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# Certification of the fatty acid profile of soya-maize oil blend BCR-162R

M. Dabrio, M. Marini, M. Ricci, F. Ulberth, H. Emons









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# BCR information

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## Summary

This report describes the production of the reference material BCR-162R, certified for its fatty acid profile. It contains all the required steps that ensure the high quality of the final product, from the processing to the value assignment, such as the homogeneity and stability studies, according to ISO Guide 34 [1]. The matrix material, a soya-maize oil blend, is packed in amber glass ampoules containing approximately 5.5 g of the oil blend and kept at a temperature of -20 ºC. Studies performed at IRMM confirmed homogeneity and stability of the fatty acids in the oil matrix under the evaluated conditions. The characterisation of the material, organised and coordinated by IRMM, was done by an interlaboratory comparison. The certified values were obtained as the unweighted mean of the laboratory means of the accepted sets of results for each analyte, and the expanded uncertainty associated ($k$ = 2) includes contributions from the characterisation, the homogeneity and the stability. The values certified for the fatty acid profile in BCR-162R are the following:

| FAME | [1]**Certified value** [g/100 g total FAME] | [2]$U_{CRM}$ [g/100 g total FAME] | p |
|---|---|---|---|
| 16:0 | 10.74 | 0.16 | 10 |
| 18:0 | 2.82 | 0.04 | 10 |
| 9c-18:1 | 25.4 | 0.4 | 7 |
| 9c,12c-18:2 | 54.13 | 0.25 | 9 |
| 9c,12c,15c-18:3 | 3.35 | 0.05 | 8 |

[1]Unweighted mean value, expressed as g of individual fatty acid methyl ester per 100 g total fatty acid methyl ester, of the accepted sets of data (p) obtained in a different laboratory and/or with a different method of determination. Monoenoic and polyunsaturated fatty acids certified correspond to the major isomers identified (oleic acid, linoleic acid and linolenic acid). The values are traceable to methodology applying gas chromatography.

[2]Expanded uncertainty with a coverage factor of $k$ = 2, according to the Guide for the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

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# 1. Glossary

| | |
|---|---|
| ANOVA | Analysis of variance |
| *b* | Slope of the linear regression |
| BCR | Community Bureau of Reference |
| CAP | Certification Advisory Panel |
| CI | Confidence interval |
| CRM | Certified Reference Material |
| *df* | Degrees of freedom |
| ERM | European Reference Material |
| *F* | *f* value |
| $F_{crit}$ | Critical *f* value |
| FAME | Fatty acid methyl ester |
| GC-FID | Gas Chromatography-Flame Ionisation Detector |
| *k* | Coverage factor |
| KFT | Karl-Fischer Titration |
| L | Laboratory code |
| meq | Milliequivalent |
| $MS_{within}$ | Mean of squares within groups (ANOVA) |
| $MS_{between}$ | Mean of squares between groups (ANOVA) |
| MS | Mass Spectrometry |
| n | Number of replicates |
| p | Number of accepted data sets |
| PTFE | Polytetrafluoroethylene |
| PUFA | Polyunsaturated fatty acid |
| *RSD* | Relative standard deviation |
| *RSE* | Relative standard error |
| *s* | Standard deviation |
| $s_b$ | Standard error of the slope of the linear regression |
| $s_{bb}$ | Between-ampoule (in)homogeneity standard deviation |
| $s_e$ | Standard error |
| $s_{wb}$ | Within-ampoule standard deviation |
| *SS* | Sum of squares (ANOVA) |
| *t* | *t* value of the Student's t distribution |
| $t_{crit}$ | Critical *t* value |
| TBHQ | *Tert*-butylhydroquinone |
| $u_{bb}$ | Standard uncertainty due to between-ampoule (in)homogeneity |
| $u_{bb}$* | Standard uncertainty due to the inhomogeneity that can be hidden by the method repeatability |
| $u_{char}$ | Standard uncertainty from characterisation |
| $U_{CRM}$ | Expanded uncertainty associated to the certified value of CRM |
| $u_{lts}$ | Standard uncertainty associated to long-term (in)stability |
| $u_{sts}$ | Standard uncertainty associated to short-term (in)stability |

# 2. Introduction

## *General*

Oils and fats (lipids) are ingredients of all foods and amount to approximately 40 % of the dietary calories in Western Europe and North America. Their composition is vital to good nutrition. Whereas lipids have been defined as fatty acids, their derivatives and substances related biosynthetically or functionally to these compounds, the fatty acids are described as compounds synthesised in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. In general fatty acids contain an even number of carbon atoms in straight chains, which typically ranges from 14 to 24 [2]. In terms of function, the dietary essential fatty acids, linoleic and linolenic acids and the longer-chain components derived from them, may be required to confer some distinctive physical properties upon the cell membranes, in addition to serving as the precursors to prostaglandins.

Lipids are of great economic importance as agricultural products and they are major items of international commerce. Evidently, health aspects related to these products are also of utmost importance. Therefore quality and authenticity are relevant terms for both health and economical aspects. Detecting adulteration with materials of lower cost is possible by the determination of the fatty acid profile, which is characteristic for the lipid materials. In this context gas chromatography with flame ionisation detection (GC-FID) is a technique that provides a convenient means of separating and determining many of the individual saturated and unsaturated fatty acids and their isomers. The determination of fatty acids by GC-FID involves initial transesterification, formation of the volatile methyl esters of the fatty acids which are separated by GC.

The reference material BCR-162R, soya-maize oil blend certified for its fatty acid profile, is produced with the aim of improving comparability of results and to ensure accuracy and traceability of the results obtained by the laboratories.

The present report covers in detail the production of BCR-162R, carried out by The Institute for Reference Materials and Measurements (IRMM).

## *Definition of the measurand*

For practical reasons fatty acids are most commonly determined as their methyl esters. By convention, the edible oil industry expresses the mass fractions of individual fatty acids in vegetable and animal oils and fats (butter fat excepted) as g of individual fatty acid methyl ester (FAME) per 100 g total fatty acid methyl esters. This special convention was taken into account throughout the report when the results for individual fatty acids are given.

The procedure employed for the expression of the results involved normalisation, i.e. the use of 100 % as the sum of all the fatty acid methyl esters detected and the levels of the individual fatty acid methyl esters as a proportion of the 100 %.

### *Nomenclature*

Four nomenclature systems are commonly accepted for fatty acids. Shorthand designation includes the carbon number, representing the total number of carbon atoms in the fatty acid, and a colon followed by a number indicating the degree of unsaturation of the molecule. As an example, 18:1 refers to n-octadecenoic acid, having one double bond. The trivial name for this compound is oleic acid, and the systematic nomenclature (IUPAC) specifies unsaturation positions in relation to the carboxyl group. In the present example, it could represent *cis*-11-octadecenoic acid that in shorthand notation is designated as 11-18:1 or 11c-18:1. The position of the double bond can alternatively be denoted in the form (*n*-x) or omega-x, where *n* represents the chain-length of the fatty acid and x is the number of carbon atoms from the double bond in the terminal region of the molecule. Again, for the given example it would be denoted as 18:1(*n*-7), having $CH_3(CH_2)_5CH=CH(CH_2)_9COOH$ as structural formula. A list of common fatty acids is presented in Table 1.

Table 1. Nomenclature of common fatty acids

| Shorthand notation | Trivial name | Systematic name* (IUPAC) |
|---|---|---|
| 14:0 | Myristic acid | Tetradecanoic acid |
| 16:0 | Palmitic acid | Hexadecanoic acid |
| 18:0 | Stearic acid | Octadecanoic acid |
| 20:0 | Arachidic acid | Eicosanoic acid |
| 9c-16:1 | Palmitoleic acid | 9-hexadecenoic acid |
| 9c-18:1 | Oleic acid | 9-octadecenoic acid |
| 9t-18:1 | Elaidic acid | *Trans*-9-octadecenoic acid |
| 11c -18:1 | Cis-vaccenic acid | 11-octadecenoic |
| 9c,12c-18:2 | Linoleic acid | 9,12-octadecadienoic acid |
| 9c,12c,15c-18:3 | $\alpha$-linolenic acid | 9,12,15-octadecatrienoic acid |
| 6c,9c,12c-18:3 | $\gamma$-linolenic acid | 6,9,12-octadecatrienoic acid |

*Unless specified, configuration of the double bond is *cis*.

Target compounds for the BCR-162R material characterisation include fatty acids designated as "major" components:

| Shorthand notation | Systematic name (IUPAC) | Major contribution |
|---|---|---|
| 16:0 | n-hexadecanoic acid | Palmitic acid |
| 18:0 | n-octadecanoic acid | Stearic acid |
| 9c-18:1 | n-octadecenoic acid | Oleic acid |
| 9c,12c-18:2 | n-octadadienic acid | Linoleic acid |
| 9c,12c,15c-18:3 | n-octadecatrienoic acid | $\alpha$-Linolenic acid |

The term "minor" in this report refers to fatty acids present at levels less than 1 % (mass fraction).

# 3. Participants

### *Processing, homogeneity and stability studies*

• European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM) , Geel, BE

### *Characterisation**

The participating laboratories were:

- Bundesforschungsanstalt für Ernährung, Kiel, DE
- Danmarks Fødevareforskning, Søborg, DK
- European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, BE
- Instituto de la Grasa (CSIC), Sevilla, ES
- Instituto del Frío (CSIC), Madrid, ES
- Istituto Sperimentale Lattiero-Caseario, Lodi, IT
- Kent County Council, Kent, GB
- Mylnefield Research Services Ltd., Dundee, GB
- Stazione Sperimentale per le Industrie degli Oli e dei Grassi, Milano, IT
- Universita' di Bologna, Food Science Department, Bologna, IT

*In alphabetical order

### *Project Management & Data evaluation*

• European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

# 4. Certification project time schedule

| Activity | Finalised by |
|---|---|
| Processing | August 2003 |
| Homogeneity testing | November 2005 |
| Short-term stability testing | December 2005 |
| Long-term stability testing | January 2006 |
| Characterisation | October 2006 |

## 5. Processing of the material

Ten litres of maize and ten litres of soya oil were obtained from Vandemoortele N. V. Izegem, Belgium, with full specification of origin including an analysis of the fatty acid profile using GC-FID and the peroxide values indicated in Table 2.

Table 2. Approximated composition of the soya and maize oils employed as raw material.

| Fatty acid [g/100 g total FAME] | soya oil | maize oil |
|---|---|---|
| 16:0 | 10.8 | 10.7 |
| 18:0 | 4.0 | 1.8 |
| 18:1 | 23.8 | 29.1 |
| 18:2 | 52.6 | 56.3 |
| 18:3 | 6.8 | 0.9 |
| 20:0 | 0.4 | 0.5 |
| 20:1 | 0.2 | 0.3 |
| 22:0 | 0.4 | 0.1 |
| Peroxide value [meq/kg] | 0.3 | 0.3 |

The experimental set-up employed for the processing of the material was made up by a re-circulating cooler/heater (Haake C40, Karlsruhe, DE) with a cryothermostat (Haake C6) connected to a 70-L glass vessel with a liquid jacket. The water was allowed to circulate at 45 °C and subsequently the vessel was filled with the oil blend. Inside the vessel a PTFE gas-line flushed nitrogen during heating and ampouling to achieve an an-oxic environment. The mixture was stirred simultaneously. Automatic ampouling was carried out in a ROTA machine (ROTA R910/PA, Wehr/Baden, DE).



Figure 1. Oil blend in a 70-L glass vessel with a liquid jacket

The oil blend was prepared by adding 6.5 kg of soya oil and 6.5 kg of maize oil into the vessel (Figure 1). In a separate container 250 g of maize oil and 250 g of soybean oil were mixed and warmed to 40 °C and 3.5 g of an antioxidant, *tert*-butylhydroquinone (TBHQ from Merck, Darmstadt, DE) were added to the 500 g of oil blend. The mixture was placed in an ultrasonic bath for 30 minutes. Once the TBHQ was completely dissolved the resulting mixture was added to the 13 kg oil-blend contained in the vessel. The blend was heated to 45 ºC and mixed by bubbling $N_2$ through it for 2 hours before ampouling. The ampouling machine was operated with argon as fill gas. The first 185 units were discarded and a total of 1800 units were labelled according to fill-order. Each unit was filled with approximately 5.5 g soybean-maize oil blend in 10-mL amber ampoule (Figure 2). The batch was stored at -20 ºC.



Figure 2. BCR-162R ampoules containing approximately 5.5 g soya-maize oil blend

### *Quality control during processing*

With regular intervals a filled and closed ampoule was collected during the filling sequence to check for the gross mass.

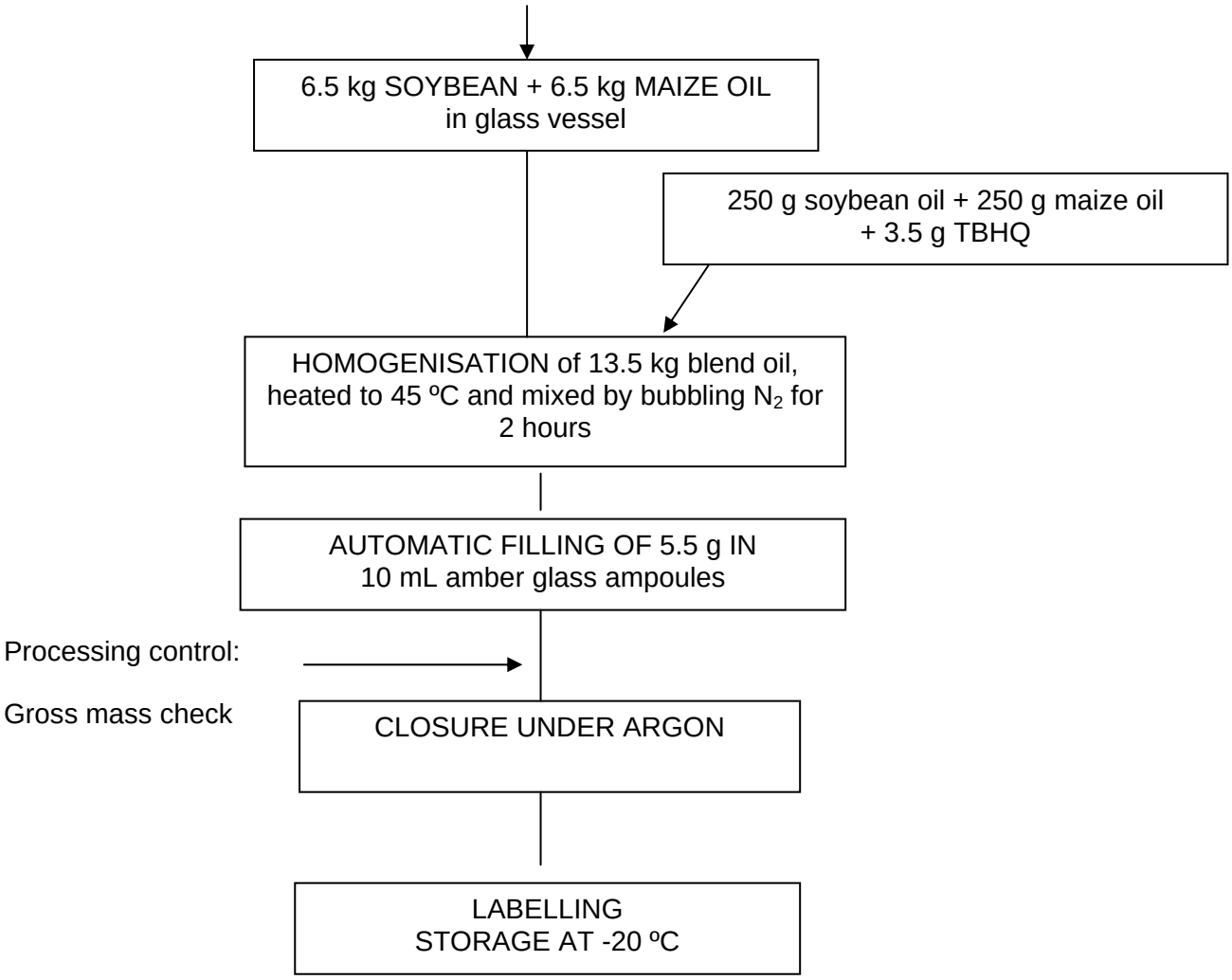
After processing three samples were randomly chosen for water analysis by Karl-Fischer titration (Table 3) confirming the expected low levels present in the oil blend material.

Table 3. Moisture content in BCR-162R as measured by Karl Fischer titration (n = 2)

| Ampoule number | Water content, [g/100 g] |
|---|---|
| 285 | 0.012 |
| 482 | 0.009 |
| 1013 | 0.008 |

The main processing steps are summarised in the following flow diagram:

## FLOW DIAGRAM FOR PROCESSING OF BCR-162R



## 6. Homogeneity

The aim of the homogeneity study was to assess the regular distribution of the components to be certified in the whole batch of reference material. That means that one has to quantify heterogeneity in terms of concentration of the analyte to be certified, among the randomly chosen ampoules [3].

To such purpose 40 ampoules from the stock batch were randomly selected and analysed in duplicate for their fatty acid profile using an in-house validated method based on GC-FID. Briefly, a portion of 100 mg of BCR-162R oil blend was submitted to a base-catalysed transesterification according to ISO 15884 [4]. Further saponification of the methyl esters was avoided by addition of sodium hydrogen sulfate. Subsequently the GC-FID analysis, based on ISO 15885 [5] allowed the determination of the fatty acid methyl esters (FAMEs) in the sample. The data obtained, shown in Annex 1, are expressed as the average mass fractions resulting from three injections of the same replicate. For monoenoic and polyunsaturated fatty acids (PUFA) major isomeric contributions were monitored.

Suspicious data (outliers), normality and unimodality were checked for all the analytes (results not shown). All the analytes followed a normal and unimodal distribution, which are important prerequisites for the analysis of the variances (ANOVA). Only one outlier was detected for 16:0, corresponding to ampoule number #394 in the processing sequence. Since the overall variability of the results obtained is very low, almost negligible, the presence of that particular outlier is not considered as technically relevant. It was not excluded for technical reasons and thus the data point was accounted for further evaluation. The homogeneity data for each individual FAME were submitted to single factor ANOVA and the corresponding results were used for the estimation of a possible inhomogeneity. The parameters employed were the so called within ($s_{wb}$) and between ampoule homogeneity ($s_{bb}$), estimated according to the following expressions:

$$s_{wb} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$

$$s_{bb} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$

Where $n$ represents the number of replicates for each sample unit, equal to 2, and $\bar{y}$ the average content of each fatty acid. The results obtained are illustrated in Table 4.

Table 4. Results obtained with SoftCRM software for each FAME based on the data given in Annex 1.

| FAME | Mass average [g/100 g total FAME] | $s_{bb}$ [g/100 g total FAME] | $s_{bb}$ relative | $s_{wb}$ [g/100 g total FAME] | $s_{wb}$ relative | $u_{bb}$* [g/100 g total FAME] | $u_{bb}$* relative |
|---|---|---|---|---|---|---|---|
| **16:0** | 10.80 | 0.01 | 0.1 | 0.01 | 0.1 | 0.004 | 0.0 |
| **18:0** | 2.80 | 0.002 | 0.1 | 0.01 | 0.2 | 0.002 | 0.1 |
| **9c-18:1** | 24.76 | 0.05 | 0.2 | 0.03 | 0.1 | 0.01 | 0.0 |
| **9c,12c-18:2** | 54.4 | 0.02 | 0.0 | 0.02 | 0.0 | 0.01 | 0.0 |
| **9c,12c,15c-18:3** | 3.27 | 0.01 | 0.4 | 0.01 | 0.3 | 0.003 | 0.1 |

Typically, the uncertainty of between-ampoule homogeneity ($u_{bb}$) is given by the larger value obtained either from $u_{bb}$*, the inhomogeneity that can be hidden by the method repeatability, or $s_{bb}$.

$$u_{bb}* = \frac{RSD_{method}}{\sqrt{n}} \sqrt[4]{\frac{2}{df}}$$

The estimated values for $u_{bb}$* are very close to zero for all FAMEs under evaluation, therefore $u_{bb}$ was given by $s_{bb}$. On the view of the results it is possible to conclude then that, according to the present evaluation, the soya-maize oil blend processed is a homogeneous material at the level of 100 mg sample intake.

For all FAMEs, the $s_{bb}$ values obtained will be taken into consideration as a contribution to the total uncertainty of the certified values of the reference material.

## 7. Minimum sample intake

The determination of the minimum sample intake was performed by testing two different portions of soya-maize oil blend, 50 and 100 mg, which where submitted to the methodology already mentioned in the previous section. Four replicates per sample intake were prepared from the same bottle and injected three times in the GC-FID. Results from the two sample intakes, presented in Annex 2, were tested for statistical difference of the variances for each analyte by performing an F-test at a 95 % of confidence level. According to the results, illustrated in Table 5, a statistically significant difference was found between the variances of the two groups (F > Fcrit) for all the fatty acids investigated. The good repeatability of the results is thus having a large influence on the estimation of F and, subsequently on the conclusion made in statistical terms which does not necessarily reflect the technical importance of the analytical data variation observed. The latter is supported by additional experiments on robustness performed during the in-house method validation (results not shown) where sample intakes from 80 to 120 mg were tested and no significant differences were detected.

Table 5. Results from the statistical analysis of the minimum sample intake experiment data, obtained from the analysis of 50 and 100 mg of oil blend BCR-162R, by GC-FID.

| | **Fatty acid** | | | | |
|---|---|---|---|---|---|
| | 16:0 | 18:0 | 9c-18:1 | 9c,12c-18:2 | 9c,12c,15c-18:3 |
| F | 6.09 | 87.45 | 31.35 | 255.61 | 24.53 |
| Fcrit | 5.99 | 5.99 | 5.99 | 5.99 | 5.99 |
| Statistical significance (95 % confidence level) | Yes | Yes | Yes | Yes | Yes |

Despite all the above mentioned, a minimum sample intake of 100 mg was established for BCR-162R material as a conservative approach.

## 8. Stability studies

The soya-maize oil blend material BCR-162R was processed under conditions designed to ensure maximum long-term stability including the use of good starting materials, the use of nitrogen as purging gas to minimise the presence of oxygen in the oils, the use of an antioxidant and the flushing of argon when sealing the amber glass ampoules. These measures reproduced those employed in the processing of the analogous and previously certified reference material BCR-162. At that time stability of the material was assessed by monitoring not only the property to be certified (the fatty acid profile) but also additional properties used as general stability indicators such as the formation of hydroperoxides and conjugate dienes, the formation of primary products of auto-oxidation and the hydrolysis of the component triglycerides to form mono- or diglycerides (by the detection of free fatty acids). The results of the studies [6] allowed concluding no degradation related to the mentioned parameters at the conditions tested (in particular at – 18 ºC, set as reference temperature). In the present certification of BCR-162R the temperature scheme was modified, -20 ºC was taken as normal storage temperature and -70 ºC as reference, diminishing even more the risk for such degradations. Thus the stability studies performed for BCR-162R benefit from previous experience and focus on the monitoring of the property to be certified, the fatty acid profile.

### *Short-term stability*

Once the material was processed, a number of ampoules were selected by random stratified sampling for the different studies to be performed, according to a previously designed project plan. For the short-term stability study, performed for the selection of the material transport conditions, an isochronous test scheme [7] was applied as follows: two ampoules were kept at a certain temperature (4 ºC, 18 ºC or 40 ºC) for 1, 2 and 4 weeks. The number of ampoules required for the complete short-term stability study was 21 (2 ampoules x 3 temperatures x 3 time point + 3 ampoules at the reference temperature (-70 ºC) corresponding to time point 0). Two independent sub-samples per ampoule were analysed by a GC-FID method described in Section 6.

The statistical evaluation of the results at each temperature (4 ºC, 18 ºC and 40 ºC) was done as follows:

For a certain temperature (4 ºC, 18 ºC or 40 ºC) the results, expressed as % for each fatty acid, are grouped by time points. The slope of the regression lines (*b*) generated by the variation of the fatty acid mass fraction *vs.* the time was calculated and the significance of the

slope was evaluated. A value for the slope which was significantly different from zero would indicate degradation of the material.

The slope was considered as statistically significant when $b/s_b > t_{\alpha,df}$

where,

$b$ is the slope of the regression line

$s_b$ is the standard error of the slope

$t_{\alpha,df}$ is the critical t-value (two-tailed) for $\alpha = 0.05$ (95 % confidence level) and $df$ degrees of freedom

The results of the isochronous testing scheme at 4 ºC, 18 ºC and 40 ºC are given in Annex 3. Figures for the slopes at the different storage temperatures as well as the estimated t values, obtained as described above, are illustrated in Table 6.

Table 6. Linear regression and statistical parameters associated to the short term stability evaluation of BCR-162R

| Fatty acid | Storage temperature [ºC] | $b$ | $s_b$ | $df$ | $\|b\|/s_b$ | $t_{(0.05;6)}$ | Statistical significance (95 % confidence level) |
|---|---|---|---|---|---|---|---|
| 16:0 | 4 | -0.001 | 0.001 | 6 | 1 | 2.45 | No |
| | 18* | -0.006 | 0.007 | 6 | 0.86 | 2.45 | No |
| | 40 | 0.000 | 0.001 | 6 | 0 | 2.45 | No |
| 18:0 | 4 | -0.001 | 0.001 | 6 | 1 | 2.45 | No |
| | 18 | 0.001 | 0.002 | 6 | 0.50 | 2.45 | No |
| | 40 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| 9c-18:1 | 4 | 0.005 | 0.005 | 6 | 1 | 2.45 | No |
| | 18 | 0.003 | 0.014 | 6 | 2.1 | 2.45 | No |
| | 40 | 0.015 | 0.008 | 6 | 1.9 | 2.45 | No |
| 9c,12c-18:2 | 4 * | 0.006 | 0.005 | 6 | 1.2 | 2.45 | No |
| | 18 | 0.002 | 0.021 | 6 | 0.10 | 2.45 | No |
| | 40 | 0.002 | 0.005 | 6 | 0.4 | 2.45 | No |
| 9c,12c,15c-18:3 | 4 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| | 18 | 0.000 | 0.002 | 6 | 0 | 2.45 | No |
| | 40 | -0.001 | 0.001 | 6 | 1 | 2.45 | No |

n/c = not calculable

* One outlier found, technically accepted value

The slope of the regression line generated by the variation of the amount of each FAME present *vs*. the time was calculated and the significance of the slope was evaluated

statistically with the aim of detecting any possible significant trend that would indicate degradation of the material. According to the criteria applied, none of the storage temperatures tested showed a statistically significant trend for any of the target compounds. Thus, taking into account that the method is precise enough to allow meaningful evaluation, one could conclude that the reference material BCR-162R, exposed to temperatures 4, 18 and 40, ºC for a period of one month, was stable. Consequently BCR-162R will be dispatched without any special cooling conditions, but the material should be stored at -20 ºC on receipt.

### *Long-term stability*

The assessment of the long-term stability was performed through the analysis of samples submitted to an isochronous scheme [7], in an analogous manner as described in the short-term stability Section.

Time-points: 0, 6, 12 and 18 months

Temperatures: -70 (reference temperature), -20 and +4 ºC

The number of bottles required for the complete study was 16 units (2 samples x 2 temperatures x 4 time points). Three replicates per sample were analysed (3 injections per replicate) and the average results are shown in Annex 4. The data was evaluated in compliance with the new ISO Guide 35 [8]. Two outliers were detected, but no technical reason was found to reject the data points. Regression analysis was performed and the

Table 7. Evaluation of long-term stability study, 18 months

| Fatty acid | Storage Temperature [ºC] | slope ($b$) | Std error slope ($s_b$) | $df$ | $\|b\|/s_b$ | $t_{(0.05;6)}$ | Statistical significance (95% CI) |
|---|---|---|---|---|---|---|---|
| 16:0 | + 4 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| | - 20 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| 18:0 | + 4* | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| | - 20 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| 9c-18:1 | + 4 | 0.002 | 0.001 | 6 | 2.0 | 2.45 | No |
| | - 20* | 0.001 | 0.002 | 6 | 0.5 | 2.45 | No |
| 9c,12c-18:2 | + 4 | 0.000 | 0.002 | 6 | 0 | 2.45 | No |
| | - 20 | -0.002 | 0.002 | 6 | 1.0 | 2.45 | No |
| 9c,12c,15c-18:3 | + 4 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |
| | - 20 | 0.000 | 0.000 | 6 | n/c | 2.45 | No |

n/c = not calculable

*One outlier per case detected and considered within the calculations.

results were summarised in Table 7, which illustrates the values of the relevant statistics obtained for the stability scheme of 18 months at the different temperatures.

The slope of the regression line generated by the variation of the percentage of each FAME *vs.* the time is calculated and the significance of the slope is evaluated statistically with the aim of detecting any possible significant trend. Since no trend was observed, it can be deduced from the results that there is no degradation of the samples at any of the temperatures in the time period under evaluation.

Furthermore the uncertainty of stability was estimated for a shelf-life of 5 years ($u_{lts}$) as illustrated in Table 8. The uncertainty introduced by the long-term stability at the normal storage temperature of the batch, -20 ºC, is considered as a contribution to the total uncertainty of the value finally assigned to the material.

Table 8. $u_{lts}$ values for BCR-162R for a shelf life of 5 years.

| | $u_{lts}$ [%] | |
|---|---|---|
| **Fatty acid** | **+4 ºC** | **-20 ºC** |
| **16:0** | 0.2 | 0.2 |
| **18:0** | 0.4 | 0.6 |
| **9c-18:1** | 0.3 | 0.6 |
| **9c,12c-18:2** | 0.2 | 0.2 |
| **9c,12c,15c-18:3** | 0.5 | 0.5 |

## 9. Characterisation of the material

Characterisation of the material was done by a collaborative study including a number of laboratories with recognised expertise in the analysis of fatty acids in food matrices. Collaborators were selected according to quality requirements criteria including a procedure description and evidence on performance for the analysis of fatty acid profile as well as previous successful participation in either national or international comparisons or in certification exercises organised by BCR/IRMM. Likewise a number of collaborators are accredited by national or international accreditation bodies.

The collaborating laboratories were allowed to apply validated methodologies of their own choice, which in all cases included flame ionisation detection (FID) coupled to a gas chromatography (GC) separation technique. Methods are described in Table 9. Briefly, a certain portion of the soya-maize oil blend was quantitatively transesterified by means of different transesterification reagents, added in excess. The efficiency of the transesterification was confirmed at IRMM during method validation by variation of the amount (3 levels) of reagent employed for the reaction. The statistical evaluation of the experimental data obtained indicated no influence of the three levels tested on the results, and thus confirmed the excess of transesterification reagent during transesterification of the fatty acids. Once the fatty acids present in the oil blend were converted into fatty acid methyl esters, the sample was injected into the gas chromatograph. A variety of GC columns having diverse dimensions and stationary phases, ranging from high to extremely high polarity, were employed by the participant laboratories. The column features, in combination with the temperature program applied, allowed the separation of the fatty acid methyl esters, which were eventually detected by the FID at various temperatures. An example of a chromatogram representing the fatty acid composition of BCR-162R is illustrated in Figure 3. The elution order and the degree of resolution of the fatty acids can vary depending on the chromatographic conditions applied.

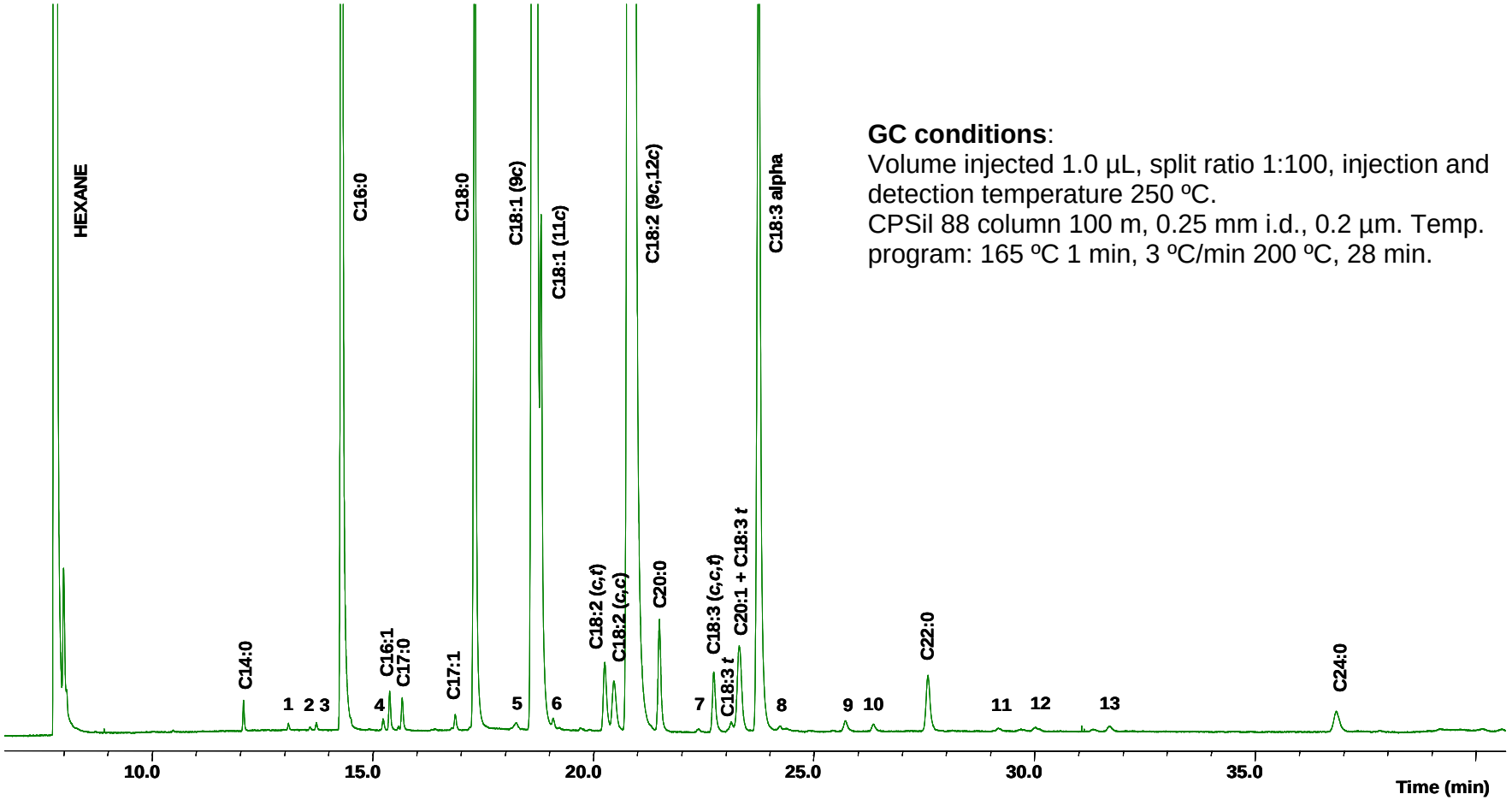



Figure 3. GC-FID chromatogram representing the fatty acid composition of BCR-162R using a CP-Sil 88 chromatographic column. Peaks 1-13, not identified.

Table 9. Methodologies employed in the characterisation of BCR-162R by the different participant laboratories

| Laboratory code | Method | Transesterification | Injection mode and T Volume injected | GC column | Temperature program | Detector (FID) temperature [ºC] |
|---|---|---|---|---|---|---|
| 1 | Analysis by ISO 5508:1995 of FAMEs (ISO 5509:2000) | potassium hydroxide methanolic solution | Split 1:100 225 ºC | SP-2380, 60 m x 0.25 mm id x 0.20 µm film thickness | 165 ºC 10 min, ramp 1.5 ºC/min to 200 ºC, 10 min | 250 |
| 2 | ISO 15884:2002 ISO 15885:2002 | sodium hydroxide methanolic solution | Split 1:100 255 ºC 0.5 µL | CP-Sil 88, 50 m x 0.25 mm id x 0.20 µm film thickness | 50 ºC 1 min, ramp 5 ºC/min to 225 ºC, 3 min at 225 ºC, ramp 1 ºC/min to 237 ºC | 255 |
| 3 | Analysis by ISO 5508:1990 of FAMEs (ISO 5509:2000) | potassium hydroxide methanolic solution | split 1:100 250°C 1 µL | CP Sil 88 50 m x 0,25mm id x 0,20 µm film thickness | 150°C 15min ramp 1,5°Cmin to 200°C | 250 |
| 4 | ISO 15885:2002 | sodium hydroxide methanolic solution | Split 1:100 250 ºC 1 µL | CP-Sil 88, 100 m x 0.25 mm id x 0.20 µm film thickness | 165 ºC 1 min, ramp 3 ºC/min to 200 ºC, 28 min at 200 ºC | 250 |
| 5 | AOCS Ce 1b-89 | sodium hydroxide methanolic solution | Split 1:50 270 ºC 1 µL | CP-Sil 88, 100 m x 0.25 mm id x 0.20 µm film thickness | 177 ºC 40 min, ramp 5 ºC/min to 220 ºC, 15 min at 220 ºC | 300 |
| 6 | | sodium hydroxide methanolic solution | Split 1:100 300 ºC 1 µL | CP-Sil 88, 100 m x 0.25 mm id | 140 ºC, ramp 2 ºC/min to 190 ºC, 10 min, ramp 2 ºC/min to 225 ºC, 12.5 min | 350 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | (EC) No 128/2002 | Diazomethane Potassium hydroxide methanolic solution | Split 1:40 250 ºC 1 µL | RTX 2330, 30 m x 0.25 mm x 0.2 µm film thickness | 100 ºC rate 4 ºC/min to 250 ºC, 5 min at 250 ºC | 250 |
| 8 | ISO 15884:2002 | Potassium hydroxide methanolic solution | On-column 1 µL | Supelcowax 10, 30 m x 0.32 mm x 0.25 µm film thickness | 40 ºC 4 min, rate 25 ºC/min to 150 ºC, 1 min 150 ºC, rate 4 ºC/min to 220 ºC, 5 min at 220 ºC, rate 4 ºC/min to 240 ºC, 10 min 240 ºC | 250 |
| 9 | | potassium hydroxide methanolic solution | Split 1:50 265 ºC 1µL | SGE BPX70, 60 m x 0.25 mm id x 0.25 µm film thickness | 120 ºC, rate 3 ºC/min to 245 ºC, 5 min at 245 ºC | 280 |
| 10 | ISO 15884:2002 | potassium hydroxide methanolic solution | Split 1:100 250 ºC 0.5 µL | CP-Sil 88, 50 m x 0.25 mm id x 0.20 µm film thickness | 180 ºC 10 min, rate 10 ºC/min to 220 ºC, 4 min | 240 |

Table 10. Approximate composition of calibration mixtures (g/100 g)

| Fatty acid Methyl ester | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|
| g/100 g | 10 | 5 | 25 | 55 | 5 |

Once optimised, the FID response of fatty acid methyl ester is directly proportional to the number of CH-groups and therefore, the molar FID response factors should be the same for the same amount of substance fraction of any of the fatty acids investigated in this CRM. In terms of calibration and response factors, the different laboratories were requested to use either response factors correction (typically normalised to palmitic acid) for the expression of their results or otherwise to confirm that relative response factors were equal to one. For such purpose collaborators employed solutions, in most cases gravimetrically prepared in their own laboratories, containing the target fatty acids approximately in the proportions expected in the reference material (Table 10).

Three BCR-162R units randomly selected from the processed batch and an additional control sample of undisclosed composition were sent to each collaborating laboratory. A total of ten collaborators were requested to analyse BCR-162R under reproducibility conditions, i.e. each bottle on a different day in two replicates plus the control sample using their own validated methodology. Individual results were reported as g/100 g of total fatty acid methyl esters, for major and minor compounds, for each injection performed.

### *Evaluation of major fatty acids*

Annex 5 summarises the average results from the BCR-162R samples sorted by laboratory code (L1 to L10) and Figures 4-8 show the average mass fraction values for the major fatty acid components detected: 16:0; 18:0; 9c-18:1; 9c,12c-18:2 and 9c,12c,15c-18:3, respectively.

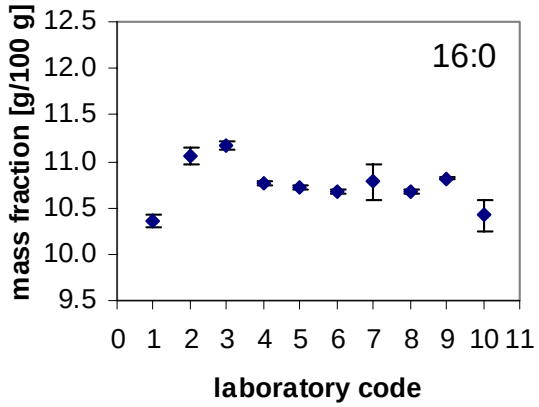



Figure 4. 16:0 sets of data, mean ± s expressed in g/100 g of total FAME, from all participating laboratories for BCR-162R.

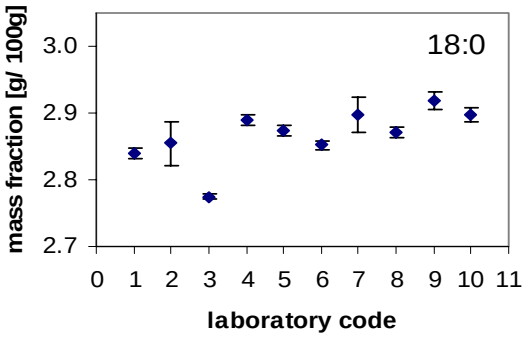



Figure 5. 18:0, mean ± s expressed in g/100 g of total FAME, sets of data from all participating laboratories for BCR-162R.

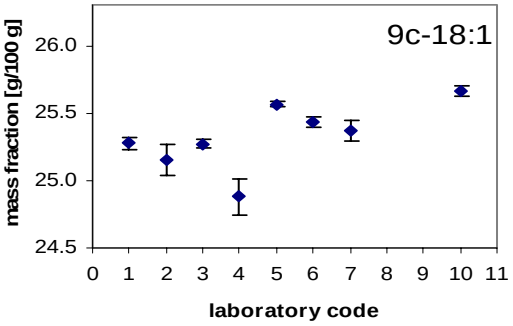



Figure 6. 9c-18:1, mean ± s expressed in g/100 g of total FAME, sets of data from all participating laboratories for BCR-162R.

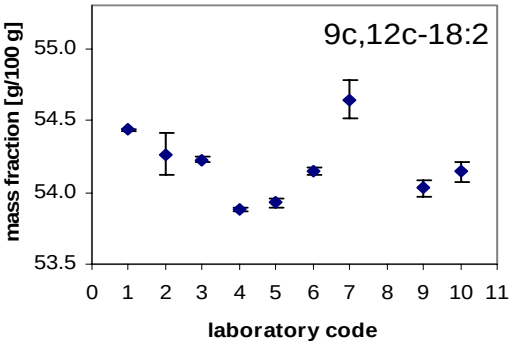



Figure 7. 9c,12c-18:2, mean ± s expressed in g/100 g of total FAME, sets of data from all participating laboratories for BCR-162R.

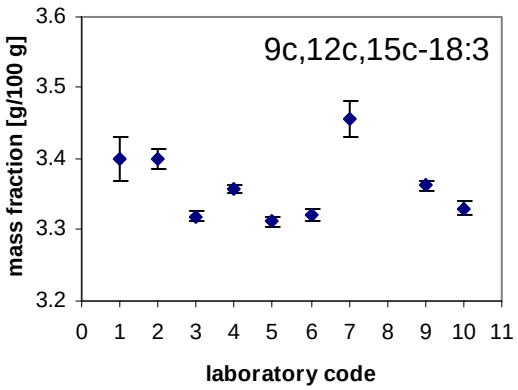



Figure 8. 9c,12c,15c-18:3, mean ± s expressed in g/100 g of total FAME, sets of data from all participating laboratories for BCR-162R.

By visual inspection of the graphs, suspicious data points were identified for 9c,12c-18:2 (Figure 7) and 9c,12c,15c-18:3 (Figure 8), both provided by L7. A detailed inspection of the corresponding chromatograms revealed possible co-elution of each of the mentioned components with minor *trans* isomers, which potentially results in an overestimation of both target compounds, thus justifying the higher values reported by the laboratory. Consequently the values were not used for further evaluation.

All sets of data were submitted for statistical analysis, including the detection of outliers by Dixon, Grubbs and Nalimov *t*-test, the normality of the data distribution, mainly the laboratory means, by a Skewness & Kurtosis test, and to the analysis of the variances. The evaluation indicated normality for all the distributions corresponding to 16:0; 18:0; 9c-18:1; 9c,12c-18:2 and 9c,12c,15c-18:3. No outlying laboratory means were identified for any of the relevant compounds according to the Dixon test at either 95 % or 99 % confidence levels. Yet, an outlier for 18:0 (L3 in Figure 5) was detected by alternatively using Nalimov *t*-test at a confidence level of 95 %. Outliers can not be eliminated on the bases of statistical grounds only and, since no technical reason was identified for justifying the low value, the data-point was accounted for value assignment. The analysis of laboratory variances by Cochran test revealed the presence of outliers for 16:0 (Figure 4) at 99 % confidence level (L1) and at 95 % confidence level (L1, L2 and L7). Likewise laboratory variance outliers for 9c,12c-18:2 (L2 in Figure 7) and for 9c,12c,15c-18:3 (L1 in Figure 8) were identified. For 9c-18:1 (Figure 6), one (L2) and two (L2 and L4) outliers were as well detected at 99 % and 95 % confidence levels respectively. The major fatty acid isomers present in BCR-162R were also measured in a control sample included in the same analytical sequence and the variances associated to each fatty acid in both materials were compared. In most cases variances were analogous

and acceptable, and therefore values were admitted for value assignment. There was only one exception, L4 data for 9c-18:1, where variance of the results obtained for BCR-162R was significantly larger than that in the control sample. A chromatogram obtained for BCR-162R by L4 showed peak overlapping between the major compound, 9c-18:1, to its isomer, 11c-18:1. A more detailed analysis of the results for the minor isomer reveals a large variance level, analogous to 9c-18:1. Furthermore when comparing the mean mass fraction for 11c-18:1 reported by all laboratories (numerical data shown in Annex 6), the L4 value seems to be an outlier on the low side. For the major isomer, the opposite effect was observed. Thus in this case there seems to be a relationship between the large variances and discrepancies in the individual isomer mass fractions reported with peak integration due to co-elution. Consequently values for individual 18:1 isomers provided by L4 were disregarded for value assignment, although they were accepted for the assignment of total 18:1 as indicative value.

Figure 9 and Table 11 illustrate the mass fraction values assigned to the major fatty acid isomers present in the soya-maize oil blend material, expressed as the mean of the means of the accepted data sets. Certified values were assigned from the characterisation, and uncertainty associated to characterisation measurements was taken as a contribution to the total uncertainty budget of the CRM.

Table 11. Characterisation of BCR-162R. Mean of the means of accepted sets of data for major fatty acids present in the soya-maize oil blend

| FAME | Mean of means [g/100 g] | p | $s_e$ mean [g/100 g FAME] | $u_{char}$ (RSE mean [%]) |
|---|---|---|---|---|
| **16:0** | 10.74 | 10 | 0.08 | 0.73 |
| **18:0** | 2.82 | 10 | 0.01 | 0.46 |
| **9c-18:1** | 25.39 | 7 | 0.07 | 0.28 |
| **9c,12c-18:2** | 54.13 | 9 | 0.06 | 0.11 |
| **9c,12c,15c-18:3** | 3.35 | 8 | 0.01 | 0.37 |

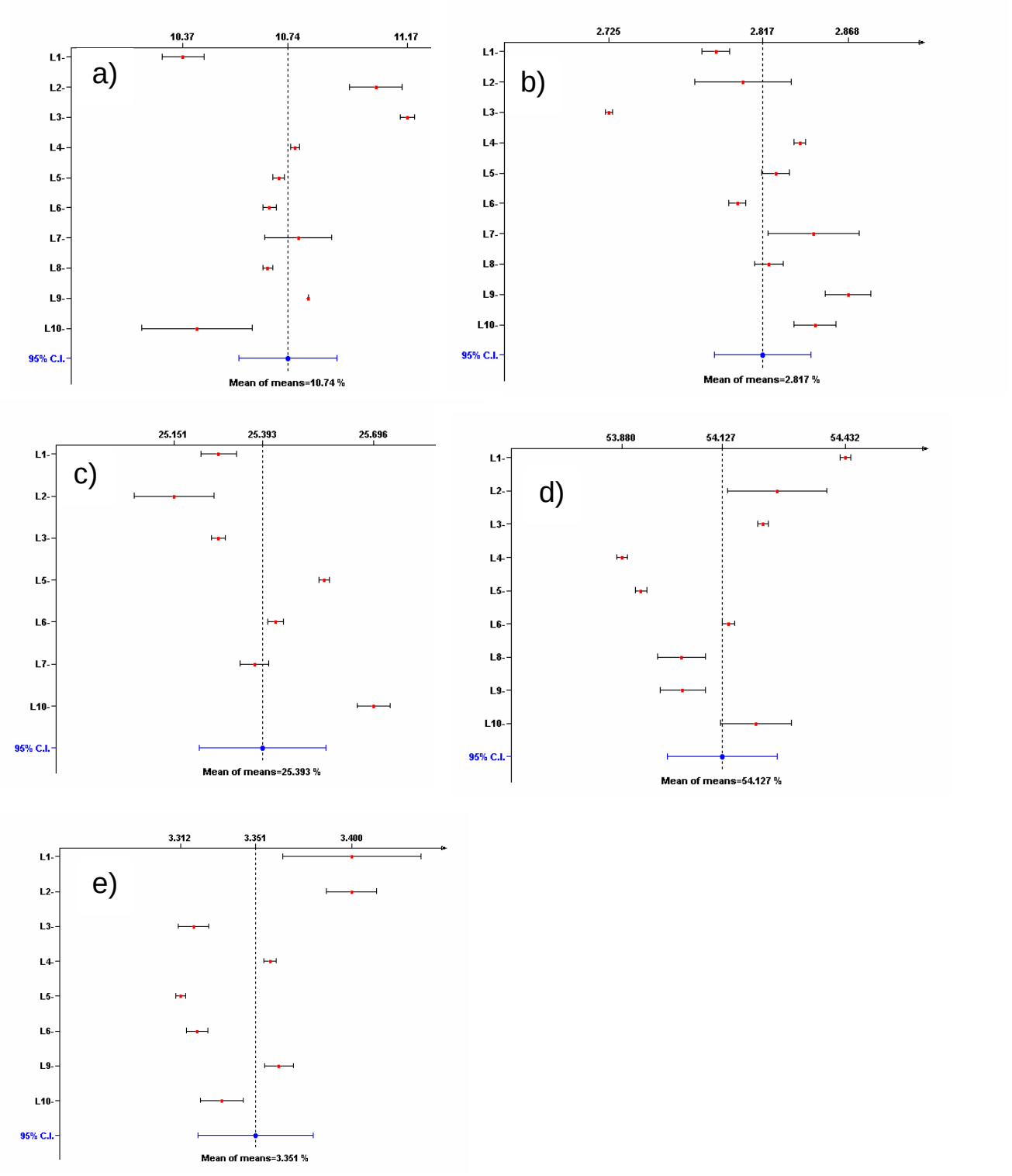



Figure 9. Characterisation of BCR-162R. Mean± standard deviations (g/100 g total FAME) from all accepted data sets, and mean of the means value with a confidence interval of 95 % for a) 16:0; b) 18:0; c) 9c-18:1; d) 9c,12c-18:2; e) 9c,12c,15c-18:3.

### *Evaluation of sum of fatty acid isomers*

A number of minor fatty acids present in the soya-maize oil blend were also reported by the participating laboratories and included in Annex 5 as laboratory mean values. Identification by chromatographic retention times was performed by a number of collaborators employing commercial pure standards. Exemplary, identity was additionally confirmed by analogy to mass spectrometric results obtained from the analysis of milk fat using equivalent GC conditions. Likewise previous experience and knowledge supported by literature data available were also tools applied for some minor peaks identification.

As a confirmatory measure and for a qualitative purpose only, transesterified BCR-162R material was analysed by GC-MS at IRMM. Annex 6 a) and b) include extracts from the resulting chromatogram showing a fatty acid profile analogous to those obtained during characterisation, which were then identified by retention times. The aim of this experiment was to confirm identification of the minor chromatographic peaks as isomers of the major unsaturated fatty acids present in the soya-maize oil blend. Application of the mass spectrometric scan mode analysis resulted in the mass spectra shown in Annex 6 c) to k). Identity of the compounds was confirmed by database search and by comparison of molecular ions of the fatty acid methyl esters with those corresponding to the major isomers, previously injected as pure standard solutions.

In order to increase the usefulness of the material it was decided to combine major and minor components reported for each unsaturated fatty acid, to provide unique figures as indicative values, that would correspond to 18:1 total, 18:2 total and 18:3 total fatty acid present in BCR-162R material.

As mentioned earlier in the text, L9 reported uniquely the major isomers associated to 18:2 and 18:3, whereas L8 reported total amounts but mainly related to the major component. Thus data sets from L8 and L9 are not accounted for evaluation of 18:2 total and 18:3 total since that would cause underestimation of the total fatty acids mass fractions. L4 was additionally discarded since co-elution was declared between a minor *trans*-18:3 isomer and 20:1. For 18:1 total, all laboratory sets of data were accepted.

Summarising, 10 data sets were considered for further statistical evaluation in the case of 18:1 total, whereas 8 sets of data were analysed for 18:2 total and seven for 18:3 total fatty acid. The results from Skewness & Kurtosis test indicated normal distribution in all three cases and no outlying laboratory means were detected according to Dixon and Nalimov tests at both 99 and 95 % confidence levels. Although outlying laboratory variances were identified by Cochran test for 18:1 total (L1 and L2), 18:2 total (L2 and L7) and 18:3 total (L1 and L7), no technical reason was identified for the high variances. Thus the values were accepted for

.

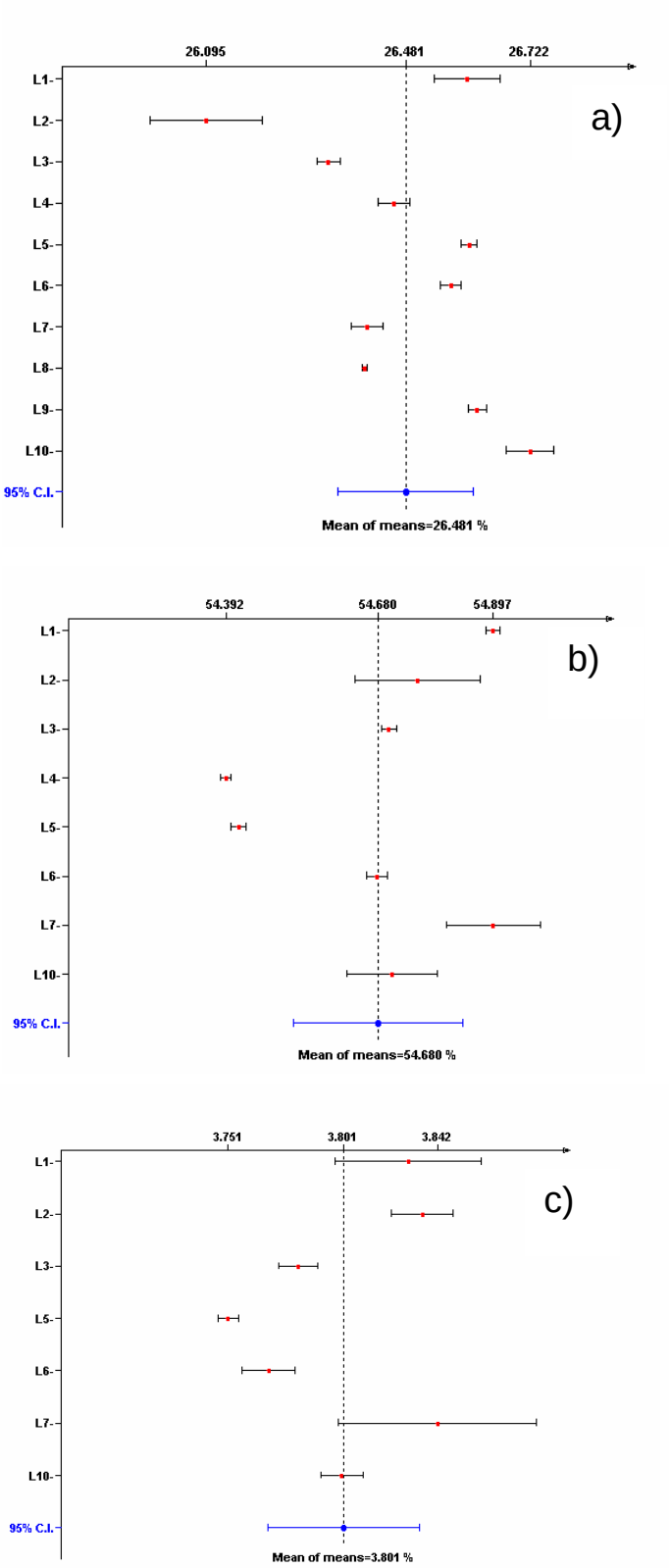



Figure 10. Mean of the means value of accepted sets of data expressed as g/100 g total fatty acid methyl ester with a confidence interval of 95 %. Laboratory means ± standard deviations from the characterisation of BCR-162R are also included for a) 18:1 total; b) 18:2 total and c)18:3 total.

value assignment. Figure 10 and Table 12 show the values corresponding to total mass fraction of unsaturated fatty acids obtained by taking into account contributions from the different *cis/trans* isomers identified in BCR-162R material. The results were included in the certificate as indicative values and the uncertainty associated to the characterisation, estimated as the relative standard error of the mean, was included as a contribution to the uncertainty budget for each FAME value.

Table 12. Characterisation of BCR-162R. Mean of the means of the accepted sets of results, standard and relative standard errors related. Monoenoic and polyunsaturated fatty acids include positional and geometrical (i.e. *cis/trans*) isomers.

| FAME | Mean of means [g/100 g FAME] | p | $s_e$ mean [g/100 g FAME] | $u_{char}$ (RSE mean [%]) |
|---|---|---|---|---|
| 18:1 total | 26.48 | 10 | 0.06 | 0.22 |
| 18:2 total | 54.68 | 8 | 0.07 | 0.12 |
| 18:3 total | 3.80 | 7 | 0.01 | 0.35 |

### *Other minor fatty acids*

The definition of the complete fatty acid profile requires having available data on the mass fractions from both major and minor components. Thus other minor compounds reported by the collaborators (Annex 6) are provided in the certificate as additional material information expressed as mean of the mean of the data sets (Table 13). No attempt has been made to attribute uncertainties to these values, which must be treated as informative only.

Table 13. Characterisation of BCR-162R. Mean values, corresponding to the minor fatty acids detected in BCR-162R

| **FAME** | **[g/100 g of total FAME]** | **p** |
|---|---|---|
| 14:0 | 0.06 | 9 |
| 16:1 | 0.10 | 10 |
| 17:0 | 0.08 | 10 |
| 17:1 | 0.04 | 7 |
| 11c-18:1 | 1.08 | 7 |
| *18:2 t | 0.46 | 8 |
| *18:3 t | 0.41 | 8 |
| 20:0 | 0.38 | 10 |
| 20:1 | 0.23 | 10 |
| 22:0 | 0.27 | 10 |
| 24:0 | 0.15 | 10 |

*Includes two *trans* isomers detected

## 10. Certified value and uncertainty budget

The certified values of fatty acid mass fractions in BCR-162R material, calculated as the mean of the means of all valid data sets, are included in Table 14 as well as the uncertainties associated [9], estimated by combining the uncertainty contributions as

$$U_{CRM} = k\sqrt{u^2_{char} + u^2_{bb} + u^2_{lts}}$$

Where $U_{CRM}$ is the expanded uncertainty of the certified quantity value

$k$ is the coverage factor (a factor of 2 is applied to give approximately 95% confidence)

$u_{char}$ is the uncertainty associated to the characterisation of the material

$u_{bb}$ is the uncertainty associated to the between bottle variation

$u_{lts}$ is the uncertainty contribution from the estimation of the long-term stability study

Table 14. Certified values and uncertainty budget for the fatty acid profile of BCR-162R, expressed as mass fraction of a particular FAME to the total FAME.

| FAME | **Certified value** [g/100 g total FAME] | p | $u_{lts}$ [%] | $u_{bb}$ [%] | $u_{char}$ [%] | $U_{CRM}$ [%] | **$U_{CRM}$** [g/100 g total FAME] |
|---|---|---|---|---|---|---|---|
| 16:0 | **10.74** | 10 | 0.2 | 0.1 | 0.73 | 1.51 | **0.16** |
| 18:0 | **2.82** | 10 | 0.6 | 0.1 | 0.46 | 1.51 | **0.04** |
| 9c-18:1 | **25.4** | 7 | 0.6 | 0.2 | 0.28 | 1.32 | **0.4** |
| 9c,12c-18:2 | **54.13** | 9 | 0.2 | 0.0 | 0.11 | 0.46 | **0.25** |
| 9c,12c,15c-18:3 | **3.35** | 8 | 0.5 | 0.4 | 0.37 | 1.24 | **0.05** |

## 11. Indicative values

Positional and geometrical isomers detected in BCR-162R oil blend were pooled to provide total amounts of the monoenoic and polyunsaturated fatty acids, as described in the Characterisation Section. The uncertainty associated to the indicative values was estimated by using the same expression applied to the certified values. An approximation was made assuming that homogeneity and stability contribution levels were analogous to those obtained for the major isomer of each of the fatty acids evaluated here. Table 15 summarised the results.

Table 15. Indicative values and uncertainty budget for the pooled unsaturated fatty acids detected in BCR-162R

| FAME | **Indicative value** [g/100 g total FAME] | p | $u_{lts}$ [%] | $u_{bb}$ [%] | $u_{char}$ [%] | $U$ [%] | ***U*** [g/100 g total FAME] |
|---|---|---|---|---|---|---|---|
| **18:1 total** | **26.5** | 10 | 0.6 | 0.2 | 0.22 | 1.28 | **0.4** |
| **18:2 total** | **54.68** | 8 | 0.2 | 0.0 | 0.13 | 0.48 | **0.26** |
| **18:3 total** | **3.80** | 7 | 0.5 | 0.4 | 0.36 | 1.23 | **0.06** |

## 12. Metrological Traceability

The fatty acid profile determination was carried out by independent laboratories with demonstrated qualification. They employed well characterised, understood and validated methodology. The starting material, a natural matrix, was weighed using calibrated balances. Subsequently a quantitative trans-esterification was performed employing different reagents. Additionally a variety of GC methodologies were followed by the participants for separation prior to detection by means of FID. Response factors (FID response of equal masses of a fatty acid and 16:0) were established by means of gravimetrically prepared mixtures of the major fatty acids in approximately the proportions expected in the reference material. Thus, the certified values for the fatty acid profile of the reference material are traceable to methodology applying gas chromatography.

## 13. Instructions for use

The certified value has been assigned to the material "as is", no dry mass correction has been applied. Karl Fischer titration measurements performed in three different units of BCR-162R randomly chosen indicated the presence of water at levels below 0.02 g/100 g.

### *Intended use*

BCR-162R is intended to be used for method validation and quality control purposes.

### *Dispatch and storage*

Samples are dispatched to the customer under no particular cooling conditions. Upon receipt, the material should be stored at a temperature equal to or lower than -20 ºC.

### *Minimum sample intake*

The material has been found sufficiently homogeneous for a sample intake of 100 mg.

### *Use of the certified value*

To asses the trueness of an analytical method, the CRM is analysed by the laboratory and the results compared to those specified in the BCR certificate as follows:

- Calculate the standard uncertainty of the certified value ($u_{CRM}$) by dividing the expanded uncertainty stated in the certificate ($U_{CRM}$) by the coverage factor ($k$ = 2).
- Estimate the measurement standard uncertainty ($u_m$) resulting from the analysis of the CRM.
- Combine the two values as:

$$u_c = k\sqrt{u^2{}_m + u^2{}_{CRM}}$$

If the difference between the certified and the measurement value is smaller than 2 x $u_c$, it can be concluded that the measurement result agrees with the certified value within the limits of the stated uncertainty.

A more detailed explanation on this procedure can be found in:
ERM application note 1 (www.erm-CRM.org), or in
www.irmm.jrc.be/html/reference_materials_catalogue/user_support/use.htm

## 14. Bibliography


[1] ISO Guide 34:2000, General requirements for the competence of reference materials producers

[2] W.W. Christie in *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*, Oily Press (2003) 3-12

[3] A.M.H. van der Veen, T. Linsinger, J. Pauwels, Uncertainty calculations in the certification of reference materials, 2. Homogeneity study. Accred. Qual. Assur., 6 (2001) 26-30

[4] ISO 15884:2002. Milk fat-Preparation of fatty acid methyl esters

[5] ISO 15885:2002. Milk fat-Determination of the fatty acid composition by gas-liquid chromatography

[6] W.D. Pocklington, J. Pearse, G. Lognay, P.J. Wagstaffe, A. Boenke, B. Schurer. The certification of the fatty acid profile and the cholesterol mass fraction of two edible oil and fat materials. EUR report CD-NA-15278-EN-C

[7] A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem., 360 (1998) 359-361

[8] ISO Guide 35:2006, Reference materials - General and statistical principles for certification

[9] T. Linsinger, J. Pauwels, A. Lamberty, H. Schimmel, AMH van der Veen, L. Siekmann. Fres. J. Anal. Chem., 370 (2001) 183-188


## 15. Acknowledgments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## Annex 1

Results from the homogeneity study of BCR-162R, expressed as g/100 g of the total fatty acid (methyl ester).

| | 16:0 | | 18:0 | | 9c-18:1 | | 9c,12c-18:2 | | 9c,12c,15c-18:3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ampoule number** | **replicate 1** | **replicate 2** | **replicate 1** | **replicate 2** | **replicate 1** | **replicate 2** | **replicate 1** | **replicate 2** | **replicate 1** | **replicate 2** |
| 17 | 10.79 | 10.80 | 2.80 | 2.81 | 24.76 | 24.78 | 54.40 | 54.39 | 3.25 | 3.25 |
| 169 | 10.79 | 10.81 | 2.79 | 2.81 | 24.74 | 24.80 | 54.41 | 54.41 | 3.26 | 3.25 |
| 490 | 10.79 | 10.81 | 2.80 | 2.80 | 24.74 | 24.79 | 54.41 | 54.38 | 3.25 | 3.25 |
| 795 | 10.77 | 10.80 | 2.79 | 2.81 | 24.73 | 24.80 | 54.43 | 54.40 | 3.26 | 3.25 |
| 1010 | 10.79 | 10.80 | 2.80 | 2.80 | 24.74 | 24.80 | 54.40 | 54.41 | 3.25 | 3.25 |
| 1125 | 10.81 | 10.82 | 2.80 | 2.81 | 24.72 | 24.79 | 54.40 | 54.38 | 3.25 | 3.25 |
| 1256 | 10.80 | 10.79 | 2.80 | 2.81 | 24.75 | 24.77 | 54.42 | 54.39 | 3.26 | 3.25 |
| 1397 | 10.80 | 10.80 | 2.81 | 2.80 | 24.75 | 24.83 | 54.40 | 54.41 | 3.26 | 3.24 |
| 1661 | 10.79 | 10.80 | 2.81 | 2.80 | 24.75 | 24.82 | 54.40 | 54.41 | 3.26 | 3.25 |
| 1795 | 10.79 | 10.80 | 2.80 | 2.80 | 24.74 | 24.81 | 54.39 | 54.38 | 3.27 | 3.25 |
| 68 | 10.82 | 10.81 | 2.81 | 2.81 | 24.87 | 24.86 | 54.35 | 54.36 | 3.30 | 3.28 |
| 322 | 10.81 | 10.82 | 2.81 | 2.80 | 24.82 | 24.84 | 54.38 | 54.45 | 3.30 | 3.29 |
| 534 | 10.82 | 10.80 | 2.81 | 2.80 | 24.85 | 24.87 | 54.39 | 54.41 | 3.29 | 3.29 |
| 741 | 10.84 | 10.83 | 2.81 | 2.80 | 24.85 | 24.86 | 54.40 | 54.39 | 3.27 | 3.27 |
| 891 | 10.82 | 10.82 | 2.80 | 2.80 | 24.83 | 24.84 | 54.43 | 54.44 | 3.29 | 3.28 |
| 929 | 10.82 | 10.79 | 2.80 | 2.80 | 24.87 | 24.82 | 54.39 | 54.41 | 3.28 | 3.29 |
| 1068 | 10.81 | 10.81 | 2.81 | 2.81 | 24.88 | 24.84 | 54.36 | 54.35 | 3.28 | 3.29 |
| 1192 | 10.77 | 10.82 | 2.81 | 2.80 | 24.81 | 24.81 | 54.34 | 54.38 | 3.28 | 3.29 |
| 1487 | 10.80 | 10.83 | 2.80 | 2.80 | 24.82 | 24.79 | 54.40 | 54.44 | 3.28 | 3.29 |
| 1776 | 10.86 | 10.81 | 2.79 | 2.79 | 24.82 | 24.80 | 54.42 | 54.45 | 3.27 | 3.28 |
| 38 | 10.80 | 10.81 | 2.81 | 2.79 | 24.73 | 24.70 | 54.39 | 54.42 | 3.26 | 3.26 |
| 205 | 10.80 | 10.80 | 2.79 | 2.80 | 24.73 | 24.74 | 54.43 | 54.42 | 3.25 | 3.26 |
| 270 | 10.77 | 10.78 | 2.79 | 2.80 | 24.70 | 24.71 | 54.42 | 54.45 | 3.28 | 3.26 |
| 394 | 10.86 | 10.84 | 2.81 | 2.81 | 24.75 | 24.67 | 54.38 | 54.39 | 3.25 | 3.27 |
| 877 | 10.80 | 10.81 | 2.79 | 2.80 | 24.68 | 24.70 | 54.43 | 54.41 | 3.28 | 3.26 |
| 919 | 10.78 | 10.79 | 2.80 | 2.79 | 24.73 | 24.68 | 54.45 | 54.44 | 3.26 | 3.25 |
| 1201 | 10.79 | 10.79 | 2.80 | 2.79 | 24.69 | 24.70 | 54.46 | 54.42 | 3.25 | 3.27 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1269 | 10.78 | 10.78 | 2.80 | 2.80 | 24.70 | 24.70 | 54.47 | 54.46 | 3.26 | 3.26 |
| 1348 | 10.81 | 10.80 | 2.80 | 2.79 | 24.77 | 24.65 | 54.41 | 54.41 | 3.28 | 3.26 |
| 1616 | 10.80 | 10.79 | 2.80 | 2.79 | 24.69 | 24.71 | 54.45 | 54.44 | 3.26 | 3.26 |
| 91 | 10.78 | 10.79 | 2.80 | 2.79 | 24.74 | 24.77 | 54.40 | 54.36 | 3.27 | 3.28 |
| 296 | 10.80 | 10.80 | 2.80 | 2.80 | 24.67 | 24.64 | 54.39 | 54.38 | 3.28 | 3.26 |
| 443 | 10.81 | 10.82 | 2.81 | 2.81 | 24.64 | 24.70 | 54.37 | 54.36 | 3.26 | 3.27 |
| 669 | 10.83 | 10.81 | 2.81 | 2.80 | 24.72 | 24.73 | 54.36 | 54.37 | 3.27 | 3.27 |
| 693 | 10.80 | 10.79 | 2.80 | 2.80 | 24.67 | 24.72 | 54.39 | 54.40 | 3.26 | 3.27 |
| 1085 | 10.80 | 10.79 | 2.81 | 2.81 | 24.74 | 24.74 | 54.38 | 54.36 | 3.29 | 3.26 |
| 1151 | 10.80 | 10.79 | 2.81 | 2.79 | 25.33 | 24.76 | 54.36 | 54.40 | 3.30 | 3.27 |
| 1441 | 10.79 | 10.81 | 2.80 | 2.80 | 24.71 | 24.70 | 54.40 | 54.39 | 3.27 | 3.27 |
| 1588 | 10.78 | 10.77 | 2.79 | 2.80 | 24.70 | 24.77 | 54.42 | 54.42 | 3.30 | 3.28 |
| 1709 | 10.79 | 10.79 | 2.80 | 2.80 | 24.65 | 24.71 | 54.40 | 54.41 | 3.28 | 3.27 |

## Annex 2

Results from the minimum sample intake evaluation of BCR-162R, expressed as g/100 g of the total fatty acid (methyl ester).

| | Fatty acid [g/100 g total FAME] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **16:0** | | **18:0** | | **9c-18:1** | | **9c,12c-18:2** | | **9c,12c,15c-18:3** | |
| Sample intake [mg] | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 |
| replicate 1 | 10.83 | 10.82 | 2.77 | 2.75 | 24.28 | 24.39 | 54.37 | 54.59 | 3.22 | 3.19 |
| replicate 2 | 10.82 | 10.80 | 2.77 | 2.75 | 24.33 | 24.43 | 54.41 | 54.65 | 3.21 | 3.20 |
| replicate 3 | 10.83 | 10.82 | 2.77 | 2.75 | 24.29 | 24.49 | 54.39 | 54.62 | 3.21 | 3.20 |
| replicate 4 | 10.82 | 10.82 | 2.77 | 2.75 | 24.27 | 24.50 | 54.39 | 54.62 | 3.21 | 3.19 |

## Annex 3

Short-term stability results. Mean g/100 g of the total fatty acid (methyl ester) values obtained from three GC-FID injections of the same preparation.

| Temperature [ºC] | Time [week] | Fatty acid [g/100 g total FAME] | | | | |
|---|---|---|---|---|---|---|
| | | 16:0 | 18:0 | 9c-18:1 | 9c,12c-18:2 | 9c,12c,15c-18:3 |
| + 4 | 1 | 10.82 | 2.76 | 24.31 | 54.40 | 3.21 |
| | | 10.82 | 2.77 | 24.26 | 54.34 | 3.21 |
| | 2 | 10.83 | 2.77 | 24.27 | 54.40 | 3.21 |
| | | 10.82 | 2.77 | 24.28 | 54.38 | 3.21 |
| | 4 | 10.82 | 2.77 | 24.32 | 54.40 | 3.21 |
| | | 10.82 | 2.76 | 24.30 | 54.41 | 3.21 |
| | reference | 10.82 | 2.77 | 24.30 | 54.39 | 3.21 |
| | | 10.83 | 2.77 | 24.28 | 54.39 | 3.21 |
| + 18 | 1 | 10.82 | 2.77 | 24.48 | 54.40 | 3.22 |
| | | 10.86 | 2.75 | 24.40 | 54.65 | 3.24 |
| | 2 | 10.84 | 2.76 | 24.44 | 54.55 | 3.23 |
| | | 10.83 | 2.77 | 24.50 | 54.50 | 3.23 |
| | 4 | 10.83 | 2.77 | 24.54 | 54.45 | 3.22 |
| | | 10.84 | 2.76 | 24.53 | 54.55 | 3.24 |
| | reference | 10.82 | 2.77 | 24.54 | 54.43 | 3.23 |
| | | 10.91 | 2.75 | 24.56 | 54.54 | 3.24 |
| + 40 | 1 | 10.83 | 2.77 | 24.61 | 54.41 | 3.23 |
| | | 10.82 | 2.77 | 24.57 | 54.43 | 3.23 |
| | 2 | 10.83 | 2.77 | 24.60 | 54.44 | 3.23 |
| | | 10.83 | 2.77 | 24.66 | 54.47 | 3.23 |
| | 4 | 10.82 | 2.77 | 24.62 | 54.42 | 3.22 |
| | | 10.83 | 2.77 | 24.69 | 54.44 | 3.23 |
| | reference | 10.83 | 2.77 | 24.59 | 54.41 | 3.23 |
| | | 10.82 | 2.77 | 24.62 | 54.45 | 3.23 |

## Annex 4

Long-term stability study. Results expressed as mean g/100 g of the total fatty acid (methyl ester) values obtained from three GC-FID injections of the same preparation.

| Temperature [ºC] | Time [month] | FAME [g/100 g total FAME] | | | | |
|---|---|---|---|---|---|---|
| | | **16:0** | **18:0** | **9c18:1** | **9c,12c-18:2** | **9c,12c,15c-18:3** |
| **+4** | **6** | 10.82 | 2.78 | 24.22 | 54.35 | 3.22 |
| | | 10.83 | 2.78 | 24.19 | 54.34 | 3.22 |
| | **12** | 10.82 | 2.78 | 24.23 | 54.39 | 3.23 |
| | | 10.82 | 2.78 | 24.25 | 54.35 | 3.22 |
| | **18** | 10.83 | 2.79 | 24.26 | 54.41 | 3.23 |
| | | 10.82 | 2.78 | 24.23 | 54.34 | 3.22 |
| | **reference** | 10.83 | 2.78 | 24.23 | 54.40 | 3.23 |
| | | 10.82 | 2.78 | 24.21 | 54.35 | 3.22 |
| **-20** | **6** | 10.82 | 2.78 | 24.25 | 54.35 | 3.22 |
| | | 10.83 | 2.79 | 24.22 | 54.34 | 3.22 |
| | **12** | 10.83 | 2.79 | 24.17 | 54.39 | 3.23 |
| | | 10.83 | 2.79 | 24.21 | 54.34 | 3.22 |
| | **18** | 10.82 | 2.78 | 24.19 | 54.37 | 3.22 |
| | | 10.83 | 2.79 | 24.23 | 54.38 | 3.22 |
| | **reference** | 10.83 | 2.78 | 24.23 | 54.42 | 3.23 |
| | | 10.84 | 2.79 | 24.11 | 54.42 | 3.22 |

## Annex 5

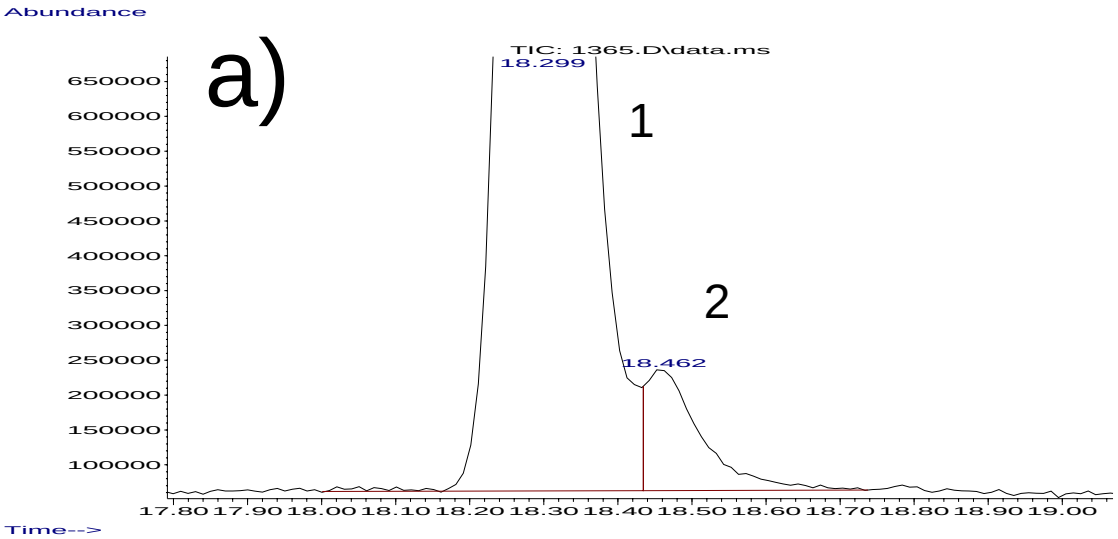
Characterisation data for the fatty acid profile of BCR-162R material. Data are expressed as the average mass fraction of the results obtained from three reference material units (N = 3) analysed in duplicate (n = 2).

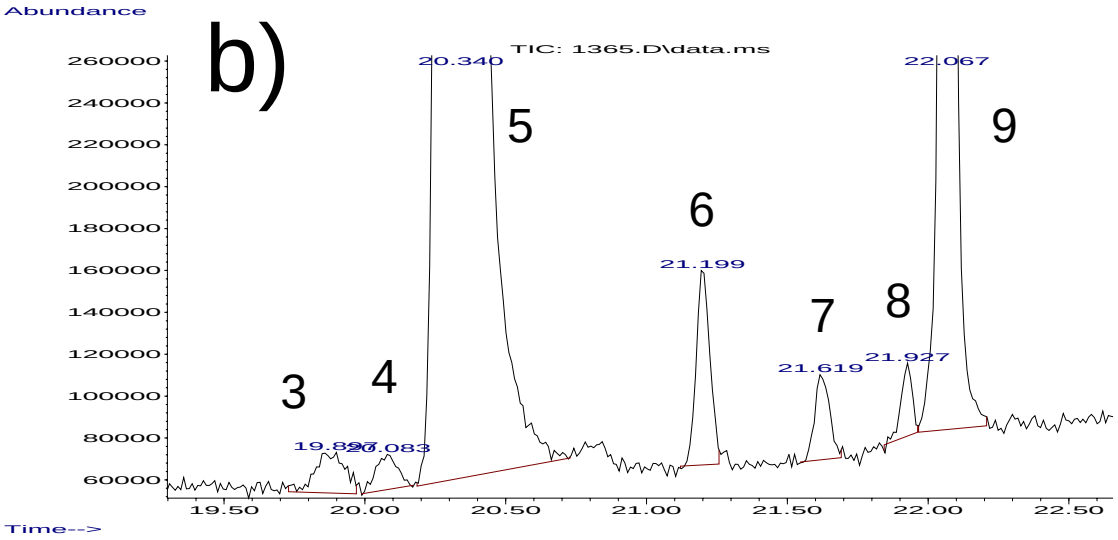
| | FAME [g/100 g total FAME] | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Laboratory code** | **14:0** | **16:0** | **16:1** | **17:0** | **17:1** | **18:0** | **9c-18:1** | ***18:1** | **9c,12c-18:2** | ***18:2** | **20:0** | **9c,12c,15c-18:3** | ***18:3** | **20:1** | **22:0** | **24:0** |
| 1 | 0.05 | 10.37 | 0.12 | 0.11 | 0.04 | 2.79 | 25.27 | 26.60 | 54.43 | 54.90 | 0.38 | 3.40 | 3.83 | 0.25 | 0.27 | 0.15 |
| 2 | 0.09 | 11.06 | 0.10 | 0.08 | 0.05 | 2.81 | 25.15 | 26.10 | 54.26 | 54.75 | 0.35 | 3.40 | 3.84 | 0.21 | 0.22 | 0.11 |
| 3 | 0.06 | 11.17 | 0.13 | 0.07 | 0.04 | 2.72 | 25.27 | 26.33 | 54.23 | 54.70 | 0.35 | 3.32 | 3.78 | 0.22 | 0.27 | 0.13 |
| 4 | 0.06 | 10.77 | 0.12 | 0.08 | 0.04 | 2.84 | 24.88 | 26.46 | 53.88 | 54.39 | 0.35 | 3.36 | 3.65 | 0.22 | 0.27 | 0.13 |
| 5 | n.r. | 10.71 | 0.11 | 0.08 | n.r. | 2.82 | 25.56 | 26.60 | 53.93 | 54.42 | 0.38 | 3.31 | 3.75 | 0.24 | 0.28 | 0.16 |
| 6 | 0.06 | 10.68 | 0.09 | 0.08 | n.r. | 2.80 | 25.43 | 26.57 | 54.14 | 54.68 | 0.40 | 3.32 | 3.77 | 0.27 | 0.30 | 0.20 |
| 7 | 0.05 | 10.78 | 0.08 | 0.07 | 0.04 | 2.85 | 25.37 | 26.41 | 54.65 | 54.90 | 0.41 | 3.46 | 3.84 | 0.21 | 0.24 | 0.14 |
| 8 | 0.06 | 10.67 | 0.09 | 0.08 | 0.05 | 2.82 | n.r | 26.40 | -[1)] | 54.03 | 0.38 | -[1)] | 3.48 | 0.26 | 0.27 | 0.14 |
| 9 | 0.05 | 10.81 | 0.09 | 0.08 | n.r. | 2.87 | n.r. | 26.62 | 54.03 | -[1)] | 0.38 | 3.36 | -[1)] | 0.24 | 0.28 | 0.15 |
| 10 | 0.05 | 10.42 | 0.08 | 0.08 | 0.04 | 2.85 | 25.67 | 26.72 | 54.14 | 54.71 | 0.40 | 3.33 | 3.80 | 0.21 | 0.30 | 0.15 |

* Values indicated for monoenoic fatty acids or PUFAs take into account the contributions from the different *cis/trans* isomers reported by the collaborators ([1)]L9 reported uniquely the value corresponding to the major 18:2 and 18:3 isomers whereas L8 declared peak co-elution)

n.r. value not reported

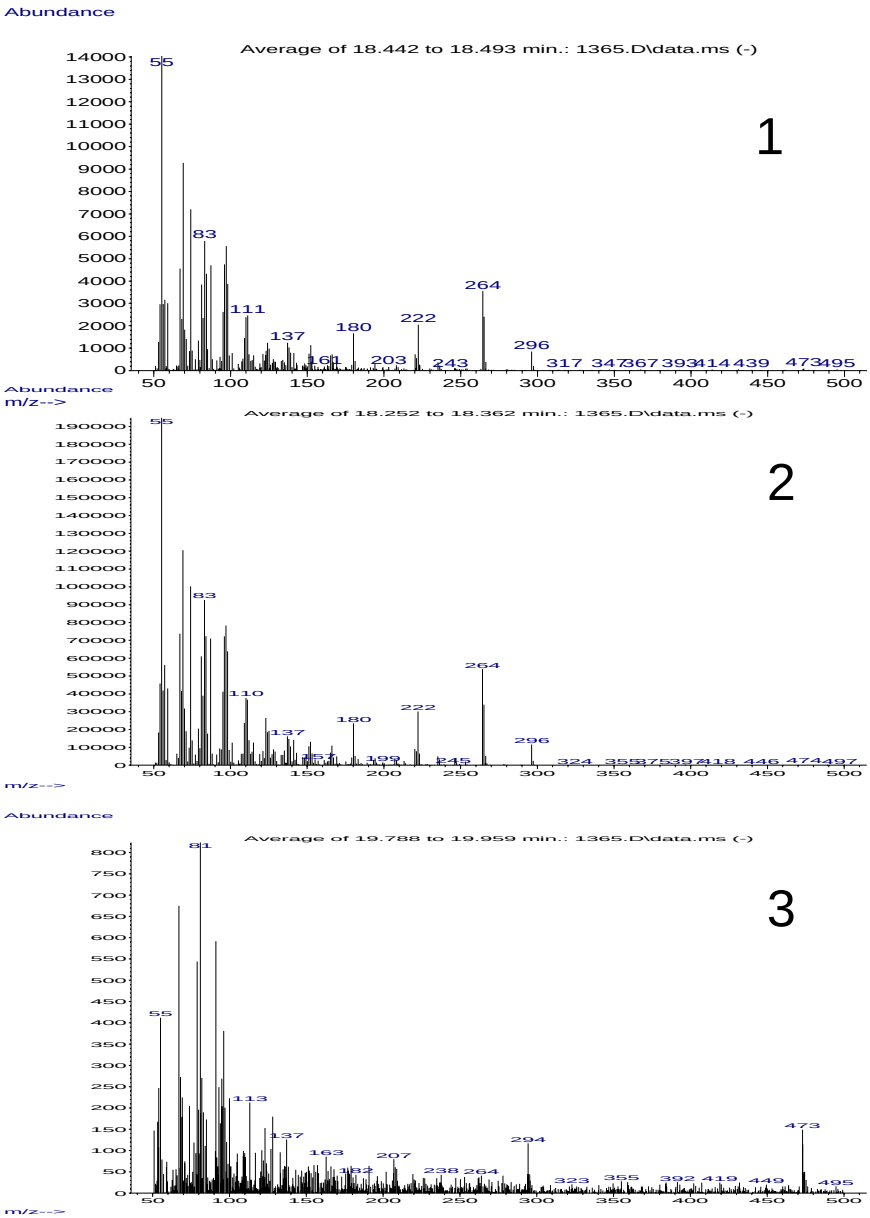
## Annex 6



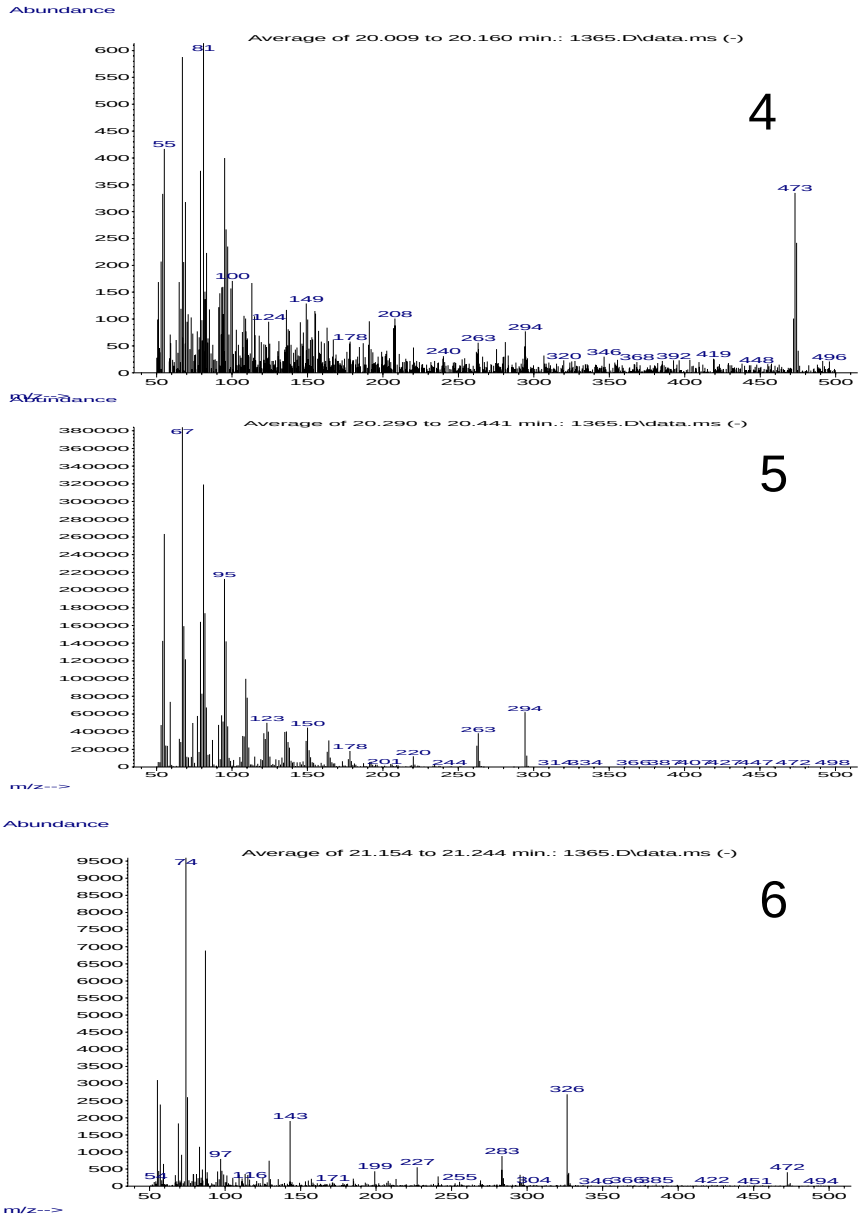




Details of chromatogram obtained for BCR-162R soya-maize oil blend by GC-MS under the following conditions: Column CB-Sil88 50m x 0.25 mm x 0.2 µm, flow 1ml/min, inlet 300°C, temperature program: 120°C (hold 0 min), 4 °C/min -> 180 (5 min), 15 °C/min -> 220 (5 min). MS source 200°C, MS quad 150 °C, full scan 50-500 amu.

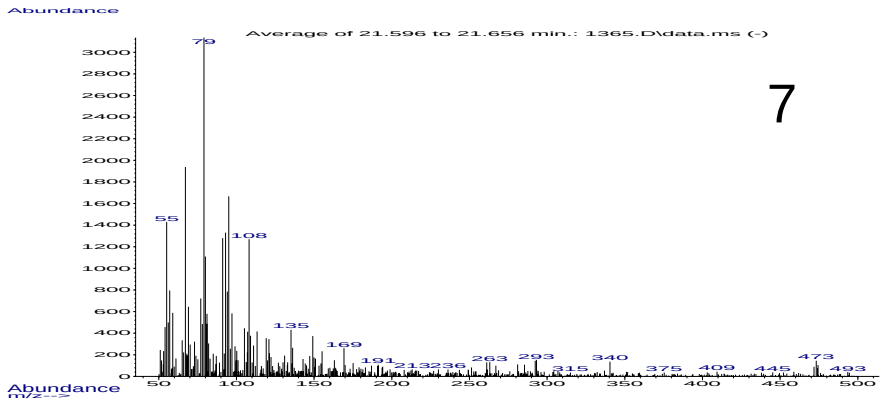
Peaks 1, 5 and 9 have been identified by retention times by previous injection of pure standards and confirmed by MS (shown below). The peaks correspond to FAMEs 9c-18:1; 9c,12c-18:2 and 9c,12c,15c-18:3 respectively. Peak 2 shows a mass spectrum qualitatively equivalent to Peak 1, which confirms both are 18:1 isomers. Peaks 3 and 4 are also confirmed as 18:2 isomers by mass spectra analogy to 9c,12c-18:2. Following the same reasoning Peaks 7 and 8 are identified as 18:3 isomers.

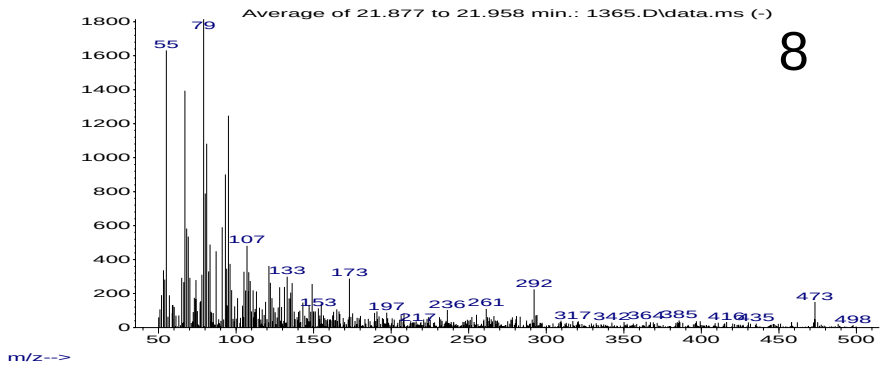


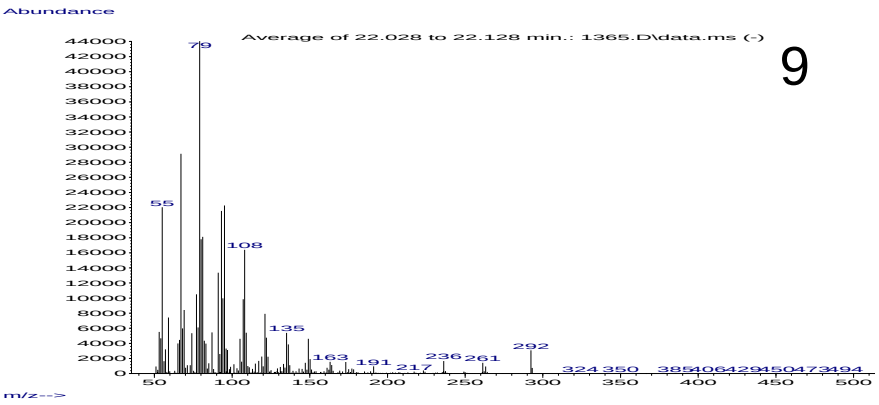

Mass spectra corresponding to chromatographic peaks 1, 2 and 3. m/z 473 has been identified as a background signal.




Mass spectra corresponding to chromatographic peaks 4, 5 and 6. m/z 473 has been identified as a background signal.




Mass spectra corresponding to chromatographic peaks 7, 8 and 9. m/z 473 has been identified as a background signal.

## Annex 7

Minor fatty acids reported for BCR-162R material, expressed as mass fraction (g/100 g of total fatty acid methyl esters).

| Laboratory code | FAME [g/100 g total FAME] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **14:0** | **16:1** | **17:0** | **17:1** | **11c-18:1** | ***18:2 trans** | ***18:3 trans** | **20:0** | **20:1** | **22:0** | **24:0** |
| 1 | 0.05 | 0.12 | 0.11 | 0.04 | 1.33 | 0.46 | 0.43 | 0.38 | 0.25 | 0.27 | 0.15 |
| 2 | 0.09 | 0.10 | 0.08 | 0.05 | 0.94 | 0.49 | 0.44 | 0.35 | 0.21 | 0.22 | 0.11 |
| 3 | 0.06 | 0.13 | 0.07 | 0.04 | 1.06 | 0.47 | 0.46 | 0.35 | 0.22 | 0.27 | 0.13 |
| 4 | 0.06 | 0.12 | 0.08 | 0.04 | 1.58 | 0.51 | 0.25 | 0.35 | 0.22 | 0.27 | 0.13 |
| 5 | n.r. | 0.11 | 0.08 | n.r. | 1.04 | 0.49 | 0.44 | 0.38 | 0.24 | 0.28 | 0.16 |
| 6 | 0.06 | 0.09 | 0.08 | n.r. | 1.14 | 0.53 | 0.45 | 0.40 | 0.27 | 0.30 | 0.20 |
| 7 | 0.05 | 0.08 | 0.07 | 0.04 | 1.03 | 0.25 | 0.39 | 0.41 | 0.21 | 0.24 | 0.14 |
| 8 | 0.06 | 0.09 | 0.08 | 0.05 | n.r. | n.r. | n.r. | 0.38 | 0.26 | 0.27 | 0.14 |
| 9 | 0.05 | 0.09 | 0.08 | n.r. | n.r. | n.r. | n.r. | 0.38 | 0.24 | 0.28 | 0.15 |
| 10 | 0.05 | 0.08 | 0.08 | 0.04 | 1.03 | 0.49 | 0.47 | 0.40 | 0.21 | 0.30 | 0.15 |

n.r. values not reported by the participant laboratories

*Taking into account contributions from different minor *trans*-isomers

European Commission

**EUR 22877 EN – Joint Research Centre – Institute for Reference Materials and Measurements**
Title: Certification of the fatty acid profile of soya-maize oil blend, BCR-162R
Author(s): M. Dabrio, M. Marini, M. Ricci, F. Ulberth, H. Emons
Luxembourg: Office for Official Publications of the European Communities
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Abstract

This report describes the production of the reference material BCR-162R, certified for its fatty acid profile. It contains all the required steps that ensure the high quality of the final product, from the processing to the value assignment, such as the homogeneity and stability studies, according to ISO Guide 34 [1]. The matrix material, a soya-maize oil blend, is packed in amber glass ampoules containing approximately 5.5 g of the oil blend and kept at a temperature of -20 ºC. Studies performed at IRMM confirmed homogeneity and stability of the fatty acids in the oil matrix under the evaluated conditions. The characterisation of the material, organised and coordinated by IRMM, was done by an interlaboratory comparison. The certified values were obtained as the unweighted mean of the laboratory means of the accepted sets of results for each analyte, and the expanded uncertainty associated ($k$ = 2) includes contributions from the characterisation, the homogeneity and the stability. The values certified for the fatty acid profile in BCR-162R are the following:

| FAME | **Certified value** [g/100 g total FAME] | $U_{CRM}$ [g/100 g total FAME] | p |
|---|---|---|---|
| 16:0 | 10.74 | 0.16 | 10 |
| 18:0 | 2.82 | 0.04 | 10 |
| 9c-18:1 | 25.4 | 0.4 | 7 |
| 9c,12c-18:2 | 54.13 | 0.25 | 9 |
| 9c,12c,15c-18:3 | 3.35 | 0.04 | 8 |

[1]Unweighted mean value, expressed as g of individual fatty acid methyl ester per 100 g total fatty acid methyl ester, of the accepted sets of data obtained in a different laboratory and/or with a different method of determination. Monoenoic and polyunsaturated fatty acids certified correspond to the major isomers identified (oleic acid, linoleic acid and linolenic acid). The values are traceable to methodology applying gas chromatography.
[2]Expanded uncertainty with a coverage factor of $k$ = 2, according to the Guide for the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

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