SLOVENSKI STANDARD
SIST EN 14105:2011

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Nadomešča:
SIST EN 14105:2003

Derivati maščob in olj - Metil estri maščobnih kislin (FAME) - Določevanje prostega in celotnega glicerola ter mono-, di- in trigliceridov

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

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

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

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

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

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 14105:2011) 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of an identical text or by endorsement, at the latest by October 2011, and conflicting national standards shall be withdrawn at the latest by October 2011.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14105:2003.

The main modifications of the standard are:

- the utilization of representative internal standards for monoglycérides, diglycerides and triglycerides in order to avoid using calibration solutions for these families of compounds;

- the introduction of a performance criteria for the gas chromatography column calculated with the response factors for the diglyceride and triglyceride internal standards.

The method has been updated to obtain better precision in general, needed for the limits required by European FAME specifications for automotive use [1]. This has been done by introducing separate internal standards for mono- (C19), di- (C38) and triglycerides (C57). Next an improvement of the integration has been incorporated and some evaluation of interference with minor components (i.e. dimers) has been done.

Via a new Round Robin study, improvement of the precision of free glycerol and diglyceride measurement has been proven. The precision statement of the former standard could be confirmed for triglyceride determination, but no improvement was made.

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

1 Scope

The purpose of this European Standard is 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 obtained results.

Under the conditions described, the quantification limits are 0,001 % (*m/m*) for free glycerol, 0,10 % (*m/m*) for all glycerides (mono-, di- and tri-). This method is suitable for FAME prepared from rapeseed, sunflower, soybean, palm, animal oils and fats and mixture of them. It is not suitable for FAME produced from or containing coconut and palm kernel oils derivatives because of overlapping of different glyceride peaks.

NOTE For the purposes of this European Standard, the term "% (*m/m*)" is used to represent respectively the mass fraction.

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 and stable silyl derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

Analysis of the sample after silylation, 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 of glycerol is carried out in presence of the internal standard 1,2,4-butanetriol.

Mono-, di- and triglycerides are directly evaluated in presence of an internal standard for each glyceride category:

— glyceryl monononadecanoate (Mono C19) for monoglycerides;

— glyceryl dinonadecanoate (Di C38) for diglycerides;

— glyceryl trinonadecanoate (Tri C57) for triglycerides.

3 Reagents

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

3.1 N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA)

3.2 Pyridine, max. 0,1 % water, stored on molecular sieve

NOTE Pyridine silyl grade (3.10) can also be used.

3.3 Tetrahydrofurane (THF)

3.4 n-Heptane

3.5 Glycerol

3.6 1,2,4-Butanetriol

3.7 1 - Glyceryl monononadecanoate (Mono C19)[1)]

3.8 1-3 Glyceryl dinonadecanoate (Di C38)[2)]

3.9 Glyceryl trinonadecanoate (Tri C57)[3)]

3.10 Pyridine, silyl grade

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 % diphenylpolysiloxane stationary phase;

— length 15 m;

— internal diameter 0,32 mm;

— film thickness 0,1 μm.

4.3 Volumetric flask, 50 ml capacity

4.4 Volumetric flasks, 20 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, 5 μl or 10 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

[1)] Monononadecaoin available from Larodan, ref. 31-1900-11 (www.larodan.se)

[2)] 1,3-dinonadecaoin available from Larodan, ref. 32-1903-8 (www.larodan.se)

[3)] Trinonadecaoin available from Larodan, ref. 33-1900-13 (www.larodan.se), or from Sigma , ref. T4632-1G (www.sigmaaldrich.com)

4.13 Carrier gas, hydrogen or helium

4.14 Auxiliary gases, such as air, hydrogen and nitrogen

5 Preparation of solutions

5.1 1,2,4-Butanetriol stock solution, 1 mg/ml

Accurately weigh approximately 50 mg (accuracy ± 0,1 mg) of 1,2,4-butanetriol (3.6) in a 50 ml volumetric flask (4.3) and make up to the mark with pyridine (3.2).

5.2 Glycerol stock solution, 0,5 mg/ml

Accurately weigh approximately 50 mg (accuracy ± 0,1 mg) of glycerol (3.5) 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).

5.3 Standard glycerides stock solution, 2,5 mg/ml

For each reference glyceride, monononadecanoate (3.7), dinonadecanoate (3.8) and trinonadecanoate (3.9), accurately weigh approximately 50 mg (accuracy ± 0,1 mg) in a unique 20 ml volumetric flask (4.4) and make up to the mark with tetrahydrofurane (3.3).

The solution shall be perfectly limpid at ambient temperature. After storage in refrigerator at 4 °C the solution might show a precipitate that must re-dissolve spontaneously when restored at ambient temperature, without any external heating.

NOTE If stored at 4 °C the solution is stable for almost 3 months.

5.4 Commercial mixture of monoglycerides

Made up of mono-palmitoylglycerol (monopalmitin), mono-stearoylglycerol (monostearin) and of mono-oleoylglycerol (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).

This solution may be used to locate the relevant peaks in GC paths.

5.5 Calibration solutions

Prepare four calibration solutions by transferring into a series of vials (4.6) the volumes of stock solutions of glycerol (5.2) and of 1,2,4-butanetriol (5.1) given in Table 1, using the 100 µl microsyringes (4.8). 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). Make sure that needle and body of the 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).

Table 1 — Preparation of calibration solutions

Stock solution	1 µl	2 µl	3 µl	4 µl	Syringe µl
glycerol solution (5.2)	10	40	70	100	100
internal butanetriol sol. (5.1)	80	80	80	80	100

6 Sampling

Samples shall be taken in accordance with the requirements of national standards or regulations for the sampling of the product under test. A recommended sampling method is given in EN ISO 5555 [2] or EN ISO 3170 [3].

7 Procedure

7.1 Operating conditions

The chromatographic analysis conditions shall 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 35 min to ensure triglycerides elution.

EXAMPLE 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 15 min;

detector temperature: 380 °C;

carrier gas pressure (hydrogen): 80 kPa;

volume injected: 1 µl.

7.2 Analysis of the calibration solutions

Using a microsyringe (4.10), add 150 µl of MSTFA (3.1) to each of the four calibration solutions (5.5), close hermetically the vials and shake vigorously. Store 15 min at room temperature, then add 8 ml of n-heptane (3.4) using a graduated cylinder (4.11).

Analyse 1 µl of each reaction mixture by gas chromatography under the conditions defined under 7.1, using only the first part of temperature programme, stopping the analysis when the temperature of 230 °C has been reached. Each reaction mixture gives rise to two chromatographic analyses. Samples are stable for some hours after derivatisation.

NOTE The silylated standard solutions are only stable one day.

7.3 Analysis of the commercial mixture of monoglycerides

Using microsyringes (4.10), transfer 200 µl of commercial mixture of monoglycerides dissolved in pyridine (3.10) and 150 µl of MSTFA (3.1) into a 10 ml vial (4.6). Avoid contact with humidity.

Hermetically close the vial and shake vigorously.

Store 15 min at room temperature, and then add 8 ml of n-heptane (3.4).

Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described under 7.1.

7.4 Preparation and analysis of the samples

Accurately weigh approximately 100 mg (accuracy ± 0,1 mg) of homogenized sample in a 10 ml vial (4.6).

Using precision microsyringes (4.8 and 4.9), add 80 µl of 1,2,4-butanetriol stock solution (5.1), 200 µl of standard glycerides stock solution (5.3), 200 µl of pyridine (3.10) and 200 µl of MSTFA (3.1). Avoid contact with humidity.

Hermetically close the vial and shake vigorously. Store 15 min at room temperature, and then add 8 ml of n-heptane (3.4). Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described under 7.1.

Carry out the determination in duplicate, by preparing two independent samples.

7.5 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 monopalmitin, monosterarin and monoolein (5.4), the latter having been previously submitted to the derivatisation reaction.

A chromatogram of a rapeseed oil methyl ester sample, obtained under the operating conditions and preparation described under 7.1 is presented in Annex A. Internal glyceride standards may be analysed under the above mentioned chromatographic conditions, after silyl derivatisation.

7.6 Calibration

For glycerol only, 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.

For mono-, di- and triglycerides it is assumed that, within the considered concentration range the detector response is regarded as linear.

7.7 Column performance control

For each analysis, evaluate the relative response factor for glyceryl dinonadecanoate (Di C38) versus glyceryl trinonadecanoate (Tri C57), by using the following equation:

$$RRF = (A_{DiC38} / M_{DiC38}) / (A_{TriC57} / M_{TriC57}) \quad (1)$$

where

A_{DiC38} is the peak area of internal standard Di C38;

M_{DiC38} is the weight of internal standard Di C38 (mg);

A_{TriC57} is the peak area of internal standard Tri C57;

M_{TriC57} is the weight of internal standard Tri C57 (mg).

The results of the calculation of RRF shall be lower than 1,8. For higher values, the gas chromatography system is not suitable for analysis and shall be verified in order to improve triglyceride detection.

8 Determination of results

8.1 Integration of the peaks

In each family of glycerides, there exist small peaks (see Annex A) which have to be integrated. This method therefore calculates the percentage of mono-, di- and triglycerides (8.4) by summing the area peaks for each family. It is advised to integrate jointly the two diglyceride peaks containing 36 atoms of carbon, major compounds of this family, on account of an insufficient resolution which may induce quantification errors if the two peaks are integrated separately. The presence of a double peak at the level of the glycerol retention time shall lead to the verification of the sylilation stage, which is probably incomplete (presence of water in the samples).

8.2 Glycerol calibration function

The calibration function is given by the following equation, obtained from the experimental data using the linear regression method as in Annex B and according to:

$$M_g / M_{ei} = a_g \left(A_g / A_{ei} \right) + b_g \quad (2)$$

where

M_g is the weight of glycerol (mg);

M_{ei} is the weight of internal standard 1,2,4-butanetriol (mg);

A_g is the peak area of glycerol;

A_{ei} is the peak area of the internal standard 1,2,4-butanetriol;

a_g , b_g are the regression coefficients of the calibration function for glycerol.

The calibration function shall be regarded as correct only if the correlation coefficient, calculated according to Annex B, is equal or greater than 0,9 (see Annex C for a worked example).

8.3 Free glycerol

Calculate the mass percentage of free glycerol (G) in % (*m/m*) in the sample using the equation:

$$G = \left[a_g \left(A_g / A_{ei1} \right) + b_g \right] \times \left(M_{ei} / m \right) \times 100 \quad (3)$$

where

A_{ei1} is the peak area of internal standard 1,2,4-butanetriol;

M_{ei1} is the weight of internal 1,2,4-butanetriol;

m is the weight of sample (mg).