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ILNAS

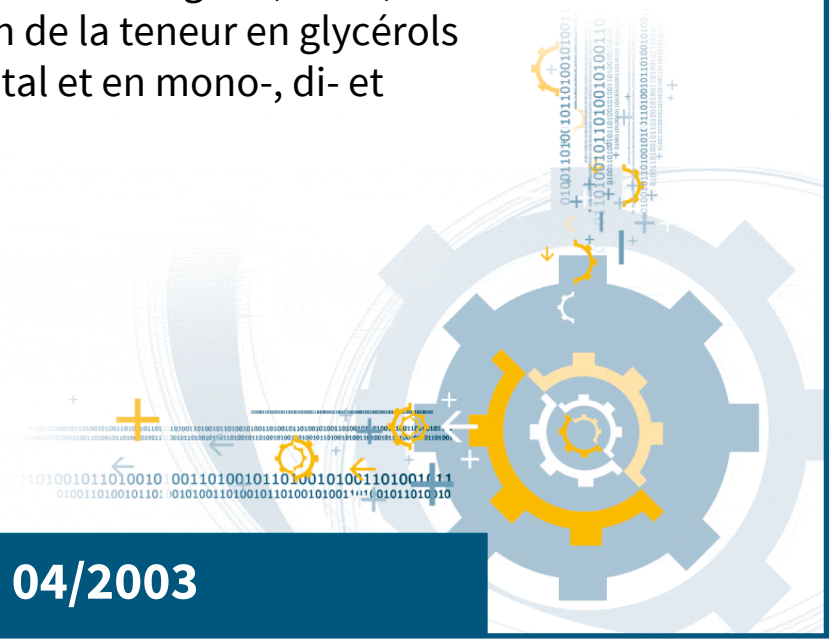
Institut luxembourgeois de la normalisation
de l'accréditation, de la sécurité et qualité
des produits et services

ILNAS-EN 14105:2003

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

Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem und Gesamtglycerin

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



04/2003

National Foreword

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EUROPEAN STANDARD **EN 14105**

NORME EUROPÉENNE

EUROPÄISCHE NORM

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ICS 67.200.10

English version

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

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

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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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.

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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).

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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.

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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.

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