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# Vegetable fats and oils — Determination of composition of triacylglycerols and composition and content of diacylglycerols by capillary gas chromatography

*Corps gras d'origine végétale — Détermination de la composition des triacylglycérols et de la teneur en diacylglycérols par chromatographie en phase gazeuse sur colonne capillaire, dans les huiles végétales*

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# Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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# Vegetable fats and oils — Determination of composition of triacylglycerols and composition and content of diacylglycerols by capillary gas chromatography

## 1 Scope

This document specifies the determination of composition of triacylglycerols and the determination of the composition and content of diacylglycerols by capillary gas chromatography in vegetable oils with a lauric acid content below 1 %.

Applying certain technological processing 1,2-diacylglycerols (1,2-DAGs) are transformed to the more stable isomeric 1,3-diacylglycerols (1,3-DAGs) due to acidic catalysed reaction. During storage, the speed and amount of this rearrangement depends on the acidity of the oil. The transformation normally reaches an equilibrium between the two isomeric forms. The relative amount of 1,2-DAGs is related to oil freshness or to a possible technological treatment. Therefore, it is possible to use the ratio of 1,2-DAGs to 1,3-DAGs as a quality criterion for vegetable fats and oils.

The triacylglycerols profile is of potential interest for the fingerprint of each vegetable oil and may help the detection of certain types of adulteration, such as the addition of high oleic sunflower oil or palm olein in olive oil.

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NOTE This document is based on Reference [3].

## 2 Normative references

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There are no normative references in this document.

## 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:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

## 4 Principle

After the addition of an internal standard the oil sample is silylated, dissolved in a suitable reagent and directly injected in the gas chromatographic apparatus. Triacyglycerols are separated into classes on the basis of their carbon atom number, while diacylglycerols are separated in function of their carbon atom number and structure, as 1,2 structures show a lower retention time than 1,3 ones.

Unsaturated diacylglycerol structures do not affect retention time. Therefore, saturated and unsaturated diacylglycerols elute together, so 1,2 and 1,3-diacylglycerol structures are identified by their peak retention time. The percentage content of 1,2 structure is determined through the ratio of 1,2-diacylglycerol areas to the sum of areas of all the diacylglycerol peaks.

The diacylglycerol total content is calculated by means of an internal standard.

The percentage content for each triacylglycerol class is calculated after normalization to 100 % of all the triacylglycerol peaks.

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## 5 Apparatus

**5.1 Analytical balance** suitable to perform weighing to an accuracy of within +/−0,1 mg.

**5.2 Gas chromatograph** for use with a capillary column, equipped with a system for direct on-column for cold injection or a programmed temperature vaporizer.

**5.3 Thermostat-controlled oven** with temperature programming.

**5.4 Cold injector** for on-column injection or programmed temperature vaporizer.

**5.5 Flame-ionization detector** and converter-amplifier.

**5.6 Recorder-integrator** for use with the converter-amplifier (5.5), with a rate of response below 1 s and variable paper speed, or any suitable device for data capture and handling.

**5.7 Capillary column, fused silica,** 6 m to 8 m length, 0,25 mm to 0,32 mm internal diameter, internally coated with SE 52, SE 54 liquid phase to a uniform thickness of 0,10 µm to 0,15 µm.

**5.8 Microsyringe,** 10 µl, with a hardened needle for on-column injector.

**5.9 Microsyringe,** 100 µl, with a hardened needle.

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**5.10 Usual laboratory glassware.**

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## 6 Reagents

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

Unless otherwise stated analytically pure reagents shall be used.

**6.1 Carrier gas:** hydrogen or helium, pure, for gas chromatography.

**6.2 Auxiliary gases:**

— hydrogen, pure, for gas chromatography;

— air, pure, for gas chromatography.

**6.3 Silylating reagent**, mix equal volumes of

— pyridine, and

— bistrimethylsilylfluoroacetamide (BSTFA)-trimethylchlorosilane (TMCS), 99:1, volume fraction.

**6.4 n-Heptane.**

**6.5 Reference samples:** pure diacylglycerols and triacylglycerols and their mixtures, with known composition.

**6.6 Methyl tert-butyl ether.**


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**6.7 Dinonadecanoin sample solution** (internal standard), 0,1 % mass/ volume in methyl tert-butyl ether.

# 7 Procedure

## 7.1 Gas chromatographic apparatus and capillary column condition

Fit the column to the gas chromatograph (5.2), connecting the inlet port to the on-column system and the outlet port to the detector. Check the gas chromatography apparatus (operation of gas loops, detector and recorder efficiency, etc.).

Run a light flow of gas through the column, then switch on the gas chromatography apparatus. Gradually heat until a temperature of 350 °C is reached after approximately 4 h.

Maintain this temperature for at least 2 h, then regulate the apparatus to the operating conditions (regulate gas flow, light flame, connect to electronic recorder, regulate oven temperature for column, regulate detector, etc.). Record the signal at a sensitivity at least twice as high as that required for the analysis. The base line should be linear, with no peaks of any kind, and shall not have any drift.

Negative straight-line drift indicates that the column connections are not correct. Positive drift indicates that the column has not been properly conditioned.

## 7.2 Choice of operating conditions

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The operating conditions are generally as follows.

— The injector temperature shall be at least 10 °C below the vaporization temperature (99 °C) of the employed solvent (n-Heptane).

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— Detector temperature: 350 °C.

— Column temperature: 80 °C at first (1 min), ramp at 20 °C/min to 220 °C, ramp at 5 °C/min to 340 °C (10 min).

— Carrier gas: hydrogen or helium at the optimal linear speed for the chosen gas.

— Amount of injected substance: 0,5 µl to 1 µl of solution prepared as in 7.3.

## 7.3 Performance of the analysis

Weigh (5.1) exactly 100 mg of oil in a glass bottom conical tube and add 1 ml of internal standard solution (6.7). Shake the sample up to a complete solution, take up with a microsyringe (5.8) 20 µl to 30 µl of solution, put it inside a new glass tube (with a stopper) and dry by nitrogen gentle stream. Add 200 µl of silylation reagent. After 20 min dry by a soft nitrogen flow, add 2 ml of n-Heptane and shake. Inject (5.4) a volume from 0,5 µl to 1 µl of solution, in the conditions given in 7.2.

## 7.4 Peak identification

The triacylglycerols peak identification is carried out from the retention times by comparing them with mixtures of known composition. They are eluted in the following order:

C46, C48, C50, C52, C54, C56, C58, C60, C62, C64.

The diacylglycerols peak identification is carried out from the retention times by comparing them with mixtures of known diacylglycerols. They are eluted in the following order:

1,2 C32, 1,3 C32, 1,2 C34, 1,3 C34, 1,2 C36, 1,3 C36 (see Figures A.1, A.2, A.3, A.4 and A.5).

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### 7.5 Determination of percentage content of each triacylglycerol class

Calculate the areas of each peak through an electronic integrator. Normalize all the triacylglycerol peaks to 100 %.

Results are expressed as a percentage of C46, C48, C50, C52, C54, C56, C58, C60, C62, C64 according to the detected triacylglycerol class in function of the vegetable oils type.

Other eventual peaks shown by gas chromatographic run do not have to be considered.

All triacylglycerols, including those lying in between the major peaks, shall be integrated. Each odd triacylglycerol (see Figure A.5) is combined to the one that precedes it in order to have the sum of all the triacylglycerols normalized to 100 %.

Express the results to two decimal places.

### 7.6 Determination of percentage content of each 1,2 diacylglycerol

Calculate the areas of each peak through an electronic integrator.

Results are expressed as a percentage of 1,2 C32, 1,3 C32, 1,2 C34, 1,3 C34, 1,2 C36, 1,3 C36 (in function of the vegetable oils type) on the sum of DAG areas.

Other eventual peaks shown by gas chromatographic run do not have to be considered.

$$\text{\% of single diacylglycerol} = \frac{A_x}{\sum A_{DAG}} \times 100 \tag{1}$$

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where

$A_x$ is the area corresponding to the peak of diacylglycerol x;

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$\sum A_{DAG}$ is the sum of areas of all diacylglycerol peaks.

Express the results to one decimal place.

### 7.7 Determination of weight percentage total content of diacylglycerols

By using the integrator, calculate the areas of internal standard and diacylglycerol compounds by considering only the peaks that are reported as a result of 7.6.

Compute each single diacylglycerol, in % weight (g/100 g) of fat matter, as shown by Formula (2):

$$\text{Single diacylglycerol, \% in weight (g/100 g)} = \frac{A_x \times m_s \times 100}{A_s \times m} \tag{2}$$

where

$A_x$ is the area corresponding to the peak of single diacylglycerol;

$A_s$ is the area corresponding to internal standard peak (6.7);

$m_s$ is the added amount (mg) of internal standard (6.7);

$m$ is the amount (mg) of sample, take up for the determination.

Express the results to one decimal place.

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## 8 Expression of results

**8.1** Single triacylglycerol percentage: compute the sum of percentages of triacylglycerols. Results shall be expressed to two decimal places.

**8.2** 1,2 diacylglycerol percentage: compute the sum of percentages of 1,2 diacylglycerols. Results shall be expressed to one decimal place.

**8.3** Weight percentage: sum the amounts of all detected diacylglycerols. Results shall be expressed to one decimal place.

## 9 Precision of the method

### 9.1 Repeatability, *r*

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, will in not more than 5 % of cases exceed the value of *r* given in Tables B.1 to B.5.

### 9.2 Reproducibility, *R*

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, will in not more than 5 % of cases exceed the value of *R* given in Tables B.1 to B.5.

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## 10 Test report

The test report shall specify the following:

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 document, i.e. ISO 21846;

d) any operating details not specified in this document, or regarded as optional, together with details of any incidents which might have influenced the test result(s);

e) the test result obtained;

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

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