SLOVENSKI STANDARD
SIST EN 14105:2003

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Derivati maġļ ob in olj - Metil estri maġļ obnih kislin (FAME) - Doloļ evanje prostih in celotnih glicerolov in mono-, di-, trigliceridov (Referenļ na metoda)

Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride contents (Reference method)

Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem und Gesamtglycerin und Mono-, Di- und Triglyceriden (Referenzmethode)

Produits dérivés des corps gras - Esters méthyliques d'acides gras (EMAG) - Détermination de la teneur en glycérols libre et total et en mono-, di- et triglycérides - Méthode de référence

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67.200.10	Rastlinske in ġivalske maġļ obe in olja	Animal and vegetable fats and oils

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Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem und Gesamtglycerin und Mono-, Di- und Triglyceriden (Referenzmethode)

This European Standard was approved by CEN on 2 January 2003.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 14105:2003) has been prepared by Technical Committee CEN /TC 307 "Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis", the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by October 2003, and conflicting national standards shall be withdrawn at the latest by October 2003.

This document has been prepared under Mandate M/245 on Fatty Acid Methylester (FAME) given to CEN by the European Commission and the European Free Trade Association.

Annexes A to D are informative.

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

1 Scope

This European Standard specifies a method to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME) intended for addition to mineral oils. The total glycerol content is then calculated from the results obtained.

This method is suitable for FAME from rapeseed, sunflower, soybean oils but is not suitable for FAME produced from or containing coconut and palm kernel oils because of overlapping of peaks.

WARNING — The use of this method may involve hazardous equipment, materials and operations. This method does not purport to address to all of the safety problems associated with its use, but it is the responsibility of the user to search and establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2 Principle

Transformation of the glycerol and of the mono- and diglycerides into more volatile silylated derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

Analysis of the silylated derivatives by gas chromatography on a short capillary column with thin film thickness, with an on-column injector or equivalent device, and flame ionization detection.

After a calibration procedure, the quantification is carried out in the presence of two internal standards:

— 1,2,4-butanetriol intended for the determination of the free glycerol;

— 1,2,3-tricaproylglycerol (tricaprin) intended for the determination of the glycerides (mono-, di- and tri-).

3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

3.1 N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA).

3.2 Pyridine, stored on molecular sieve.

3.3 n-Heptane.

3.4 1,2,4-Butanetriol, (internal standard No.1).

3.5 1,2,3-Tricaproylglycerol (tricaprin), (internal standard No.2).

3.6 Reference substances : glycerol, 1-monooleoylglycerol (monoolein), 1,3-dioleoylglycerol (diolein), 1,2,3-trioleoylglycerol (triolein), pure - GLC standard grade.

3.7 Internal standard No. 1 stock solution, 1 mg/ml.

Accurately weigh approximately 50 mg (to the nearest 0,1 mg) of 1,2,4-butanetriol (3.4) in a 50 ml volumetric flask (4.4) and make up to the mark with pyridine (3.2).

3.8 Internal standard No. 2 stock solution, 8 mg/ml.

Accurately weigh approximatively 80 mg (to the nearest 0,1 mg) of 1,2,3-tricaproylglycerol (3.5) in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

3.9 Glycerol stock solution, 0,5 mg/ml.

Accurately weigh approximately 50 mg (to the nearest 0,1 mg) of glycerol (3.6) in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2). Using a pipette (4.7) transfer 1 ml of this solution into a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

3.10 Glyceride stock solution, 5 mg/ml.

For each reference glyceride, mono-, di- and triolein (3.6), accurately weigh approximately 50 mg (to the nearest 0,1 mg) in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

3.11 Monoglycerides[1)], commercial mixture.

Made up of monopalmitoylglycerol (monopalmitin), monostearoylglycerol (monostearin) and of monooleoylglycerol (monoolein), present in quantities having an identical mass.

Prepare a stock solution of this mixture by weighing approximately 100 mg in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

3.12 Calibration solutions

Prepare daily four calibration solutions by transferring into a series of vials (4.6) the volumes of stock solutions of reference substances (3.9 and 3.10) and of internal standards (3.7 and 3.8) given in Table 1, using microsyringes (4.8 and 4.9). The choice of the appropriate syringe shall be done according to Table 1. Do not use syringe at maximum capacity, but dispense the half volume twice (i. e.: in case of 100 µl dosing using a 100 µl syringe, load 50 µl twice). Be sure that needle and body of syringe are free from air bubbles, and measure volumes only by difference (i. e.: when dispensing 80 µl, fill syringe up to 100 µl and supply solution up to the 20 µl mark).

NOTE The silylated standard solutions are only stable one day.

Table 1 — Preparation of calibration solutions

Calibration solution	1	2	3	4	Syringe, µl
µl of glycerol solution	10	40	70	100	100
µl of monoolein solution	50	120	190	250	500
µl of diolein solution	10	40	70	100	100
µl of triolein solution	10	30	60	80	100
µl of internal std sol. No. 1	80	80	80	80	100
µl of internal std sol. No. 2	100	100	100	100	500

3.13 Carrier gas, hydrogen or helium.

3.14 Auxiliary gases:

— air;

— hydrogen.

1) Products available commercially from SIGMA, reference 178-8 (for example). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Gas chromatograph, equipped with an on-column injector or equivalent device, a temperature-programmable oven and a flame ionization detector.

4.2 Capillary column, capable of being programmed up to 400 °C ("high temperature" type) for which the following characteristics are advised:

— 100 % dimethylpolysiloxane or 95 % dimethyl-5 % diphenyl polysiloxane stationary phase;

— length 10 m;

— internal diameter 0,32 mm;

— film thickness 0, 1 µm.

4.3 Operating conditions

The chromatographic analysis conditions will be chosen taking into account the characteristics of the column being used and the type of carrier gas (hydrogen or helium). It is however recommended to observe an analysis time of about 30 min to ensure triglycerides elution.

By way of indication, an example of analysis conditions is described below:

— column temperature: 50 °C hold for 1 min, programmed at 15 °C/min up to 180 °C, programmed at 7 °C/min up to 230 °C, programmed at 10 °C/min up to 370 °C, final temperature hold for 5 min;

— detector temperature: 380 °C;

— carrier gas pressure (hydrogen): 80 kPa;

— volume injected: 1 µl.

4.4 Volumetric flask, 50 ml capacity.

4.5 Volumetric flasks, 10 ml capacity.

4.6 Screw-cap vials with PTFE-faced septa, 10 ml capacity.

4.7 Precision pipette, 1 ml capacity.

4.8 Microsyringe, 100 µl capacity.

4.9 Microsyringe, 500 µl capacity.

4.10 Microsyringe, 1 µl capacity specially designed for on-column operation.

4.11 Graduated cylinder, 10 ml capacity.

4.12 Analytical balance, with an accuracy of ± 0,1 mg.

5 Procedure

5.1 Preparation and analysis of the calibration solutions

Using a microsyringe (4.8), add 100 µl of MSTFA (3.1) to each of the four calibration solutions (3.12), close hermetically the vials and shake vigorously and avoid contact with moisture. Store 15 min at room temperature, then add 8 ml of heptane (3.3) using a graduated cylinder (4.11).

Analyse 1 µl of each reaction mixture by gas chromatography under the conditions defined above (4.3). Each reaction mixture gives rise to two chromatographic analysis. Samples are stable for some hours after derivatisation.

5.2 Preparation and analysis of the commercial mixture of monoglycerides

Using microsyringes (4.8 and 4.9), transfer 200 µl of commercial mixture of monoglyceride dissolved in pyridine (3.11) and 100 µl of MSTFA (3.1) into a 10 ml vial (4.6). Avoid contact with moisture. Close hermetically the vial and shake vigorously. Store 15 min at room temperature, then add 8 ml of heptane (3.3). Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described above (4.3).

5.3 Sampling

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 5555 [1].

5.4 Preparation and analysis of the samples

Accurately weigh approximately 100 mg of homogenized sample in a 10 ml vial (4.6). Using a syringe (4.8 and 4.9), add 80 µl of internal standard N° 1 stock solution (3.7), 100 µl of internal standard No. 2 stock solution (3.8) and 100 µl of MSTFA (3.1). Avoid contact with moisture. Close hermetically the vial and shake vigorously. Store 15 min at room temperature, then add 8 ml of heptane (3.3). Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described in (4.3).

For each sample, two test portions are submitted to the derivatisation reaction and give rise, each one, to two chromatographic analyses. Samples are stable for some hours after derivatisation.

5.5 Identification

The analysis of the calibration solutions under the same operating conditions as those used for the analysis of the sample allows the identification of the peaks by comparison of the retention times. Due to the overlapping of the elution zones of the methyl esters and of the monoglycerides, it is therefore advised, in order to identify the monoglyceride peaks, to inject the commercial mixture composed of monopalmitine, monostearin and monooleine (3.11), the latter having been previously submitted to the derivatisation reaction (5.2).

A chromatogram of a rapeseed oil methyl ester sample, obtained under the operating conditions and preparation described above (4.3 and 5.4) is presented in annex C (Figures C.1 to C.4). The relative retention times corresponding to the different peaks to be integrated are given in Table 2.

Table 2 — Relative retention times of glycerol and glycerides

Compounds	RRT / IS 1	RRT / IS 2
Glycerol	0,75	
1,2,4-Butanetriol (IS 1)	1,00	
Monopalmitin		0,61
Monoolein, monolinolein, monolinolenin		0,68
Monostearin		0,69
Tricaprin (IS 2)		1,00
Diglycerides		1,19 to 1,30
Triglycerides		1,56 to 1,65

5.6 Calibration

For each reference substance (monoolein, diolein and triolein), the study of the variation of weight ratio versus area ratio makes it possible to verify the linearity of the response and to work out a calibration function.

6 Expression of results

6.1 Integration of the peaks

The calibration functions given below (6.2 and 6.3) can only be used in the range of contents specified (Table 3).

Table 3

Compounds	Mass ratio (m/m_{ei})	Content (%)
Glycerol	0,06 to 0,62	0,005 to 0,05
Monoglycerides	0,31 to 1,56	0,25 to 1,25
Diglycerides	0,06 to 0,62	0,05 to 0,5
Triglycerides	0,06 to 0,50	0,05 to 0,4

In each family of glycerides, there exist small peaks (see annex B), which have to be integrated and for which the calibration functions cannot be applied on account of their validity ranges. This method therefore calculates the percentage of mono-, di- and triglycerides (6.5) by summing the area peaks for each family, whereas in theory, it would be necessary to calculate the percentage of each glyceride peak taken individually.

It is advised to integrate jointly the two diglyceride peaks containing 36 atoms of carbon, major compounds of this family, an account of an insufficient resolution, which may induce quantification errors if the two peaks are integrated separately. The same integration procedure may be applied for the diglyceride peaks containing 34 atoms of carbons, and for the diglyceride peaks containing 38 atoms of carbon.

The presence of a double peak at the level of the glycerol retention time shall lead to the verification of the silylation stage, which is probably incomplete (presence of water in the samples).

6.2 Glycerol calibration function

The calibration function is given by the following expression, obtained from the experimental data using the linear regression method (see annex A for data evaluation) :

$$M_g/M_{ei1} = a_g\,(A_g/A_{ei1}) + b_g$$

where

M_g is the mass of glycerol (mg);

M_{ei1} is the mass of internal standard No. 1 (mg);

A_g is the peak area of glycerol;

A_{ei1} is the peak area of the internal standard No. 1.

a_g and b_g are constants coming from regression method for glycerol.

The calibration function can be regarded as acceptable only if the correlation coefficient, calculated according to annex A, is equal or higher than 0,95.

6.3 Glycerides calibration function

The calibration functions are given by the following expressions, obtained from the experimental data using the linear regression method (see annex A for data evaluation):

$$M_m/M_{ei2} = a_m\,(A_m/A_{ei2}) + b_m$$

$$M_d/M_{ei2} = a_d\,(A_d/A_{ei2}) + b_d$$

$$M_t/M_{ei2} = a_t\,(A_t/A_{ei2}) + b_t$$

where

M_m, M_d, M_t are respectively the mass of monoolein, diolein and triolein (milligrams);

M_{ei2} is the mass of internal standard No. 2 (milligrams);

A_m, A_d, A_t are the peak areas, respectively, of monoolein, diolein and triolein;

A_{ei2} is the peak area of the internal standard No. 2.

a_m and b_m are constants coming from regression method for monoglycerol;

a_d and b_d are constants coming from regression method for diglycerol;

a_t and b_t are constants coming from regression method for triglycerol.

The calibration function can be regarded as acceptable only if the correlation coefficient, calculated according to annex A, is equal or higher than 0,95.

6.4 Calculation of the percentage of free glycerol

Calculate the percentage (m/m) of free glycerol in the sample using the expression:

$$G = [a_g\,(A_g/A_{ei1}) + b_g] \times (M_{eil}/m) \times 100$$

where

G is the percentage (m/m) of free glycerol in the sample;

A_g is the peak area of the glycerol;

A_{eil} is the peak area of internal standard No. 1;

M_{eil} is the mass of internal standard No. 1 (milligrams);

m is the mass of sample (milligrams).

a_g and b_g are constants coming from regression method for glycerol.

6.5 Calculation of the percentage of glycerides

Calculate the percentage (m/m) of the mono-, di- and triglycerides using the expressions:

$$M = [a_m\,(\Sigma A_{mi}/A_{ei2}) + b_m] \times (M_{ei2}/m) \times 100$$

$$D = [a_d\,(\Sigma A_{di}/A_{ei2}) + b_d] \times (M_{ei2}/m) \times 100$$

$$T = [a_t\ (\Sigma A_{ti}/A_{ei2}) + b_t] \times (M_{ei2}/m) \times 100$$

where

M, D, T are the mono-, di- and triglyceride percentage (m/m) in the sample;

$\Sigma A_{mi}, \Sigma A_{di}, \Sigma A_{ti}$ are the sums of the peak areas of the mono-, di- and triglycerides;

A_{ei2} is the peak area of internal standard No 2;

M_{ei2} is the mass of internal standard No 2 (milligrams);

m is the mass of sample (milligrams).

a_m and b_m are constants coming from regression method for monoglycerol;

a_d and b_d are constants coming from regression method for diglycerol;

a_t and b_t are constants coming from regression method for triglycerol.

6.6 Calculation of the percentage of total glycerol

Calculate the percentage (m/m) of total glycerol in the sample using the expression :

$$G_T = G + 0{,}255\ M + 0{,}146\ D + 0{,}103\ T$$

where

G_T is the percentage (m/m) of total glycerol (free and bound) in the sample;

G is the percentage (m/m) of free glycerol in the sample;

M is the percentage (m/m) of monoglycerides in the sample;

D is the percentage (m/m) of diglycerides in the sample;

T is the percentage (m/m) of triglycerides in the sample.

6.7 Expression of results

All the contents are expressed in percentages (m/m), to the nearest 0,01 %.

7 Precision

7.1 Interlaboratory test

An interlaboratory test organized in 1988 at European level with the participation of eleven laboratories, each having carried out two determinations on each sample, gave the statistical results indicated in annex D.

7.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short time interval, shall not be greater more than once out of 20 determinations than :