



**INTERNATIONAL
OLIVE
COUNCIL**

COI/T.20/Doc. No 32
November 2013

ENGLISH
Original: ENGLISH

Príncipe de Vergara, 154 – 28002 Madrid – España Telef: +34 915 903 638 Fax: +34 915 631 263 - e-mail: iooc@internacionaloliveoil.org - <http://www.internacionaloliveoil.org/>

DETERMINATION OF COMPOSITION OF TRIACYLGLYCEROLS AND COMPOSITION AND CONTENT OF DI-ACYLGLYCEROLS BY CAPILLARY GAS CHROMATOGRAPHY, IN VEGETABLE OILS

1 SCOPE

This method describes determination of composition and content of the composition of triacylglycerol and of the composition and content of di-acylglycerols (DAG) by capillary gas chromatography in vegetable oils with a lauric acid content below 1 %.

2 PRINCIPLE OF THE METHOD

The fatty substance, after adding the internal standard and silylating reagent, is solved in a suitable reagent and directly injected in the gas chromatographic apparatus. Triacylglycerols are resolved on the basis of their carbon atom number, while di-acylglycerols are resolved in function of their Carbon atom number and structure. 1,2 structures show a lower retention time than 1,3 ones.

Unsaturated di-acylglycerol structures don't affect retention time therefore saturated and unsaturated diglycerides are eluted together. 1,2 and 1,3 di-acylglycerol structures are identified through retention time and the percentage content of 1,2 structure is determined through the ratio of 1,2 diglyceride peaks on the sum of all di-acylglycerol peaks; total diglycerides are quantified through the ration between the sum of all di-acylglycerol peaks areas and the internal standard.

3 MEANING OF DETERMINATION

The profile of triglycerides is characteristic for each kind of oil, as it depends on the fatty acid composition, as well as on the biosynthesis rules, so that it can be used to check for the presence of extraneous oils.

Triglyceride biosynthesis pathway develops through 1,2 di-acylglycerols. However this reaction isn't 100 % completed therefore little 1,2 diglyceride amounts remain. During storage or the technological processes, partial hydrolysis can occur with also 1,3 diglyceride origin; it is also possible to get this result through the transposition from 1,2 to 1,3 di-acylglycerol. The speed of this transposition depends on oil acidity. During technological treatments a transposition from 1,2 to 1,3 usually occurs up to reach the equilibrium (\approx 33% of 1,2-DAG - 66% of 1,3-DAG). Therefore by assessment of 1,2 percentage content it's related the olive oil freshness or eventual carried out treatments.

4 **APPARATUS**

- 4.1 **Analytical balance** suitable to perform weighing to an accuracy of within +/- 0,1 mg.
- 4.2 **Gas chromatograph** for use with a capillary column, equipped with a system for direct on-column for cold injection
- 4.2.1 **Thermostat-controlled oven** with temperature programming.
- 4.2.2 **Cold injector** for on-column injection or PTV injector with on-column insert" (liner guiding the syringe needle into the column inlet).
- 4.2.3 **Flame-ionisation detector** and converter-amplifier.
- 4.2.4 **Recorder-integrator** for use with the converter-amplifier (4.2.3), rate of response below 1 second, with variable paper speed.
- 4.2.5 **Capillary column, fused silica, 3 – 7 m length, 0,25 – 0,32 mm internal diameter, internally coated with SE 52, SE 54 liquid phase (5% diphenyl dimethyl polysiloxane) to a uniform thickness of 0,10 – 0,15 µm.**
- (note: thermal degradation may affect analysis by diminishing recovery on C58 an C60, if this happens a shorter column should be used) – Normally a 5 m column leads to acceptable results.
- 4.3 **Microsyringe**, 10 µl, suitable for on-column injection.
- 4.4 **Microsyringe**, 100 µl
- 4.5 Usual laboratory glassware.

5 **REAGENTS**

- 5.1 **Carrier gas** : hydrogen or helium, pure, for gas chromatography.
- 5.2 **Auxiliary gases** :
- Hydrogen, pure, for gas chromatography;
 - Air, pure, for gas chromatography;
 - Nitrogen, pure for gas chromatography.
- 5.3 **Silylating reagent** : mix equal volumes of:
- 1) pyridine
 - 2) bistrimethylsilylfluoroacetamide/trimethylchlorosilane (BSTFA/TMCS 99:1, v/v).
- Note: ready to use solution are available; the silylating solution should be freshly made.
- 5.4 **n-Heptane** for analysis
- 5.5 **Reference samples** : pure diglycerides and triglycerides and their mixtures, with known composition.
- 5.6 **Methyl-terbutyl-ether**
- 5.7 **Dinonadecanoine sample solution** (internal standard), 0,1 % w/v in methyl-terbutyl-ether.

6.4 Peak identification

The triglycerides peaks identification is carried out from the retention times by comparing them with mixtures of known composition; they are eluted in the following order: C50, C52, C54, C56, C58, C60, C62.

The diglycerides peak identification is carried out from the retention times by comparing them with mixtures of known di-acylglycerols. Diglycerides are eluted according to the following order : 1,2 C32; 1,3 C32; 1,2 C34; 1,3 C34; 1,2 C36 1,3 C36 (see figures 1,2,3 and 4).

6.5 Determination of percentage content of each triacylglycerol

Calculate the areas of each peak through an electronic integrator.
Results are expressed as percentage of C50, C52, C54, C56, C58, C60, C62
Other peaks showed by gas chromatographic run haven't to be considered.

All triacylglyceroles, including those lying in between the major peaks, must be integrated.
Each odd triglycerides (see figure 5) is combined to the one that precedes in order to have the sum of all triglycerides normalized to 100%.

6.6. Determination of percentage content of each 1,2 di-acylglycerol

Calculate the areas of each peak through electronic integrator.
Results are expressed as percentage of 1,2 C32, 1,3 C32, 1,2 C34, 1,3 C34, 1,2 C36, 1,3 C36 on the sum of DAG areas. Other eventual peaks showed by gas chromatographic run haven't to be considered :

$$\% \text{ of single diglyceride} = \frac{A_x}{\sum A_{\text{DAG}}}$$

Where :

A_x : area corresponding to the peak of diglyceride x,

$\sum A_{\text{DAG}}$: sum of areas of all diglyceride peaks.

Express these results to one decimal place.

6.7 Determination of weigh percentage total content of di-acylglycerols

By using the integrator, calculate the areas of internal standard and diglycerides compounds, by considering only the peaks that are reported in previous 6.5 point.

You compute each single diglyceride, in % weight (gr/100g) of fat matter, as follows :

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

where :

A_x : area corresponding to the peak of single diglyceride,

A_s : area corresponding to internal standard peak,

m_s : added amount (mg) of internal standard,

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

Express these results to one decimal place.

7 EXPRESSION OF RESULTS

Single triglyceride percentage. Compute the sum of percentages of triglycerides. Results have to be expressed to two decimal place.

1,2 diglyceride percentage. Compute the sum of percentages of 1,2 diglycerides. Results have to be expressed to one decimal place.

Weight percentage. Sum the amounts of all detected diglycerides. Results have to be expressed to one decimal place.

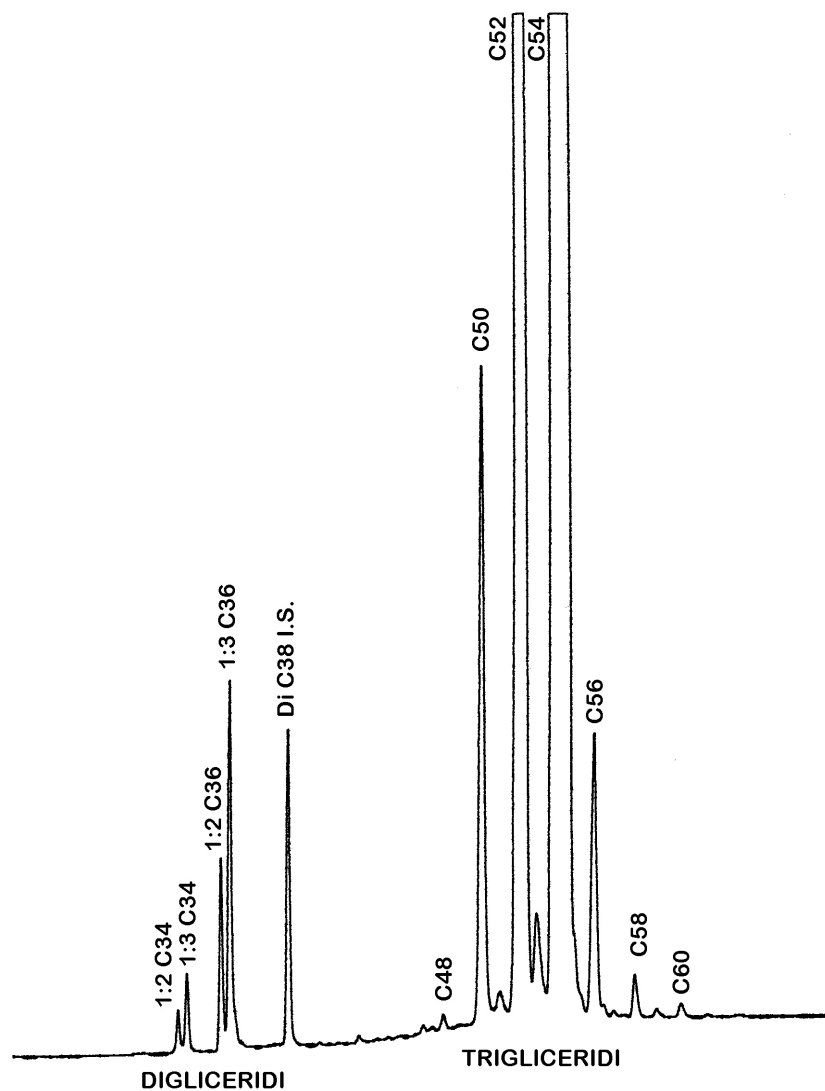


Figure 1: GLC trace of diacylglycerol and triacylglycerol of an olive oil sample

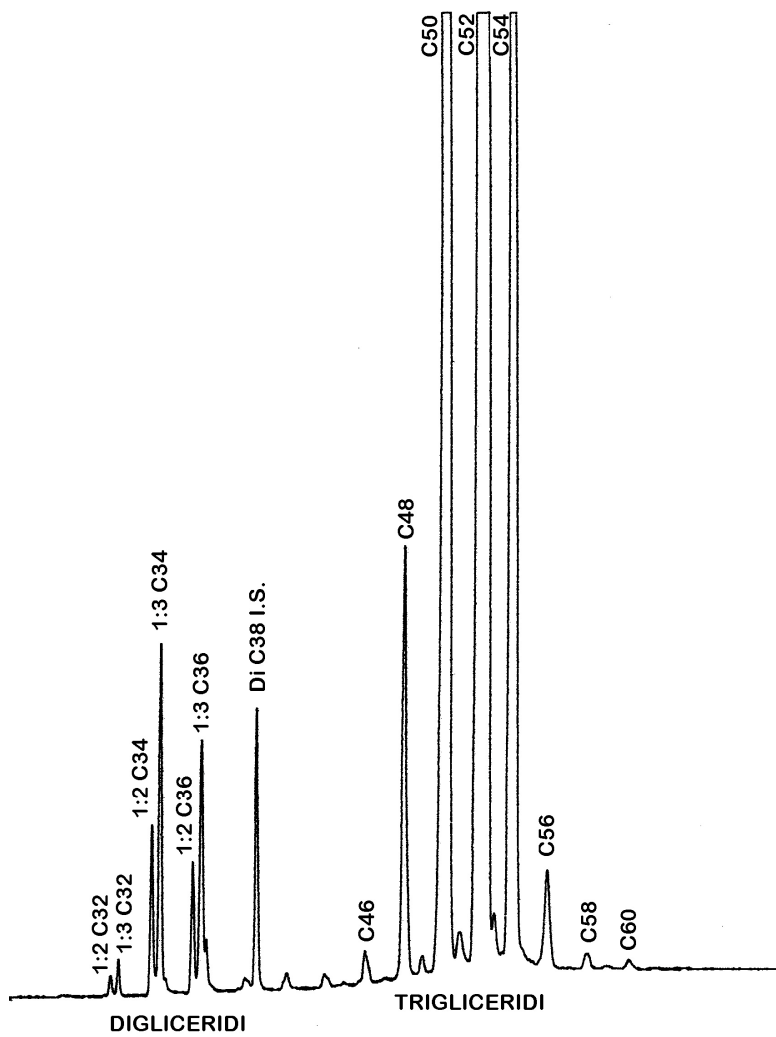


Figure 2: GLC trace of diacylglycerol an triacylglycerol of a palm olein sample

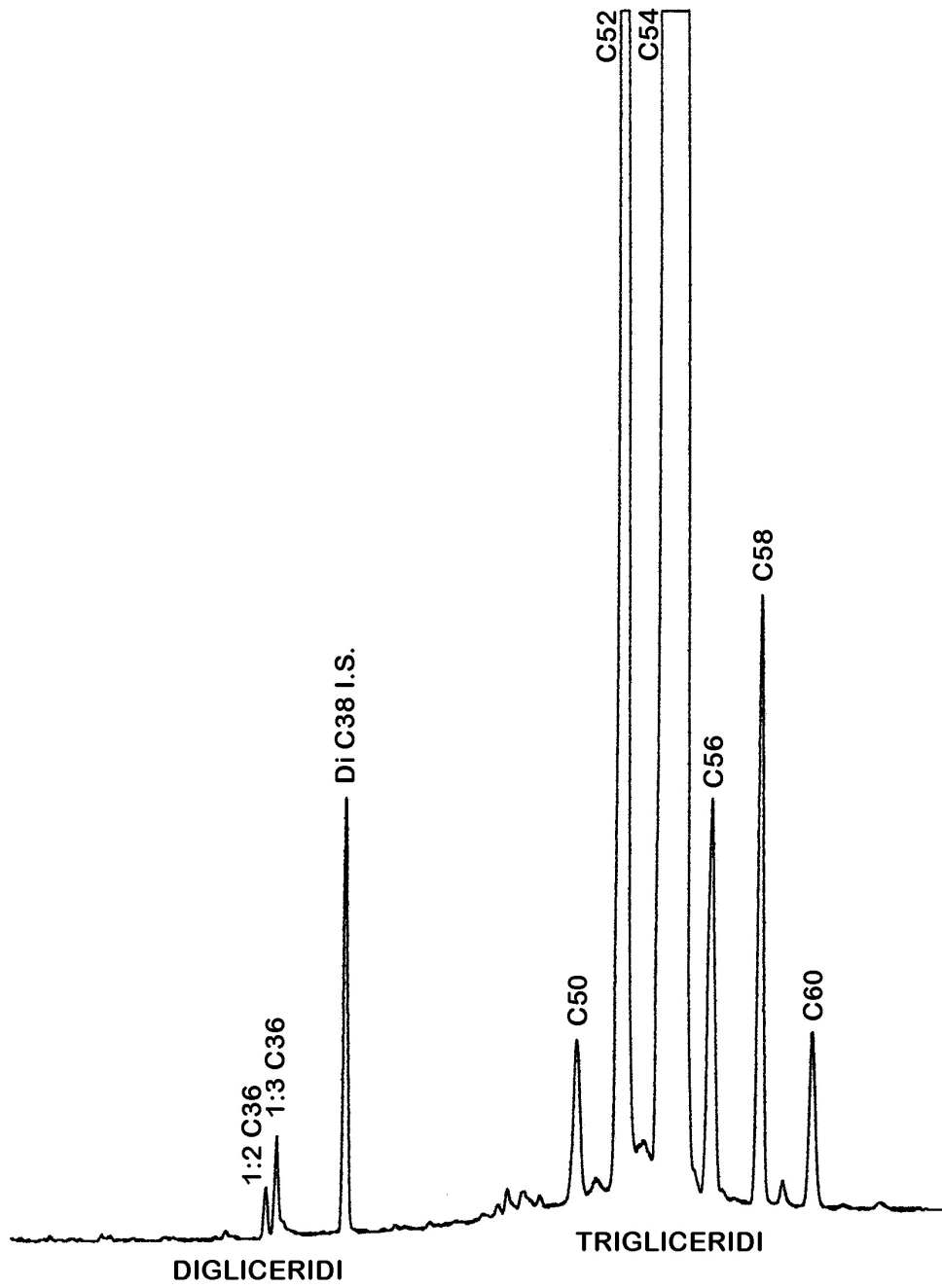


Figure 3: GLC trace of diacylglycerol an triacylglycerol of an high oleic sunflower oil sample

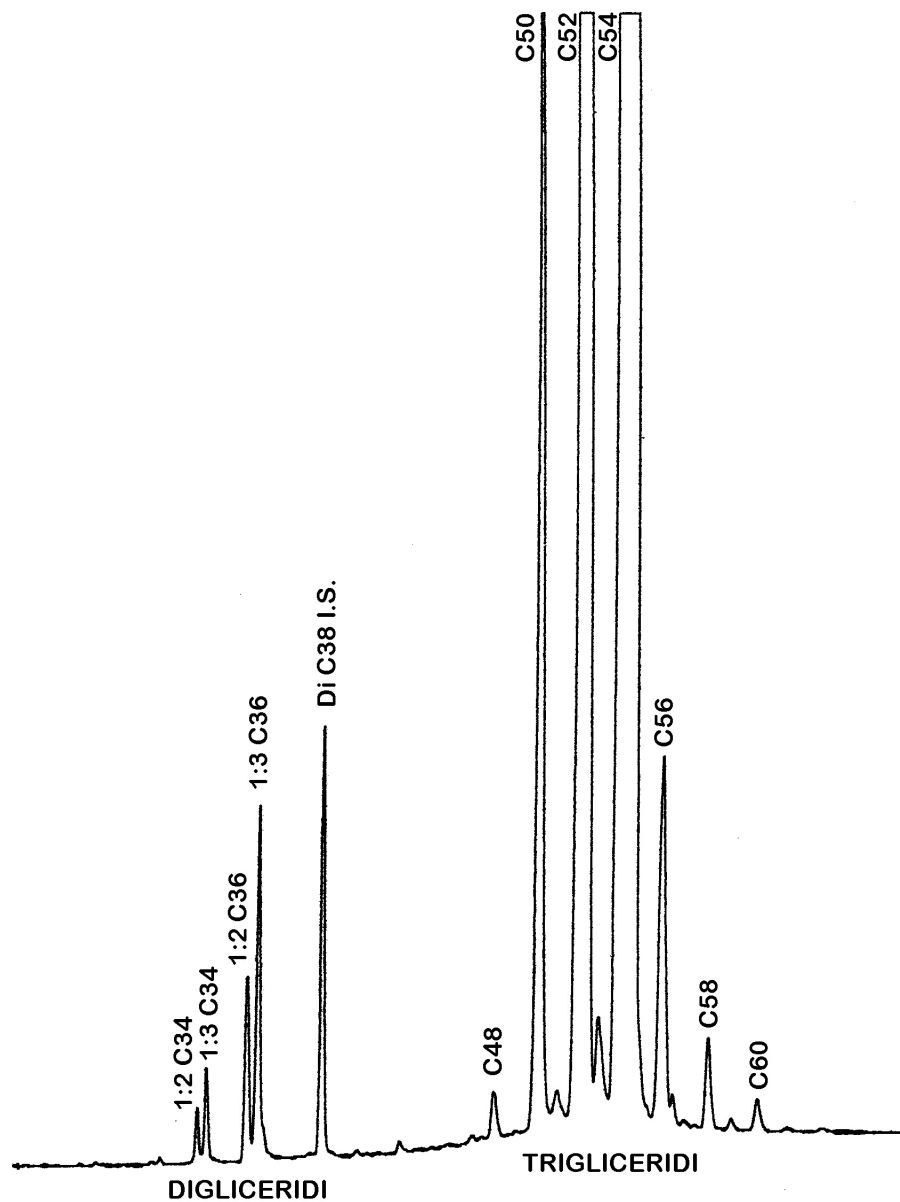


Figure 4: GLC trace of diacylglycerol an triacylglycerol of an olive oil mixed with palm olein and high oleic sunflower oil

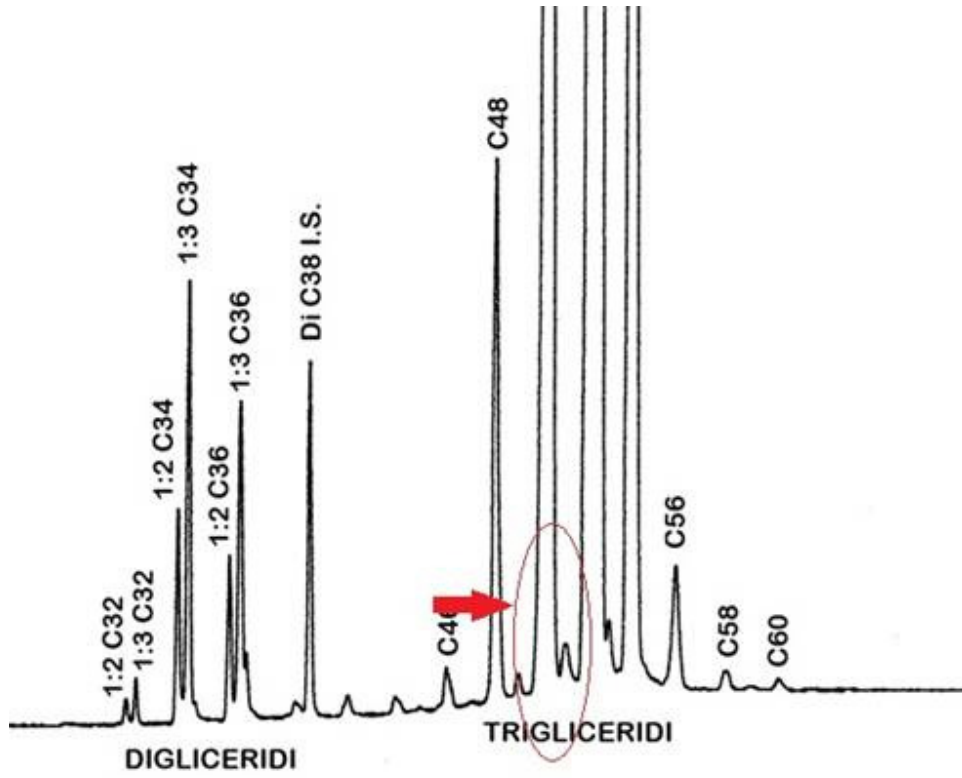


Figure 5: [example of peaks integration](#)

PRECISION VALUES OF THE METHOD FOR DETERMINATION OF COMPOSITION OF TRIACYLGLYCEROLS AND COMPOSITION AND CONTENT OF DI-ACYLGLYCEROLS

1. Analysis of the collaborative test results

The precision values of the method are given in the table overleaf.

Ten laboratories took part in the collaborative test arranged by Executive Secretariat in 2013. The laboratories were from five countries.

The test was performed in duplicate on five samples:

Sample 1 – Extra virgin olive oil with 5% of extraneous oils (desterolate sunflower, avocado, peanut, rapeseed and hazelnut oils)

Sample 2 – Extra virgin olive oil with 10% of extraneous oils (desterolate sunflower, avocado, peanut, rapeseed and hazelnut oils)

Sample 3 – High quality extra virgin olive oil

Sample 4 – Extra virgin olive oil from retail

Sample 5 – Extra virgin olive oil with palm, desterolate sunflower, avocado and hazelnut oils

The results of the collaborative test organised by the IOC Executive Secretariat underwent statistical analysis according to ISO 5725 “Accuracy (trueness and precision) of measurement methods and results”. Outliers were examined by applying Cochran’s and Grubbs’s tests to the laboratory results for each determination (replicates a and b) .

The tables lists:

| | |
|----------------------------|---|
| n | number of participating laboratories |
| outliers | number of laboratories with outlying values |
| mean | mean of the accepted results |
| r | value below which the absolute difference between two single independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time may be expected to lie with a probability of 95% |
| S_r | repeatability standard deviation |
| RSD_r (%) | repeatability coefficient of variation ($S_r \times 100 / \text{mean}$) |
| R | value below which the absolute difference between two single test results obtained with the same method on identical test material in different laboratories with different operators using different equipment may be expected to lie with a probability of 95%. |
| S_r | reproducibility standard deviation |
| RSD_r (%) | reproducibility coefficient of variation ($S_r \times 100 / \text{mean}$) |

Single triglyceride percentage (%)

TAG 48

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---------------------------|----------|----------|----------|----------|----------|
| N | 10 | 10 | 10 | 10 | 10 |
| outliers | 1 | 1 | 2 | 2 | 1 |
| mean | 0,088 | 0,115 | 0,044 | 0,055 | 0,224 |
| r | 0,023 | 0,021 | 0,020 | 0,020 | 0,044 |
| S_r | 0,0083 | 0,0074 | 0,0070 | 0,0070 | 0,0157 |
| RSD_r(%) | 9,4% | 6,5% | 16,0% | 12,8% | 7,0% |
| R | 0,0545 | 0,0808 | 0,0683 | 0,0589 | 0,0858 |
| S_R | 0,0195 | 0,0289 | 0,0244 | 0,0210 | 0,0306 |
| RSD_R(%) | 22,0% | 25,2% | 55,9% | 38,4% | 13,7% |

TAG 58

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---------------------------|----------|----------|----------|----------|----------|
| N | 10 | 10 | 10 | 10 | 10 |
| outliers | 0 | 0 | 0 | 0 | 1 |
| mean | 0,305 | 0,405 | 0,255 | 0,221 | 0,369 |
| r | 0,034 | 0,062 | 0,077 | 0,029 | 0,040 |
| S_r | 0,012 | 0,022 | 0,028 | 0,010 | 0,014 |
| RSD_r(%) | 3,9% | 5,5% | 0,8% | 4,7% | 3,8% |
| R | 0,15 | 0,19 | 0,15 | 0,14 | 0,19 |
| S_R | 0,052 | 0,068 | 0,054 | 0,050 | 0,068 |
| RSD_R(%) | 17,0% | 16,8% | 21,2% | 22,6% | 18,4% |

1,2 diglyceride percentage (%)

1,2 DAG

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---------------------------|----------|----------|----------|----------|----------|
| N | 10 | 10 | 10 | 9 | 8 |
| outliers | 1 | 1 | 1 | 1 | 0 |
| mean | 36,83 | 31,36 | 49,85 | 29,43 | 29,24 |
| r | 1,5 | 4,3 | 4,8 | 4,8 | 1,7 |
| S_r | 0,53 | 1,5 | 1,7 | 1,7 | 0,62 |
| RSD_r(%) | 1,4% | 4,9% | 3,5% | 5,9% | 2,1% |
| R | 16 | 11 | 6,9 | 7,9 | 9,4 |
| S_R | 5,8 | 4,0 | 2,5 | 2,8 | 3,4 |
| RSD_R(%) | 15,7% | 12,8% | 5,0% | 9,5% | 11,6% |

Diglycerides content (g/100g)

Total DAG

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---------------------------|----------|----------|----------|----------|----------|
| N | 10 | 10 | 10 | 10 | 10 |
| outliers | 1 | 1 | 1 | 1 | 1 |
| mean | 1,48 | 1,62 | 1,23 | 1,47 | 2,33 |
| r | 0,16 | 0,21 | 0,19 | 0,15 | 0,21 |
| S_r | 0,058 | 0,074 | 0,067 | 0,053 | 0,076 |
| RSD_r(%) | 3,9% | 4,6% | 5,5% | 3,6% | 3,2% |
| R | 0,45 | 0,59 | 0,48 | 0,50 | 0,67 |
| S_R | 0,16 | 0,21 | 0,17 | 0,18 | 0,24 |
| RSD_R(%) | 10,8% | 13,0% | 13,8% | 12,2% | 10,3% |

3. References

ISO 5725 – Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

ISO 5725 - 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.

ISO 5725 – Accuracy (trueness and precision) of measurement methods and results – Part 5: Alternative methods for the determination of the precision of a standard measurement method.

ISO 5725 – Accuracy (trueness and precision) of measurement methods and results – Part 6: Use in practice of accuracy values.
