SLOVENSKI STANDARD
SIST EN 14105:2021

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Nadomešča:
SIST EN 14105:2011

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 (EMAG) - Détermination de la teneur en glycérols libre et total et en mono-, di- et triglycérides

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This European Standard was approved by CEN on 2 November 2020.

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

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European foreword

This document (EN 14105:2020) 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of an identical text or by endorsement, at the latest by June 2021, and conflicting national standards shall be withdrawn at the latest by June 2021.

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

This document supersedes EN 14105:2011.

In comparison with the previous edition, the following technical modifications have been made:

— document revised editorially;

— improvement of the quality of the figures;

— addition of figures in Annex A for clarification.

According to the CEN-CENELEC Internal Regulations, the national standards organisations 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, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This document specifies a method to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME). 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 1 For the purposes of this document, the term "% (*m*/*m*)" is used to represent the mass fraction.

NOTE 2 Under the common EN 14105 GC conditions squalene can coelute with alpha glycerol monostearate. If the presence of squalene is suspected, EN 17057 can be used to discriminate between squalene and glycerol monostearate.

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all of the safety problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 5555, *Animal and vegetable fats and oils — Sampling (ISO 5555)*

EN ISO 3170, *Petroleum liquids — Manual sampling (ISO 3170)*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

4 Principle

The glycerol and the mono- and diglycerides are transformed into more volatile and stable silyl derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

The sample after silylation is analysed 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.

5 Chemicals

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

5.1 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

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

Pyridine silyl grade (5.10) can also be used.

5.3 Tetrahydrofurane (THF).

5.4 n-Heptane.

5.5 Glycerol.

5.6 1,2,4-Butanetriol.

5.7 1-Glyceryl monononadecanoate (Mono C19).

5.8 1-3 Glyceryl dinonadecanoate (Di C38).

5.9 Glyceryl trinonadecanoate (Tri C57).

5.10 Pyridine, silyl grade.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

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

6.2 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.

6.3 Volumetric flask, 50 ml capacity, Grade A.

6.4 Volumetric flasks, 20 ml capacity, Grade A.

6.5 Volumetric flasks, 10 ml capacity, Grade A.

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

6.7 Precision pipette, 1 ml capacity.

6.8 Precision pipette or syringe, 100 µl capacity.

6.9 Precision pipette or syringe, 500 µl capacity.

6.10 Graduated cylinder, 10 ml capacity.

6.11 Analytical balance, with a weighing accuracy of ± 1 mg or better and a readability of ± 0,1 mg or better.

6.12 Carrier gas, hydrogen or helium.

6.13 Auxiliary gases, such as air, hydrogen and nitrogen.

7 Preparation of solutions

7.1 1,2,4-Butanetriol stock solution, 1 mg/ml

Weigh (50,0 ± 0,1) mg 1,2,4-butanetriol (5.6) in a 50 ml volumetric flask (6.3) and fill up to the mark with pyridine (5.2 or 5.10).

7.2 Glycerol stock solution, 0,5 mg/ml

Weigh (50,0 ± 0,1) mg glycerol (5.5) in a 10 ml volumetric flask (6.5) and fill up to the mark with pyridine (5.2 or 5.10). Transfer 1 ml of this solution into a 10 ml volumetric flask (6.5) using a pipette (6.7) and fill up to the mark with pyridine (5.2 or 5.10).

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

For each reference glyceride, mononononadecanoate (5.7), dinonadecanoate (5.8) and trinonadecanoate (5.9), weigh (50,0 ± 0,1) mg in a unique 20 ml volumetric flask (6.4) and fill up to the mark with tetrahydrofurane (5.3).

The solution shall be perfectly transparent at ambient temperature. After storage in a refrigerator at 4 °C the solution can show a precipitate that shall 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.

7.4 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 (6.5) and fill up to the mark with pyridine (5.2 or 5.10).

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

7.5 Calibration solutions

Prepare four calibration solutions by transferring the volumes given in Table 1 of the glycerol stock solutions (7.2) and of the 1,2,4-butanetriol (7.1) into a series of vials (6.6), using the 100 µl precision pipette (6.8). If using a syringe, make sure that the needle and the body of the syringe are free from air bubbles.

Table 1 — Preparation of calibration solutions

Stock solution	1 (µl)	2 (µl)	3 (µl)	4 (µl)	syringe (µl)
glycerol solution (7.2)	10	40	70	100	100
internal butanetriol solution (7.1)	80	80	80	80	100

8 Sampling

Samples shall be taken in accordance with EN ISO 5555 or EN ISO 3170.

9 Procedure

9.1 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 in Table 2:

Table 2 — Example of analysis conditions

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 or constant flow (about 2 ml/min)
volume injected:	1 µl

9.2 Analysis of the calibration solutions

Using a precision pipette (6.9), add 150 µl of MSTFA (5.1) to each of the four calibration solutions (7.5), close hermetically the vials and shake vigorously. Store 15 min at room temperature. Then open the vial and add 8 ml of n-heptane (5.4) using a graduated cylinder (6.10). Close the vial hermetically again.

Analyse 1 µl of each reaction mixture by gas chromatography e.g. under the conditions indicated in 9.1, using only the first part of the temperature programme, stopping the analysis when the temperature of 230 °C has been reached. Samples are stable for some hours after derivatisation.

NOTE The silylated standard solutions are only stable for one day.

9.3 Analysis of the commercial mixture of monoglycerides

Using precision pipette (6.9), transfer 200 µl of commercial mixture of monoglycerides dissolved in pyridine (5.10) and 150 µl of MSTFA (5.1) into a 10 ml vial (6.6). Avoid contact with humidity.

Hermetically close the vial and shake vigorously.

Store 15 min at room temperature. Then open the vial and add 8 ml of n-heptane (5.4) using a graduated cylinder (6.10). Close the vial hermetically again.

Analyse 1 µl of the reaction mixture by gas chromatography e.g. according to the conditions indicated in 9.1.

9.4 Preparation and analysis of the samples

Weigh (100,0 ± 0,1) mg of homogenized sample in a 10 ml vial (6.6).

Using precision pipettes or syringes (6.8 and 6.9), add 80 µl of 1,2,4-butanetriol stock solution (7.1), 200 µl of standard glycerides stock solution (7.3), 200 µl of pyridine (5.10) and 200 µl of MSTFA (5.1). Avoid contact with humidity.

Hermetically close the vial and shake vigorously. Store 15 min at room temperature. Then open the vial and add 8 ml of n-heptane (5.4) using a graduated cylinder (6.10). Close the vial hermetically again. Analyse 1 µl of the reaction mixture by gas chromatography e.g. according to the conditions indicated in 9.1.

9.5 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 (7.4), the latter having been previously submitted to the derivatisation reaction.

Chromatograms of a rapeseed oil and a palm oil methyl ester sample, obtained under the operating conditions in 9.1 are presented in Annex A. In addition the chromatogram of a second palm oil methyl ester sample containing fatty acid methyl ester dimers is presented. Internal glyceride standards may be analysed under the above mentioned chromatographic conditions, after silyl derivatisation.

9.6 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.

9.7 Column performance control

For each analysis, evaluate the relative response factor (*RF*) for glyceryl dinonadecanoate (Di C38) versus glyceryl trinonadecanoate (Tri C57), by using Formula (1):

$$RF = \left(A_{\mathrm{DiC38}} / M_{\mathrm{DiC38}}\right) / \left(A_{\mathrm{TriC57}} / M_{\mathrm{TriC57}}\right) \quad (1)$$

where

$A_{\mathrm{Di\ C38}}$ is the peak area of internal standard Di C38;

$M_{\mathrm{Di\ C38}}$ is the weight of internal standard Di C38, in mg;

$A_{\mathrm{Tri\ C57}}$ is the peak area of internal standard Tri C57;

$M_{\mathrm{Tri\ C57}}$ is the weight of internal standard Tri C57, in mg.

The results of the calculation of *RF* 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.

10 Determination of results

10.1 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 (10.4) 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 silylation stage, which is probably incomplete (presence of water in the samples).

10.2 Glycerol calibration function

The calibration function is given by Formula (2), obtained from the experimental data using the linear regression method as in Annex B and according to:

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

where

M_g is the weight of glycerol, in mg;

M_{ei} is the weight of internal standard 1,2,4-butanetriol, in 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).

10.3 Free glycerol

Calculate the mass percentage of free glycerol (G) in % (m/m) in the sample using Formula (3):

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

where

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

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

m is the weight of the sample, in mg.