BRITISH STANDARD

BS ISO
29822:2009

Vegetable fats and oils — Isomeric diacylglycerols — Determination of relative amounts of 1,2- and 1,3-diacylglycerols

ICS 67.200.10,

British Standards

National foreword

This British Standard is the UK implementation of ISO 29822:2009.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oil seeds, animal and vegetable fats and oils and their by products.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2009

ISBN 978 0 580 59912 5

Amendments/corrigenda issued since publication

Date	Comments

INTERNATIONAL
STANDARD

ISO
29822

First edition
2009-03-15

Vegetable fats and oils — Isomeric diacylglycerols — Determination of relative amounts of 1,2- and 1,3-diacylglycerols

Corps gras d'origine végétale — Diacylglycérols isomériques — Détermination des teneurs relatives en 1,2- et 1,3-diacylglycérols

Reference number
ISO 29822:2009(E)

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

COPYRIGHT PROTECTED DOCUMENT

© ISO 2009

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 29822 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Vegetable fats and oils — Isomeric diacylglycerols — Determination of relative amounts of 1,2- and 1,3-diacylglycerols

1 Scope

This International Standard specifies the determination of the degree of isomerization of diacylglycerols in vegetable fats and oils. 1,2-Diacylglycerols are transformed to the more stable 1,3-isomers during storage or due to acidic catalysed reaction.

The mass fraction of 1,2-diacylglycerols can be used as a quality criterion for vegetable fats and oils.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

3.1
degree of isomerization
mass fraction of the peak areas of all 1,2-diacylglycerols (C_{32}, C_{34}, C_{36}) relative to the sum of the peaks of all diacylglycerols (C_{32}, C_{34}, C_{36}).

NOTE The mass fraction is expressed as a percentage to one decimal place.

4 Principle

A miniaturized column chromatography on a silica gel column is used to separate the isomeric diacylglycerols as the more polar fraction from the major part of other lipids. The peak areas of 1,2- and 1,3-isomers are determined by gas chromatography after silylation. Only C_{32}-, C_{34}- and C_{36}-diacylglycerols are taken into account.

5 Reagents

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Silica gel 60 [1], for column chromatography, (0,063 to 0,100 mm).

5.1.1 Silica gel 60, with a moisture content, w_{H_2O} = 5 % mass fraction.

Activate the silica gel by heating overnight at (160 ± 5) °C. After heating, place the silica gel in a desiccator for cooling and then transfer the silica gel to a stoppered flask. Add 5 % mass fraction of water and shake until no lumps can be seen and the powder flows freely (1 h in an automatic shaking machine). Store the conditioned silica gel overnight before use.

5.2 Cotton wool, defatted.

5.3 Reference substances [2].

5.3.1 Dilaurin (1,3-dilauroyl-*sn*-glycerol).

5.3.2 Dipalmitin (1,2- and 1,3-dipalmitoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.3.3 Distearin (1,2- and 1,3-distearoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.3.4 Diolein (1,2- and 1,3-dioleoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.4 Reference solutions:

For the reference substances dipalmitin and distearin, prepare a solution in toluene (mass concentration, ρ = 1 mg/ml).

5.5 Isooctane (2,2,4-trimethylpentane).

5.6 Diethyl ether, eluent.

5.7 Diisopropyl ether.

5.8 Solvent mixture: isooctane/diisopropyl ether [volume fraction φ(isooctane) = 85 ml/100 ml and φ(diisopropyl ether) = 15 ml/100 ml].

5.9 1-Methylimidazole.

5.10 *N*-Methyl-*N*-(trimethyl-silyl)heptafluorobutyramide (MSHFBA).

5.11 Silylating reagent: 50 µl 1-methylimidazole in 1 ml of MSHFBA.

5.12 Toluene.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,001 g and displaying 0,000 1 g.

---

1) Merck Art.-No 612-1484 or 613-2623 (www.merck.de) are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

2) A suitable supplier is Sigma-Aldrich (www.sigmaaldrich.com). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this supplier.

6.2 **Desiccator**, for storing adsorbents after heating.

6.3 **Pipette tip** [3], capacity 5 ml, length approx. 150 mm.

6.4 **Pointed flask**, capacity 10 ml, with ground-glass neck and rounded bottom.

6.5 **Beaker**, capacity 10 ml.

6.6 **Rotary evaporator**.

6.7 **Gas chromatograph for capillary columns**, split injector, flame ionization detector (FID) and suitable integration system.

Do not use on-column or splitless injectors, as this results in isomerization of 1,2-diacylglycerols.

6.8 **Fused silica capillary column**, for gas chromatography (internal diameter, 0,25 mm or 0,32 mm, length 12 m, 15 m, 30 m or 60 m) coated with 5 % diphenyl-, 95 % dimethylpolysiloxane, 0,1 µm film thickness.

Other columns of similar polarity and selectivity may also be used.

7 Sample

7.1 Sampling

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

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

7.2 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

8 Procedure

8.1 Preparation of the silica gel chromatography column

Insert a small stopper of cotton wool (5.2) approx. 5 mm high, in the lower part of a pipette tip (6.3) followed by 1 g silica gel 60 (5.1). Cover the silica layer with a 5 mm high stopper of cotton wool. Compress the filling by slight pressing with a flat ended rod.

8.2 Separation of the fraction containing non polar lipids

8.2.1 Weigh, to the nearest 0,1 mg, about 100,0 mg of the test sample into a beaker (6.5) and add 1 ml of toluene (5.12).

8.2.2 Transfer the test portion on to the column, carefully purging the flask with 1 ml solvent mixture (5.8). Wash the column with two 3,5 ml portions of the solvent mixture (5.8). Rinse the end of the pipette tip with solvent mixture (5.8) and discard the solvent.

---

[3] VWR/Merck Art.-No 1.15101.1000 (www.merck.de) is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

8.2.3 Elute the diacylglycerols with two 3,5 ml portions of diethyl ether (5.6) and collect the eluate in a pointed flask (6.4).

8.2.4 Remove the solvent from the eluate to about 1 ml with a rotary evaporator (6.6) at 20 °C. Transfer the remaining solution into a reaction vial. Blow off the solvent in the reaction vial with a stream of nitrogen.

8.3 Preparation of trimethylsilyl ethers (silylation)

8.3.1 Add 200 µl of the silylation reagent (5.11) to the reaction vial containing the diacylglycerols, seal and allow the mixture to react for 20 min at room temperature.

8.3.2 After silylation, add 1 ml acetone and use 1 µl to 2 µl of the solution for the gas chromatography.

8.4 Gas chromatography

The conditions in Table 1 for the gas chromatograph have been found to give useful chromatograms.

Table 1 — Gas chromatographic conditions

Function	Condition
Capillary GC column	Restek RTX5 [4], 60 m; internal diameter 0,25 mm, film thickness 0,1 µm
Injection volume	1 µl (split 1:50)
Carrier gas	Hydrogen at 2 ml/min, constant flow
Gas for FID	Hydrogen at 33 ml/min and air at 420 ml/min
Injector temperature	340 °C
Detector temperature	340 °C
Temperature oven	240 °C; maintain for 1 min, heat at 10 °C/min to 320 °C, maintain for 10 min

Optimize the temperature programme and the velocity of the carrier gas flow so that chromatograms similar to Figure A.1 are obtained. Test the separation with silylated diacylglycerol fractions.

8.5 Identification of diacylglycerol isomers

Prepare a solution of dipalmitin (1,2-C_{32},1,3-C_{32}) (5.3.2), distearin (1,2-C_{36}, 1,3-C_{36}) (5.3.3), and diolein (1,2-C_{36}, 1,3-C_{36}) (5.3.4), each with a mass concentration, ρ = 1 mg/ml toluene. Add 100 µl of this solution to a derivatization vial. Blow off the solvent in the reaction vial with a stream of nitrogen and silylate.

Do not add acetone to the reference solutions.

9 Result of the determination

9.1 Identification of diacylglycerols

To identify the 1,2- and 1,3-diacylglycerols in the test portion, determine the retention times of the reference standards.

---

4) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

NOTE The retention times of the diacylglycerols containing unsaturated fatty acids can differ slightly from those of the corresponding saturated fatty acids. For the purpose of this method, it is assumed that the response factors of all diacylglycerols are equal.

9.2 Determination of the peak areas of 1,2- and 1,3- diacylglycerols in the oil

Calculate the percentage mass fraction, $w_{1,2}$, of the 1,2-diacylglycerols as follows:

$$w_{1,2} = \frac{A_{1,2}}{\sum A} \times 100$$

where

$A_{1,2}$ is the peak area of all 1,2-diacylglycerols (C_{32}, C_{34}, C_{36}) present in the test portion;

ΣA is the sum of the peak areas of the individual 1,2- and 1,3-diacylglycerols (C_{32}, C_{34}, C_{36}).

Report the result to one decimal place.

10 Precision of the method

10.1 Interlaboratory test

Details of the test and the precision of the method are summarized in Annex B. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, shall in not more than 5 % of cases exceed the value of r given in Table B.1.

10.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, shall in not more than 5 % of cases exceed the value of R given in Table B.1.

11 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

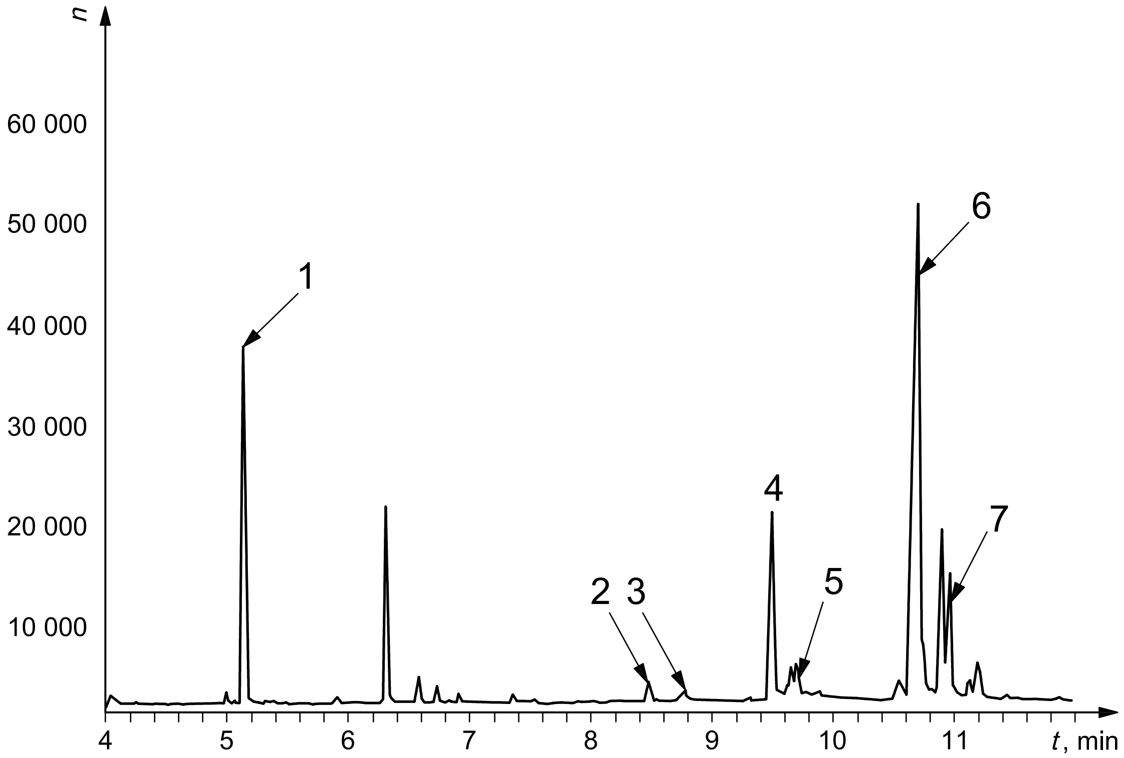
d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents occurred when performing the method, which may have influenced the test result(s);

e) the test result obtained;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Example of a typical chromatogram



Key

1 1,3-DG C_{24} (dilaurin)
2 1,2-DG C_{32}
3 1,3-DG C_{32}
4 1,2-DG C_{34}
5 1,3-DG C_{34}
6 1,2-DG C_{36}
7 1,3-DG C_{36}

Conditions

Column	SGE, HT-5, 12 m × 0,22 mm, 0,25 µm film thickness	
Split	1:10	
Oven programme	1 min	140 °C
	8 °C/min	320 °C
	20 °C/min	420 °C
	3 min	420 °C

Figure A.1 — Gas chromatogram obtained from an olive oil

Annex B
(informative)

Results of an interlaboratory test

The precision of the method is the result of an interlaboratory study organized by the Joint Committee for the Analysis of Fats, Oils, Fat Products, Related Products and Raw Materials (DIN/DGF) on an international basis. The study was carried out in 2005 on five samples, evaluated according to DGF Standard Method A-II [4], ISO 5725-1:1994 [2] and ISO 5725-2:1994 [3]. In this test, 19 laboratories from France, Germany and Italy participated.

Table B.1 — Summary of statistical results for olive oils

Sample	A	B	C	D	E
Number of participating laboratories, n_{P}	19	19	19	19	19
Number of laboratories retained after eliminating outliers, n_{p}	16	19	18	19	19
Number of individual test results of all laboratories on each sample, n_{Z}	32	38	36	38	38
Mean value, $\bar{w}_{1,2}$, %	**42,18**	**34,91**	**60,27**	**55,44**	**35,14**
Repeatability standard deviation, s_r, %	0,51	0,76	1,24	1,63	0,72
Repeatability coefficient of variation, CV(r), %	1,2	2,2	2,0	2,9	2,0
Repeatability limit, r, (= 2,8 s_r), %	**1,42**	**2,14**	**3,46**	**4,55**	**2,00**
Reproducibility standard deviation, s_R, %	1,56	1,60	2,15	2,19	2,95
Reproducibility coefficient of variation, CV(R), %	3,7	4,6	3,6	4,0	8,4
Reproducibility limit, R, (= 2,8 s_R), %	**4,36**	**4,48**	**6,02**	**6,14**	**8,27**

Bibliography

[1] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] DGF Standard Method A-II 1, *Execution and evaluation of ring tests*

ICS 67.200.10

Price based on 8 pages

This page has been intentionally left blank

BSI - British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001. Fax: +44 (0)20 8996 7001 Email: orders@bsigroup.com You may also buy directly using a debit/credit card from the BSI Shop on the Website http://www.bsigroup.com/shop

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact Information Centre. Tel: +44 (0)20 8996 7111 Fax: +44 (0)20 8996 7048 Email: info@bsigroup.com

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002 Fax: +44 (0)20 8996 7001 Email: membership@bsigroup.com

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsigroup.com/BSOL

Further information about BSI is available on the BSI website at http://www.bsigroup.com

Copyright

BSI Group
Headquarters 389
Chiswick High Road,
London, W4 4AL, UK
Tel +44 (0)20 8996 9001
Fax +44 (0)20 8996 7001
www.bsigroup.com/
standards