maintaining the desired temperature to within 1 °C with a packed column and within 0,1 °C with a capillary column. The last requirement is particularly important when a fused silica tube is used.

The use of temperature-programmed heating is recommended in all cases, and in particular for fatty acids with less than 16 carbon atoms.

4.1.3 Packed column.

4.1.3.1 Column, constructed of a material inert to the substances to be analysed (i.e. glass or stainless steel) having the following dimensions.

a) Length: 1 m to 3 m. A relatively short column should be used when long-chain fatty acids (above C_{20}) are present. When analysing acids with 4 or 6 carbon atoms, it is recommended that a column 2 m in length is used.

b) Internal diameter: 2 mm to 4 mm.

NOTES

3 If polyunsaturated components with more than three double bonds are present, they may be decomposed in a stainless steel column.

4 A system with packed twin columns may be used.

4.1.3.2 Packing, comprising the following elements.

a) Support: Acid-washed and silanized diatomaceous earth, or other suitable inert support with a narrow range of grain size (25 µm range between the limits 125 µm to 200 µm), the average grain size being related to the internal diameter and length of the column.

b) Stationary phase: Polyester type of polar liquid (e.g. diethylene glycol polysuccinate, butanediol polysuccinate, ethyleneglycol polyadipate, etc.), cyanosilicones or any other liquid permitting the chromatographic separation required (see clause 5). The stationary phase should amount to 5 % (*m*/*m*) to 20 % (*m*/*m*) of the packing. A non-polar stationary phase can be used for certain separations.

4.1.3.3 Conditioning of the column.

With the column disconnected, if possible, from the detector, gradually heat the oven to 185 °C and pass a current of inert gas through the freshly prepared column at a rate of 20 ml/min to 60 ml/min for at least 16 h at this temperature, and for a further 2 h at 195 °C.

4.1.4 Capillary column.

4.1.4.1 Tube, made of a material inert to the substances to be analysed (usually glass or fused silica). The internal diameter shall be between 0,2 mm and 0,8 mm. The internal surface shall undergo an appropriate treatment (e.g. surface preparation, inactivation) before receiving the stationary phase coating. A length of 25 m is sufficient in most cases.

4.1.4.2 Stationary phase, usually of the type polyglycol [poly(ethylene glycol) 20 000], polyester (butanediol polysuccinate) or polar polysiloxane (cyanosilicones). Bonded (cross-linked) columns are suitable.

NOTE 5 There is a risk of polar polysiloxanes giving rise to difficulties in the identification and separation of linolenic acid and C_{20} acids.

The coatings shall be thin, i.e. 0,1 µm to 0,2 µm.

4.1.4.3 Assembly and conditioning of the column.

Observe the normal precautions for assembling capillary columns, i.e. arrangement of the column in the oven (support), choice and assembly of joints (leak tightness), positioning of the ends of the column in the injector and the detector (reduction of dead-spaces). Place the column under a flow of carrier gas [e.g. 0,3 bar (30 kPa) for a column of length 25 mm and internal diameter 0,3 mm].

Condition the column by temperature programming of the oven at 3 °C/min from ambient temperature to a temperature 10 °C below the decomposure limit of the stationary phase. Maintain the oven at this temperature for 1 h until stabilization of the baseline. Return it to 180 °C to work under isothermal conditions.

NOTE 6 Suitably pre-conditioned columns are available commercially.

4.1.5 Detector, preferably capable of being heated to a temperature above that of the column.

4.2 Syringe.

The syringe shall have a maximum capacity of 10 µl, and be graduated in 0,1 µl divisions.

4.3 Recorder.

If the recorder curve is to be used to calculate the composition of the mixture analysed, an electronic recorder of high precision, compatible with the apparatus used, is required. The recorder shall have the following characteristics:

a) rate of response, below 1,5 s, preferably 1 s (the rate of response is the time taken for the re-

cording pen to pass from 0 % to 90 % following the sudden introduction of a 100 % signal);

b) width of the paper, 20 cm minimum;

c) paper speed, adjustable to values between 0,4 cm/min and 2,5 cm/min.

4.4 **Integrator** or **calculator** (optional).

Rapid and accurate calculation can be performed with the help of an electronic integrator or calculator. This shall give a linear response with adequate sensitivity, and the correction for deviation of the base-line shall be satisfactory.

5 Procedure

The operations described in 5.1 to 5.3 relate to the use of a flame-ionization detector.

As an alternative a gas chromatograph employing a catharometer detector (working on the principle of thermal conductivity changes) may be used. The operating conditions are then modified as described in clause 7.

5.1 Test conditions

5.1.1 Selection of optimum operating conditions

5.1.1.1 Packed column

In the selection of the test conditions, the following variables should be taken into account:

a) the length and diameter of the column;

b) the nature and amount of the stationary phase;

c) the temperature of the column;

d) the carrier gas flow;

e) the resolution required;

f) the size of the test portion, selected in such a way that the assembly of the detector and electrometer gives a linear response;

g) the duration of analysis.

In general, the values given in table 1 and table 2 will lead to the desired results, i.e. at least 2 000 theoretical plates per metre of column length for methyl stearate and its elution within about 15 min.

Where the apparatus allows it, the injector should be at a temperature of about 200 °C and the detector at a temperature equal to or higher than that of the column.

As a rule, the ratio of the flow-rate of the hydrogen supplied to the flame-ionization detector to that of the carrier gas varies from 1:2 to 1:1 depending on the diameter of the column. The flow of oxygen is about 5 to 10 times that of the hydrogen.

Table 1

Internal diameter of column mm	Carrier gas flow ml/min
2	15 to 25
3	20 to 40
4	40 to 60

Table 2

Concentration of stationary phase % (*m*/*m*)	Column temperature °C
5	175
10	180
15	185
20	185

5.1.1.2 Capillary column

The properties of efficiency and permeability of capillary columns mean that the separation between constituents and the duration of the analysis are largely dependent on the flow-rate of the carrier gas in the column. It will therefore be necessary to optimize the operating conditions by acting on this parameter (or more simply on the headloss of the column), according to whether one wishes to improve the separations or to make a rapid analysis.

5.1.2 Determination of the number of theoretical plates (efficiency) and resolution

(See figure 1.)

Carry out the analysis of a mixture of methyl stearate and methyl oleate in about equivalent proportions (for example, methyl esters from cocoa butter).

Choose the temperature of the column and the carrier gas flow so that the maximum of the methyl stearate peak is recorded about 15 min after the solvent peak. Use a sufficient quantity of the mixture of methyl esters that the methyl stearate peak occupies about three-quarters of the full scale.

Calculate the number of theoretical plates, n (efficiency), using the formula

$$n = 16\left(\frac{d_{r(I)}}{\omega_{(I)}}\right)^2$$

and the resolution, R, using the formula

$$R = \frac{2\Delta}{\omega_{(I)} + \omega_{(II)}}$$

where

$d_{r(I)}$ is the retention distance, in millimetres, from the start of the chromatogram to the maximum of the peak for methyl stearate;

$\omega_{(I)}$ and $\omega_{(II)}$ are the widths, in millimetres, of the peaks for methyl stearate and methyl oleate respectively, measured between the points of intersection of the tangents at the points of inflexion of the curve with the base-line;

Δ is the distance, in millimetres, between the peak maxima for methyl stearate and methyl oleate.

The operating conditions to be selected are those which will afford at least 2 000 theoretical plates per metre of column length for methyl stearate and a resolution of at least 1,25.

5.2 Test portion

Using the syringe (4.2), take 0,1 µl to 2 µl of the solution of methyl esters prepared according to ISO 5509 and inject them into the column.

In the case of esters not in solution, prepare a solution of approximately 100 mg/ml in heptane of chromatographic quality, and inject 0,1 µl to 1 µl of this solution.

If the analysis is for constitutents present only in trace amounts, the size of the test portion may be increased (up to ten-fold).

5.3 Analysis

Generally, the operating conditions shall be those defined in 5.1.1.

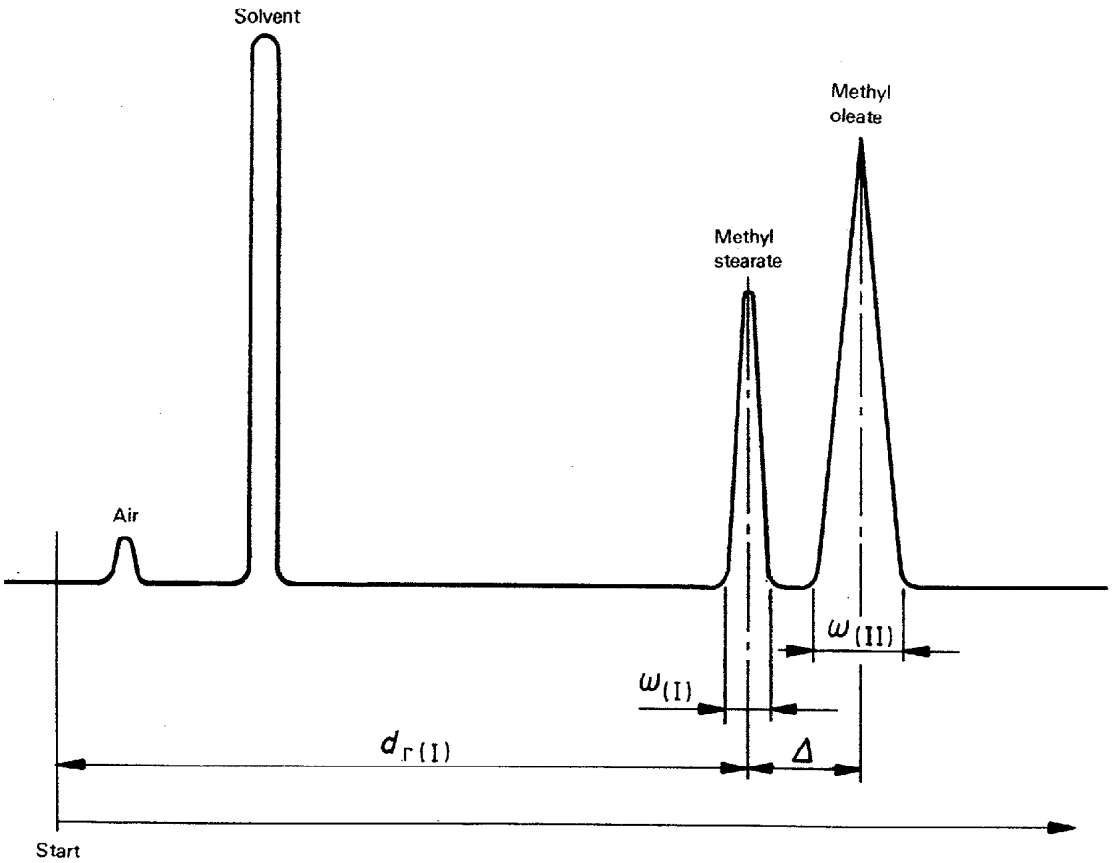

Figure 1 — Chromatogram for determining the number of theoretical plates (efficiency) and resolution

Nevertheless, it is possible to work with a lower column temperature when the determination of fatty acids with fewer than 12 carbon atoms is required, or at a higher temperature when determining fatty acids with more than 20 carbon atoms. On occasion, it is possible to employ temperature programming in both these cases. For example, if the sample contains the methyl esters of fatty acids with fewer than 12 carbon atoms, inject the sample at 100 °C (or at 50 °C to 60 °C if butyric acid is present) and immediately raise the temperature at a rate of 4 °C/min to 8 °C/min to the optimum. In certain cases, the two procedures can be combined.

After the programmed heating, continue the elution at a constant temperature until all the components have been eluted. If the instrument does not have programmed heating, use it at two fixed temperatures between 100 °C and 195 °C.

If necessary, it is recommended that an analysis be carried out on two fixed phases with different polarities to verify the absence of masked peaks, for example for fish oils or in the case of the simultaneous presence of $C_{18:3}$ and $C_{20:0}$, or $C_{18:3}$ and $C_{18:2}$ conjugated.

5.4 Preparation of the reference chromatogram and reference graphs

Analyse the reference standard mixture (3.3), using the same operating conditions as those employed for the sample, and measure the retention times or retention distances for the constituent fatty acids. Construct on semi-logarithmic paper, for any degree of unsaturation, the graphs showing the logarithm of retention time or distance as a function of the number of carbon atoms. In isothermal conditions, the graphs for straight-chain acids of the same degree of unsaturation should be straight lines. These lines should be approximately parallel.

It is necessary to avoid conditions such that "masked peaks" exist, i.e. where the resolution is insufficient to separate two constituents.

6 Expression of results

6.1 Qualitative analysis

Identify the methyl ester peaks for the sample from the graphs prepared in 5.4, if necessary by interpolation.

6.2 Quantitative analysis

6.2.1 Determination of the composition

Apart from exceptional cases, use the internal normalization method, i.e. assume that the whole of the components of the sample are represented on the chromatogram, so that the total of the areas under the peaks represents 100 % of the constituents (total elution).

If the equipment includes an integrator, use the figures obtained therefrom. If not, determine the area under each peak by multiplying the height of the peak by its width at mid-height, and where necessary take into account the various attenuations used during the recording.

6.2.2 Method of calculation

6.2.2.1 General case

Calculate the content of a given component i, expressed as a percentage by mass of methyl esters, by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks, using the following formula:

$$\frac{A_i}{\sum A} \times 100$$

where

A_i is the area under the peak corresponding to component i;

$\sum A$ is the sum of the areas under all the peaks.

Give the result to one decimal place.

NOTE 7 In this general case, the result of the calculation based on relative areas is considered to represent a percentage by mass. For the cases in which this assumption is not allowed, see 6.2.2.2.

6.2.2.2 Use of correction factors

In certain cases, for example in the presence of fatty acids with fewer than 8 carbon atoms or of acids with secondary groups, when using thermal conductivity detectors or where the highest degree of accuracy is particularly required, correction factors should be used to convert the percentages of peak areas into mass percentages of the components.

Determine the correction factors with the help of a chromatogram derived from the analysis of a reference mixture of methyl esters of known composition, carried out under operating conditions identical with those used for the sample.

For this reference mixture, the percentage by mass of component i is given by the formula

$$\frac{m_i}{\sum m} \times 100$$

where

m_i is the mass of component i in the reference mixture;

$\sum m$ is the total of the masses of the various components of the reference mixture.

From the chromatogram of the reference mixture (5.4), calculate the percentage (area/area) for component i as follows:

$$\frac{A_i}{\sum A} \times 100$$

where

A_i is the area under the peak corresponding to component i;

$\sum A$ is the sum of the areas under all the peaks.

The correction factor is then calculated as

$$K_i = \frac{m_i \times \sum A}{A_i \times \sum m}$$

Commonly, the correction factors are expressed relative to K_{C16}, so that the relative factors become

$$K'_i = \frac{K_i}{K_{C16}}$$

For the sample, the content of each component i, expressed as a percentage by mass of methyl esters, is

$$\frac{K'_i \times A_i}{\sum (K'_i \times A_i)} \times 100$$

Give the results to one decimal place.

6.2.2.3 Use of an internal standard

In certain analyses (for example where not all of the fatty acids are quantified, such as when acids with 4 and 6 carbons are present alongside acids with 16 and 18 carbons, or when it is necessary to determine the absolute amount of a fatty acid in a sample) it is necessary to use an internal standard. Fatty acids with 5, 15 or 17 carbons are frequently used. The correction factor (if any) for the internal standard should be determined.

The percentage by mass of component i, expressed as methyl esters, is then given by the formula

$$\frac{m_s \times K'_i \times A_i}{m \times K'_s \times A_s} \times 100$$

where

A_i is the area under the peak corresponding to component i;

A_s is the area under the peak corresponding to the internal standard;

K'_i is the correction factor for component i (relative to K_{C16});

K'_s is the correction factor for the internal standard (relative to K_{C16});

m is the mass, in milligrams, of the test portion;

m_s is the mass, in milligrams, of the internal standard.

Give the results to one decimal place.

6.2.3 Precision

The values for repeatability and reproducibility given below cover the preparation of the methyl esters according to ISO 5509, together with the gas chromatographic analysis described in this International Standard. The figures have been accepted historically.

6.2.3.1 Repeatability

The difference between the values of two determinations, carried out in rapid succession by the same operator using the same apparatus on the same test sample and for constituents present in excess of 5 % (m/m), shall not exceed 3 % (relative) of the determined value, with a maximum of 1 % (m/m). For constituents present in smaller quantities, the difference shall not exceed a value of 0,2 % (m/m).

6.2.3.2 Reproducibility

The difference between the values of the final result obtained by two different laboratories, using this method for the analysis of the same laboratory sample for constituents present in excess of 5 % (m/m), shall not exceed 10 % (relative) of the determined value, with a maximum of 3 % (m/m). For constituents present in smaller quantities, this difference shall not exceed a value of 0,5 % (m/m).

7 Special case — Use of a catharometer detector (working on the principle of thermal conductivity changes)

A gas chromatograph employing a detector working on the principle of thermal conductivity changes (a catharometer) may also be used for the determination of the qualitative and quantitative composition of a mixture of fatty acid methyl esters. If it is

used, the conditions specified in clause 4 and clause 5 should be modified as shown in table 3.

For quantitative analysis, use the correction factors defined in 6.2.2.2.

Table 3

Variable	Value/condition
Column	Length: 2 m to 4 m Internal diameter: 4 mm
Support	Grain size between 160 µm and 200 µm
Concentration of stationary phase	15 % (*m/m*) to 25 % (*m/m*)
Carrier gas	Helium or, failing this, hydrogen, with as low an oxygen content as possible
Auxiliary gases	None
Injector temperature	From 40 °C to 60 °C above that of the column
Column temperature	180 °C to 200 °C
Flow of carrier gas	Usually between 60 ml/min and 80 ml/min
Size of test portion injected	Usually between 0,5 µl and 2 µl

8 Test report

The test report shall specify the methods used for the preparation of the methyl esters and for the gas chromatographic analysis, and the results obtained. It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all information necessary for the complete identification of the sample.

Publication(s) referred to

See national foreword.

BS 684 : Section 2.35 : 1990
ISO 5508 : 1990

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the Agriculture and Food Standards Policy Committee (AFC/-) to Technical Committee AFC/18, upon which the following bodies were represented:

AFRC Institute of Food Research
Association of Public Analysts
British Food Manufacturing Industries Research Association
Co-operative Union
Department of Trade and Industry (Laboratory of the Government Chemist)
FOSFA International
International Association of Seed Crushers
Margarine and Shortening Manufacturers' Association
Ministry of Agriculture, Fisheries and Food
Overseas Development Natural Resources Institute
Royal Society of Chemistry
Seed Crushers' and Oil Processors' Association
Soap and Detergent Industry Association
Tropical Growers' Association
United Kingdom Renderers' Association Ltd.

This British Standard, having been prepared under the direction of the Agriculture and Food Standards Policy Committee, was published under the authority of the Board of BSI and comes into effect on 31 December 1990

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Amendments issued since publication

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BSI, 2 Park Street, London W1A 2BS **BSI, Linford Wood, Milton Keynes MK14 6LE**

AMD 8672

Amendment No. 1
published and effective from 15 July 1995
to BS 684 : Section 2.35 : 1990

Methods of analysis of fats and fatty oils
Part 2. Other methods
Section 2.35 Analysis by gas chromotography of methyl esters of fatty acids

NOTE. The European Committee for Standardization (CEN) has accepted ISO 5508 : 1990 as a European Standard designated as EN ISO 5508 : 1995. This amendment implements EN ISO 5508 : 1995 as a British Standard in the BS EN ISO series.

Implementation of European Standard

Front cover

Delete the outside cover page and substitute the attached.

AMD 8762/July 1995

National foreword

At the end of paragraph 1, insert the following new paragraph.

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AMD 8672/July 1995

New EN title page and foreword

Immediately after the contents list insert the attached new EN title page and foreword page.

AMD 8672/July 1995

9507—0.5k—PP

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Foreword

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