ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(6): 973-985
doi: 10.25083/rbl/24.6/973.985

Received for publication, December, 2, 2018
Accepted, July, 1, 2019

Original paper

Evaluation of fatty acid profile of oils/fats by GC-MS through two quantification approaches

ADRIANA LAURA MIHAI, MIOARA NEGOIȚĂ, NASTASIA BELC

National Research & Development Institute for Food Bioresources, IBA Bucharest, Chromatography Department, 6 Dinu Vintilă Street, District 2, 021102, Bucharest, Romania

Abstract For simultaneous separation, detection, identification and quantification of fatty acid methyl esters from oils/fats, a GC-MS method was developed and validated. The method is based on fat extraction and transesterification of fatty acids to fatty acid methyl esters. Samples of sunflower oil, refined non-hydrogenated palm oil, fish oil and lard, demonstrated the applicability of the proposed method. Fatty acid methyl esters determination and quantification was realized by using internal standards, and applying relative response factors, and without using internal standards by applying correction factors. Linearity, sensitivity, precision, accuracy, recovery and robustness were determined. The method is sensitive enough to simultaneously quantify 26 compounds, when using internal standard (absolute concentration), and 40 fatty acids without using internal standard (relative concentration). Accuracy was achieved by using a reference material, peanut butter (SRM®2387). The results have shown that the proposed method could be considered an effective tool for analyzing the fatty acid profile of food.

Keywords Fat, fatty acids quantification, fatty acid methyl esters, GC-MS, cooking oil.

To cite this article: MIHAI AL, NEGOIȚĂ M, BELC N. Evaluation of fatty acid profile of oils/fats by GC-MS through two quantification approaches. *Rom Biotechnol Lett*. 2019; 24(6): 973-985. DOI: 10.25083/rbl/24.6/973.985

*Corresponding author: Dr. MIOARA NEGOIȚĂ, National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, 021102, Bucharest, Romania; phone/fax numbers 004031-620.58.33, 004031-620.58.34
E-mail: mioaranegoita@yahoo.com

Introduction

The main sources of lipids in our diet are oils and fats of vegetable and/or animal origin. In order to analyse the fatty acid (FA) profiles of food, it is needed the extraction of lipids from samples followed by separation, identification and determination through different techniques. For the determination of FAs various methods were developed using different devices such as high-performance liquid chromatography (HPLC) (de la MATA-ESPINOSA, 2011), silver-ion-HPLC (Ag^+-HPLC) (LUNA, 2008), attenuated total reflection- Fourier transformer infrared spectroscopy (ATR-FTIR) (VONGSVIVUT, 2012; Da COSTA FILHO, 2014; LUCARINI, 2018; KARUNATHILAKA, 2019), capillary zone electrophoresis (De CASTRO BARRA, 2012), nuclear magnetic resonance (NMR) (CASTEJÓN, 2013; MIHAI, 2018) but the the most used method is gas chromatography (GC) (CHOWDHURY, 2007; DIKBAS, 2011; MANZANO, 2012; CHEN, 2014; FIRL, 2014; PINTILIE, 2014; DEMIREL, 2016; ADJEPONG, 2017; MAZUREK, 2017). GC coupled with mass spectrometry (GC-MS) helps to be a better separation and identification of FA isomers and less overlapping compared to GC-FID (ZHANG H., 2015). In GC analysis, fatty acids identification is based on conversion of FAs into corresponding fatty acid methyl esters (FAME) with higher volatility.

An aspect that should be taken into consideration when validating the method for fatty acids determination, is the procedure for derivatisation of fatty acids after extraction of lipids from food samples. The methods for fatty acids derivatisation involve acid or base catalysis. The most used base reagents for fast transformation of FAs into FAMEs are NaOH or KOH in methanol (SIMIONATO, 2010; CHEN, 2014; SALIMON, 2014; ZHANG M., 2015).

Another important aspect of GC method development and validation is the choice of GC column. From the polarity of the column depends the peak resolutions, a highly polar capillary column being a good choice for the separation of geometric isomers of unsaturated FAMEs (DELMONTE, 2016).

For fatty acids quantification, it can be used the internal or the external standard procedure. In the case of external calibration procedure, the analyte peak area is compared with the peak area of the reference standards of fatty acids with known concentration (SOBRADO, 2016). In the method which uses internal standards of triglycerides or fatty acids, which has been used mostly, the results are being expressed in weight (absolute concentration, g/100 g). For expressing the absolute concentration, it is required to use the response factors, which are dependent on the detector response. The response factors for quantification of individual fatty acids are different, being proportional with the number of active carbons in the fatty acid chain (SIMIONATO, 2010; FIRL, 2014). Results can also be expressed in weight percentage (relative concentration, weight %). When the results are expressed like this, there are used correction factors which can be theoretical or experimentally determined (SIMIONATO, 2010).

The aim of this study was to validate a GC-MS method for simultaneous determination and quantification of fatty acids from oils/fats of vegetable and/or animal origin, by expressing the results in relative and absolute concentration. In our study it were used both the internal and the external standard procedure in order to quantify the FA content and the results were compared. To demonstrate the suitability of the proposed method, a certified reference material (SRM®2387- peanut butter) was used to study the accuracy and the results were compared.

Materials and Methods

Reference standards, reagents

Two reference standards were used in the validation procedure: F.A.M.E. Mix, C4-C24 (mixture of 37 FAME, Bellefonte, PA, USA) and SRM®2377 (mixture of 26 FAME, NIST certified, USA). Internal standards of triglycerides (TAG-IS, C11:0, C15:0) and fatty acid methyl ester (FAME-IS C23:0) purchased from Sigma-Aldrich (St. Louis, MO, USA) and Larodan AB (Solna, Sweden) were used during the validation procedure.

A standard reference material (SRM®2387- peanut butter, Gaithersburg, MD 20899), NIST certified for fat (extractable), 12 fatty acids, for the sum of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids was used to assess the accuracy of the method and the fat content.

All solvents and reagents were of analytical grade, specially for chromatography and were used for the preparation and analysis of FAME: petroleum ether, 40-60 °C, solvent used for fat extraction (VWR Chemicals, France), 5.4 M methanolic solution of sodium hydroxide (Acros, New Jersey), 14% methanolic solution of boron trifluoride (Sigma Aldrich, Switzerland), sodium chloride (Sigma Aldrich, Denmark), methanol picograde and 2,2,4-trimethylpentane picograde (LGC Standards GmbH, Germany).

Food matrices

Samples from the category of oils/fats of vegetable and/or animal origin were purchased from several local supermarkets and were as follows: vegetable origin oil of sunflower – produced in Romania; refined non-hydrogenated palm oil – produced in Malaysia, animal origin oil of pure fish with omega-3 and lemon flavour – produced in Iceland, vegetable origin fat of peanut butter (SRM®2387) and also animal origin fat of lard, obtained from rural households in the county of Brăila, Romania.

Calibration solution preparation

Calibration solutions from the F.A.M.E. Mix, C4-C24 (N_1-N_6) and SRM®2377 (S_{1a}-S_{6a}) were realised in order to

determine correction factors (CFs). From the SRM®2377, calibration solutions (S_{1a}-S_{6a}) with FAME-IS C23:0 were prepared. The calibration curves were performed by plotting the linear graphical representation of the peak area ratio ($A_{FAME}/A_{FAME\text{-}IS}$) versus FAME concentration (C_{FAME}) or graphical representation of the peak area ratio ($A_{FAME}/A_{FAME\text{-}IS}$) versus concentration ratio (C_{FAME}/C_{IS}) of analyte/internal standard (average response).

Calibration curves were performed for 26 FAME components of the reference standard SRM®2377, in relation to FAME-IS C23:0. The concentration of FAME-IS in each calibration level was 50 µg/mL, and 2,2,4-trimethylpentane picograde was used as a solvent to prepare the stock and working solutions of the reference standards.

Determination of correction factors (CFs) and relative response factors (RRFs)

Determination of correction factors (CFs)

The 6 calibration level solutions (S_{1a}-S_{6a}; N_1-N_6) were injected into GC-MS, each in 3 replicates. Correction factors were calculated using the formula from the equation (1).

$$CF_i = \frac{p}{100} \times \frac{\sum A_i}{A_i} \quad (1)$$

where: p - $FAME_i$ mass percent from the mix of reference standards based on the certificate of analysis (%); $\sum Ai$ - sum of FAME areas for all FAME from the chromatogram of reference standards; A_i - area of $FAME_i$ from the chromatogram of reference standards.

Determination of relative response factors (RRFs)

For the determination of experimental RRF_i, the calibration levels S_{1a}-S_{6a} were prepared by using the certified reference standard (SRM®2377) and the FAME-IS C23:0. RRFs were determined for the 26 FAMEs of the reference standard SRM®2377, in relation with FAME-IS C23:0.

S_{1a}-S_{6a} calibration solutions were injected into GC-MS in 3 replicates for each level, and it were calculated the RRF_i means determined for the 6 calibration levels. These RRF_i values were calculated using the formula from the equation (2).

$$RRF_i = \frac{Area\ ratio}{Amount\ ratio} = \frac{A_{FAMEi}/A_{FAME-IS}}{C_{FAMEi}/C_{FAME-IS}} = \frac{A_{FAME} \times C_{FAME-IS}}{A_{FAME-IS} \times C_{FAMEi}} \quad (2)$$

where: Area ratio - the ratio between A_{FAMEi} and $A_{FAME\text{-}IS}$ from the chromatogram of the calibration solution; Amount ratio - the ratio between C_{FAMEi} and $C_{FAME\text{-}IS}$ from the calibration solution level; A_{FAMEi} - area of $FAME_i$ from the chromatogram of calibration solution; $A_{FAME\text{-}IS}$ - area of FAME-IS from the chromatogram of calibration solution; C_{FAMEi} - quantity of $FAME_i$ from the calibration solution (µg); $C_{FAME\text{-}IS}$ - quantity of FAME-IS from the calibration solution (µg).

Preparation of FAMEs from oils/fats of vegetable and/or animal origin

Samples of sunflower oil, non-hydrogenated refined palm oil, fish oil and lard were prepared in accordance with ISO 661 (2003) and the fat of peanut butter sample was extracted based on ISO 17189 (2003). FAMEs were prepared by transesterification of fat extracted from the studied matrices according to ISO 12966-2 (2017) by using the transmethylation procedure with boron trifluoride (BF_3) catalyst.

Recovery determination

Recovery (Rec.) was determined by using an internal standard of triglyceride (TAG-IS) which is added to the fat sample extracted from the food matrix, before converting it into FAME.

The selection of IS is critical when using GC-MS, TAG-IS and FAME-IS must accomplish the following conditions: not to be included in the sample to be analyzed, to be completely separated from the rest of the components, and not to differ too much from the concentration of the component to be determined.

To determine recovery, a 4 mg/mL TAG-IS (C11:0/C15:0) solution was used. Over the amount of fat weighted into the 50 mL flask was added 250 µL of TAG-IS solution, and the preparation of FAME was performed as described in the procedure.

Recovery, expressed in %, is calculated based on equation (3).

$$\text{Rec.}\,(\%) = \frac{V \times D \times A_{TAG-IS} \times C_{FAME-IS} \times F_{TAG}}{A_{FAME-IS} \times RRF_i \times C_{TAG-IS}} \times 100 \quad (3)$$

where: V - volume of the final extract of $FAME_i$ (3 mL); D - dilution of final extract; $A_{TAG\text{-}IS}$ - peak area of TAG-IS from the chromatogram of final extract; $C_{FAME\text{-}IS}$ - quantity of internal standard added to the diluted final extract (µg); F_{TAG} - stoichiometric conversion factor of TAG-IS into FAME-IS; $A_{FAME\text{-}IS}$ - area of FAME-IS from the chromatogram of final extract; RRF_i - relative response factor of TAG-IS in relation to FAME-IS; $C_{TAG\text{-}IS}$ - quantity of TAG-IS added to the mass of fat taken into analysis (g).

Quantification of FAMEs/FAs composition of food samples analyzed

Quantification of FAMEi/FAi composition of food samples based on CFs

CFs determined according to equation (1) were used to quantify $FAME_i/FA_i$ (relative concentration expressed as weight % of total identified FAMEs/FAs) in the food matrices studied. The obtained values correspond to the weight percentage of $FAME_i/FA_i$ individually determined, expressed as triacylglycerol per 100 g fat and calculated using the equation (4).

$$p_i\% = \frac{CF_i \times A_i}{\sum(CF_i \times A_i)} \times 100 \quad (4)$$

where: p - weight percent of individually $FAME_i$, calculated as triacylglycerol per 100 g fat (%); CF_i - correction factors corresponding to each $FAME_i$ from the reference standard; A_i - peak area of $FAME_i$ from the chromatogram of fat extract; $\sum(CF_i x A_i)$ - sum of the results of CF_i xA_i for the FAME peaks from the extracted fat.

Quantification of $FAME_i/FA_i$ composition of food samples based on RRFs

The RRFs determined according to equation (2) were used for the quantification of $FAME_i/FA_i$ (absolute concentration expressed as g/100 g) in the food matrices studied. The values obtained are expressed as weight fraction in g $FAME_i/FA_i$ per 100 g sample and given by equations (5) and (6):

$$\text{g FAME}_i/100\text{g} = \frac{\text{V x D x Area ratio x C}_{\text{FAME-IS}} \text{ x L}}{\text{w x RRF}_i \text{ x F}_{tr}} \quad (5)$$

$$\text{g FA}_i = \text{g FAME}_i \text{ x F}_{\text{FAi}} \quad (6)$$

where: V - volume of the final extract of $FAME_i$ (3 mL); D - dilution of final extract; Area ratio - ratio between A_{FAMEi} and $A_{FAME\text{-}IS}$ from the final extract chromatogram; $C_{FAME\text{-}IS}$ - quantity of internal standard FAME-IS added to the diluted final extract (µg); L - lipid content of the food matrice (%); w - weight of sample taken into analysis (g); RRF_i - relative response factor of $FAME_i$ in relation to FAME-IS determined from the reference standard; F_{tr} - transformation factor from µg in g (10^6); F_{FAi} - stoichiometric conversion factor of $FAME_i$ into FA_i.

GC-MS analysis

Chromatographic analysis was performed on a Trace GC Ultra/TSQ Quantum XLS system (Thermo Fisher Scientific, USA), a gas chromatograph coupled with a mass spectrometer (MS) TSQ Quantum XLS, autosampler, TriPlus AS. FAMEs/FAs separation was realized on a high polarity capillary column, TR-FAME (60 m x 0.25 mm x 0.25 µm film thickness) of 70% cyanopropyl and 30% polysilphenyl-siloxane. Analysis of calibration solutions and the derivatized extract samples was performed in the positive electron impact ionization (EI^+) mode, selected ion monitoring (SIM) mode, using 24 segments. The ion source temperature was 250°C, the oven temperature was programmed at 100 °C for 0.2 min, increased to 240°C with 2 °C/min and hold for 15 min. The mobile phase was He of purity 99.9995% (5.0), at a constant flow rate of 1 mL/min. A volume of 0.5 µL of extract was injected at 240 °C in split mode with a 1:50 split ratio and a 50 mL/min splitting rate. Instrument control, data acquisition and processing were performed using the Xcalibur Program. The total run time of a GC-MS chromatogram was 85.20 min.

Validation procedure

The evaluation of the performance parameters of the GC-MS method to determine FAMEs/FAs in oils/fats of vegetable and/or animal origin was made based on the guidelines and recommendations for the validation of chromatographic methods (ISO 5725-6 (1994); Commission Decision 2002/657/EC; Taverniers, 2004; ICH, 2005).

The internal validation procedure of the method, implied the evaluation and optimisation of the performance parameters, by repeated measurements and the interpretation of the results obtained according to the established acceptability criteria. The following performance parameters of the method were evaluated: method linearity, working range, sensitivity, precision, accuracy, recovery, and robustness.

Statistical analysis

The significance of difference was performed by using one-way ANOVA (analysis of variance) followed by Tukey's test and was considered significant when p value was less than 0.05. Data are expressed as mean ± standard deviation. Analyses were performed by using the SPSS software program (IBM SPSS Statistics 24).

Results and Discussions

The aim of this study was to validate a precise and robust method for $FAME_i/FA_i$ determination from oils/fats of vegetal and/or animal origin. For the identification of FAME, the retention times (RT) of FAME were confirmed by comparing their RT and the mass/charge (*m/z*) ratio characteristic of each compound with those obtained for each individual FAME from the reference materials.

The elution order of the FAME of the SRM®2377 is given in the chromatogram from Fig. 1.

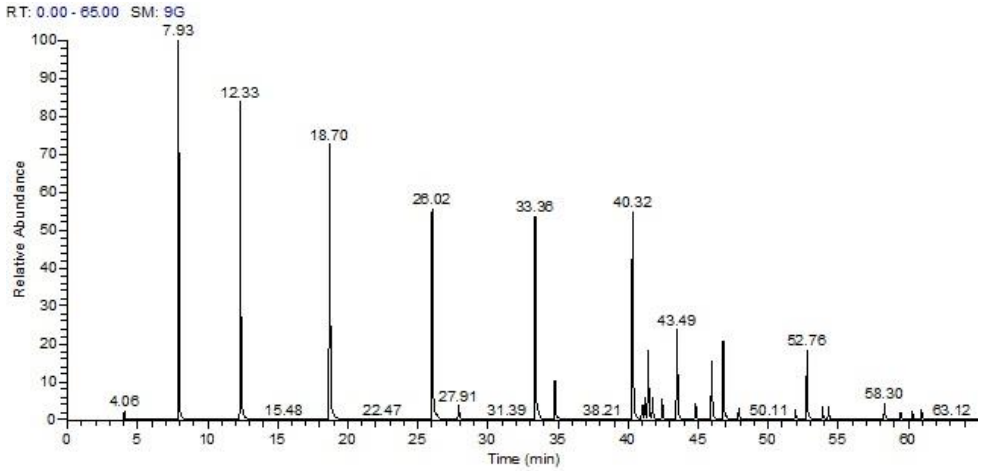

Figure 1. Chromatogram of 26 separated and identified FAMEs from the reference standard SRM®2377

The correction factors (CFs) and relative response factors (RRFs) were determined in order to quantify the concentration of each $FAME_i/FA_i$ present in the sample.

CFs and RRFs experimentally determined

The detector's response is different based on the carbon chains of methyl esters and will respond differently, which determines that experimentally determined CF_i or RRF_i should be used when quantifying $FAME_i/FA_i$.

CFs were calculated in each calibration level as well the CF mean for the 6 concentration levels was obtained. By evaluating the CFi values obtained by using the two reference standards (F.A.M.E. Mix, C4-C24 and SRM®2377), it is noted that for the FAMEs common to the both standards (23 FAME) there are no major differences between them, and therefore can be determined up to 40 FAME from the food samples (23 FAMEs are common to the reference standards F.A.M.E. Mix, C4-C24 and SRM®2377, 3 are SRM®2377 specific, and 14 are F.A.M.E. Mix, C4-C24 specific). Based on CFs experimentally determined, FAMEs in the food matrices studied can be quantified as relative concentration, expressed as weight %.

RRFs that were obtained experimentally, were calculated based on the equation (2), for each $FAME_i/FA_i$ of the SRM®2377 standard, in relation to FAME-IS C23:0. Based on the experimentally determined RRF_i, 26 FAMEs can be quantified (absolute concentration, expressed in g/100 g) from the food matrices studied.

Evaluation of performance characteristics

The following performance characteristics of the method were evaluated: linearity, working range, sensitivity (LOD and LOQ), precision, accuracy, recovery, and robustness.

Linearity

Linearity was determined by performing the calibration curves (linear) from different serial dilutions of the FAME stock solutions. Good linearity of the MS detector response was found for all 26 FAME with linear regression coefficients (R^2) higher than 0.99. The detailed results are summarized in Table 1.

Working range

The working range for quantification of $FAME_i/FA_i$ is the lowest working limit equal to the LOQ and the upper working limit given by the maximum concentration of S_{6a} solution in the standard reference material SRM®2377.

Sensitivity

The sensitivity of the method was evaluated by limit of detection (LOD) and limit of quantification (LOQ), calculated based on calibration curves and estimated using the ICH approach (2005). LOD and LOQ were calculated based on the residual standard deviation of the calibration curve (SD) and the slope of the calibration curve (b) for each $FAME_i/FA_i$ of the reference standard SRM®2377, in relation with FAME-IS C23:0, where LOD = 3.3xSD/b and LOQ = 10xSD/b (Table 1).

Precision

To estimate precision, repeatability (equipment precision and method precision) and intra-laboratory reproducibility were determined. The equipment precision was achieved by consecutive injections (n= 9) of the same sample on RT. The CV(r) values obtained experimentally were between 0.01 and 0.04% for all the $FAME_i/FA_i$ and correspond to laboratory requirements ($CV(r) \leq 0.05\%$), for all of the tested samples.

The intra-day precision was determined on 5-6 replicates, from each matrix, in repeatability conditions, by evaluating the statistical parameters: mean of absolute concentration (g/100 g), mean of relative concentration (%); standard deviation under repeatability conditions, SD(r); coefficient of variation under repeatability conditions, CV(r); the repeatability limit (*r*) for both individual $FAME_i/FA_i$ and sum of SFA, MUFA and PUFA.

The mean values of the concentration and repeatability limits (*r*) for the food matrices taken into study are presented in Table 2 and correspond to the required conditions, according to ISO 12966-4 (2017). The repeatability limit (*r*) for each $FAME_i/FA_i$, sum of SFA, MUFA and PUFA was below 1.32% for sunflower oil, palm oil and lard quantified based on CFs and was below 8.15 g/100 g when it were calculated based on RRFs. Fish oil was quantified just

Table 1. Performance characteristics of the method (linearity, sensitivity) in relation to FAME-IS C23:0

No.	Compound name	Identification		Regression equations[a]	R^2	Sensitivity	
		RT (min)	*m/z*	(y=ax+b)/linearity range (µg/mL)		LOD (µg/mL)	LOQ (µg/mL)
1	C8:0 caprylic acid	7.93	74	y = 0.0402x – 0.1054/ 37.50 – 284.74	0.9966	12.50	37.50
2	C10:0 capric acid	12.33	74	y = 0.0414x – 0.1262/ 38.59 – 293.07	0.9989	12.00	36.37
3	C12:0 lauric acid	18.70	74	y = 0.0392x – 0.0305/ 40.81 – 309.91	0.9999	4.36	13.30
4	C14:0 myristic acid	26.02	74	y = 0.0359x + 0.0056/ 36.59 – 277.87	0.9999	4.03	12.22
5	C14:1n5 myristoleic acid	27.91	55	y = 0.0105x – 0.0006/ 9.75 – 74.02	0.9998	1.42	4.29
6	C16:0 palmitic acid	33.36	74	y = 0.0338x – 0.063/ 37.981 – 288.42	0.9995	7.44	22.54
7	C16:1n7 palmitoleic acid	34.77	55	y = 0.0088x – 0.01/ 25.89 – 196.62	0.9991	7.23	21.92
8	C18:0 stearic acid	40.32	74	y = 0.0311x – 0.1238/ 39.53 – 300.14	0.9993	9.87	29.90
9	C18:1n9t elaidic acid	41.00	55	y = 0.0073x – 0.0104/ 10.39 – 78.94	0.9988	3.28	9.94
10	C18:1n7t *trans*- vaccenic acid	41.19	55	y = 0.0088x – 0.0122/ 12.19 – 92.54	0.9990	3.59	10.86
11	C18:1n9 oleic acid	41.43	55	y = 0.0087x – 0.0233/ 36.08 – 273.96	0.9989	10.94	33.15
12	C18:1n7 vaccenic acid	41.74	55	y = 0.0089x + 0.005/ 11.89 – 90.28	0.9959	3.96	11.89
13	C18:2n6t linolelaidic acid	42.43	67	y = 0.0117x - 0.0237/ 10.35 – 79.96	0.9992	2.72	8.25
14	C18:2n6 linoleic acid (LA)	43.49	67	y = 0.0119x - 0.0049/ 37.72 – 286.46	0.9990	10.86	32.92
15	C18:3n6 γ-linolenic acid (GLA)	44.81	79	y = 0.0105x - 0.0112/ 9.24 – 70.19	0.9992	2.43	7.36
16	C18:3n3 α-linolenic acid (ALA)	45.97	79	y = 0.0152x - 0.0387/ 21.92 – 166.49	0.9991	6.15	18.64
17	C20:0 arachidic acid	46.78	74	y = 0.0272x - 0.069/ 18.90 – 143.04	0.9987	6.24	18.90
18	C20:1n9 gondolic acid	47.90	55	y = 0.0080x - 0.0115/ 9.87 - 75	0.9985	3.29	9.87
19	C20:4n6 arachidonic acid (ARA)	51.94	79	y = 0.0097x - 0.0104/ 7.78 – 59.05	0.9990	2.26	6.84
20	C22:0 behenic acid	52.76	74	y = 0.0231x - 0.0703/ 22.03 – 167.27	0.9978	7.34	22.03
21	C22:1n9 erucic acid	53.88	55	y = 0.0072x – 0.0112/ 11.34 – 86.14	0.9979	3.78	11.34
22	C20:5n3 eicosapentaenoic acid (EPA)	54.30	79	y = 0.0102x – 0.0153/ 7.85 – 59.64	0.9988	2.25	7.73
IS	C23:0 tricosanoic acid	55.54	74	-	-	-	-
23	C24:0 lignoceric acid	58.30	74	y = 0.0183x – 0.0337/ 9.30 – 70.62	0.9973	3.10	9.30
24	C24:1n9 nervonic acid	59.43	55	y = 0.0065x – 0.0109/ 8.96 - 68	0.9976	2.99	8.96
25	C22:5n3 docosapentaenoic acid (DPA)	60.29	79	y = 0.0092x – 0.0137/ 7.34 – 55.73	0.9987	2.45	7.34
26	C22:6n3 docosahexaenoic acid (DHA)	60.94	79	y = 0.0090x – 0.0168/ 8.34 – 63.35	0.9987	2.78	8.34

[a] y- FAMEi peak area/FAME-IS peak area; x- [FAMEi]/[FAME-IS]; IS- internal standard (C23:0); R^2 – regression coefficient

based on CFs and the repeatability limit was below 2.68%. This sample was not quantified based on RRFi, as the internal standard, FAME-IS C23:0, was found in this matrix.

The same statistical parameters were also evaluated for intra-laboratory reproducibility by repeated measurements (n= 5-9), by different analysts, on identical samples from each food matrix. The results obtained (Table 3) correspond to the requirements for reproducibility according to ISO 12966-4 (2017). The reproducibility limit (*R*) for each $FAME_i/FA_i$, sum of SFA, MUFA and PUFA was below 2.55% for sunflower oil, palm oil and lard quantified based on CFs and below 8.91 g/100 g when it were calculated based on RRFs. Fish oil was quantified just based on CFs and the reproducibility limit was below 2.10%.

As it can be seen in Table 2, oleic acid (C18:1n9) and linoleic acid (C18:2n6) were the major FAs of sunflower oil, followed by palmitic acid (C16:0). Similar results were obtained by Chowdbury (2007) and Kostik (2013) who studied the composition of sunflower oil and showed a high content of oleic and lino leic acids, and a lower content of palmitic acid. Sunflower oil has a low content of SFA, making it a suitable oil for consumption.

Regarding the fatty acid composition of palm oil, our results are in accordance with the one obtained by Chowdhury (2007), Kamatou (2017), and Montoya (2014) who showed that the major FAs are palmitic and oleic acids, followed by linoleic and stearic acids (C18:0).

Table 2. Mean concentration and repeatability limit (*r*) for FA_i/SFA/MUFA/PUFA from the composition of food samples taken into study

Compound name	Sunflower oil, n = 5		Palm oil, n=6		Fish oil, n = 6		Lard, n=5	
	Concentration (*), [**]	*r* (*), [**]	Concentration (*), [**]	*r* (*), [**]	Concentration (*)	*r* (*)	Concentration (*), [**]	*r* (*), [**]
C4:0 butyric acid	-	-	-	-	(0.01)	(0.01)	-	-
C8:0 caprylic acid	-	-	-	-	-	-	(0.01), [0.01]	(0.01), [0.01]
C10:0 capric acid	-	-	-	-	(0.02)	(0.01)	(0.08), [0.06]	(0.02), [0.01]
C12:0 lauric acid	-	-	-	-	(0.17)	(0.12)	(0.12), [0.11]	(0.02), [0.01]
C13:0 tridecanoic acid	-	-	-	-	(0.06)	(0.05)	-	-
C14:0 myristic acid	(0.07), [0.06]	(0.01), [0.01]	(0.92), [1.06]	(0.06), [0.06]	(5.66)	(0.40)	(1.99), [1.80]	(0.36), [0.25]
C14:1n5 myristoleic acid	-	-	-	-	(0.26)	(0.18)	-	-
C15:0 pentadecanoic acid	-	-	-	-	(0.84)	(0.57)	-	-
C15:1n5 pentadecenoic acid	-	-	-	-	(0.06)	(0.04)	-	
C16:0 palmitic acid	(5.69), [5.58]	(0.15), [0.43]	(42.67), [50.62]	(1.26), [4.43]	(11.87)	(0.62)	(25.33), [24.23]	(1.32), [2.82]
C16:1n7 palmitoleic acid	(0.12), [0.12]	(0.01), [0.02]	-	-	(7.40)	(0.51)	(2.72), [2.67]	(0.13), [0.37]
C17:0 margaric acid	-	-	-	-	(1.08)	(0.68)	-	-
C18:0 stearic acid	(2.40), [2.38]	(0.15), [0.21]	(3.83), [4.61]	(0.18), [0.71]	(2.19)	(0.23)	(10.71), [10.39]	(0.60), [1.99]
C18:1n9 oleic acid	(46.92), [47.84]	(0.51), [4.29]	(41.73), [51.48]	(0.71), [6.38]	(5.71)	(0.49)	(43.40), [43.21]	(0.74), [7.15]
C18:1n7 vaccenic acid	-	-	(0.69), [0.86]	(0.08), [0.13]	(2.48)	(0.25)	(3.22), [3.2]	(0.06), [0.53]
C18:2n6 linoleic acid (LA)	(43.60), [41.95]	(0.33), [3.44]	(9.72), [11.33]	(0.45), [1.70]	(0.98)	(0.10)	(11.08), [10.44]	(0.74), [2.12]
C18:3n6 γ-linolenic acid (GLA)	-	-	-	-	(0.45)	(0.27)	-	-
C18:3n3 α-linolenic acid (ALA)	(0.05), [0.05]	(0.02), [0.02]	(0.16), [0.19]	(0.02), [0.04]	(1.42)	(0.87)	(0.37), [0.35]	(0.06), [0.11]
C20:0 arachidic acid	(0.18), [0.18]	(0.07), [0.03]	(0.27), [0.33]	(0.04), [0.08]	(0.29)	(0.18)	(0.15), [0.15]	(0.01), [0.03]
C20:1n9 gondolic acid	(0.15), [0.15]	(0.01), [0.01]	-	-	(1.63)	(0.96)	(0.71), [0.72]	(0.10), [0.15]
C21:0 heneicosanoic acid	-	-	-	-	(0.06)	(0.04)	-	-
C20:2n6 eicosadienoic acid	-	-	-	-	(0.31)	(0.19)	-	-
C20:3n6 dihomo-γ-linolenic (DGLA)	-	-	-	-	(0.56)	(0.34)	-	-
C20:4n6 arachidonic acid (ARA)	-	-	-	-	(2.55)	(1.53)	(0.12), [0.12]	(0.01), [0.02]
C20:3n3 eicosatrienoic acid (ETE)	-	-	-	-	(0.15)	(0.09)	-	-
C22:0 behenic acid	(0.58), [0.57]	(0.09), [0.10]	-	-	(0.16)	(0.10)	-	-
C22:1n9 erucic acid	-	-	-	-	(0.26)	(0.16)	-	-
C20:5n3 eicosapentaenoic acid (EPA)	-	-	-	-	(27.72)	(0.86)	-	-
C23:0 tricosanoic acid	-	-	-	-	(0.04)	(0.02)	-	-
C22:2n6 docosadienoic acid	-	-	-	-	(0.03)	(0.02)	-	-
C24:0 lignoceric acid	(0.23), [0.23]	(0.03), [0.05]	-	-	(0.07)	(0.04)	-	-
C24:1n9 nervonic acid	-	-	-	-	(0.99)	(0.60)	-	-
C22:5n3 docosapentaenoic acid (DPA)	-	-	-	-	(1.88)	(0.25)	-	-
C22:6n3 docosahexaenoic acid (DHA)	-	-	-	-	(22.62)	(2.68)	-	-
SFA	(9.1), [9.00]	(0.25), [0.66]	(47.7), [56.62]	(1.11), [4.83]	(22.5)	(1.30)	(38.4), [36.75]	(1.12), [5.05]
MUFA	(47.2), [48.11]	(0.50), [4.30]	(42.4), [52.34]	(0.70), [5.91]	(18.8)	(0.95)	(50.0), [49.80]	(0.68), [8.15]
PUFA	(43.7), [42.00]	(0.32), [3.45]	(9.9), [11.52]	(0.46), [1.59]	(58.7)	(2.24)	(11.6), [10.91]	(0.69), [2.21]

(*) – relative concentration (% FAi from total FAs); [**] – absolute concentration (g FAi/100 g); *r* - limit of repeatability; $r = 2.8 \times SD(r)$.

Similar results were also obtained by Al-Khusaibi (2012) who studied the fatty acid composition of palm olein.

The fish oil taken into study had a high content of omega-3 fatty acids, eicosapentaenoic acid (C20:5n3 – EPA) and docosahexaenoic acid (C22:6n3 – DHA) being the major FAs found.

In the case of lard, it can be noticed (Table 2) that it has a high content of oleic acid, followed by palmitic, linoleic and stearic acids. Piasentier (2009) studied the fatty

acid composition of heavy pig back fat and the results are in agreement with the one obtained in this study.

By evaluating the two FAMEs quantification approaches, with and without IS in the matrices studied, it was noticed that the values are close. RRFs quantification is more accurate, but the cost of the method is higher due to IS used. Sample preparation and processing by using IS is much more expensive than quantification based on CFs (without IS).

Table 3. Mean concentration and reproducibility limit (*R*) for FA_i/SFA/MUFA/PUFA from the composition of food samples taken into study

Compound name	Sunflower oil, n = 5		Palm oil, n=6		Fish oil, n = 6		Lard, n=5	
	Concentration (*), [**]	*R* (*), [**]	Concentration (*), [**]	*R* (*), [**]	Concen-tration (*)	*R* (*)	Concentration (*), [**]	*R* (*), [**]
C4:0 butyric acid	-	-	-	-	(0.01)	(0.01)	-	-
C8:0 caprylic acid	-	-	-	-	-	-	(0.01), [0.01]	(0.00), [0.01]
C10:0 capric acid	-	-	-	-	(0.01)	(0.01)	(0.07), [0.07]	(0.02), [0.02]
C12:0 lauric acid	-	-	-	-	(0.15)	(0.08)	(0.11), [0.11]	(0.02), [0.02]
C13:0 tridecanoic acid	-	-	-	-	(0.06)	(0.03)	-	-
C14:0 myristic acid	(0.07), [0.06]	(0.01), [0.01]	(0.94), [1.07]	(0.15), [0.11]	(5.62)	(0.10)	(1.92), [1.98]	(0.17), [0.33]
C14:1n5 myristoleic acid	-	-	-	-	(0.23)	(0.12)	-	-
C15:0 pentadecanoic acid	-	-	-	-	(0.74)	(0.39)	-	-
C15:1n5 pentadecenoic acid	-	-	-	-	(0.05)	(0.03)	-	-
C16:0 palmitic acid	(5.84), [5.37]	(0.21), [0.29]	(42.79), [51.08]	(2.55), [6.03]	(11.88)	(0.22)	(25.17), [26.76]	(1.61), [4.57]
C16:1n7 palmitoleic acid	(0.13), [0.12]	(0.01), [0.02]	-	-	(7.40)	(0.21)	(2.73), [2.98]	(0.11), [0.50]
C17:0 margaric acid	-	-	-	-	(0.92)	(0.48)	-	-
C18:0 stearic acid	(2.33), [2.17]	(0.03), [0.16]	(3.84), [4.72]	(0.36), [0.67]	(2.22)	(0.13)	(10.83), [11.65]	(0.62), [2.04]
C18:1n9 oleic acid	(47.07), [45.06]	(0.19), [3.04]	(41.57), [52.13]	(1.47), [6.93]	(5.82)	(0.34)	(43.36), [47.94]	(0.37), [7.70]
C18:1n7 vaccenic acid	-	-	(0.70), [0.87]	(0.05), [0.13]	(2.53)	(0.15)	(3.20), [3.56]	(0.12), [0.57]
C18:2n6 linoleic acid (LA)	(43.39), [39.22]	(0.07), [2.68]	(9.72), [11.59]	(0.89), [1.69]	(1.01)	(0.06)	(11.25), [11.75]	(0.82), [2.15]
C18:3n6 γ-linolenic acid (GLA)	-	-	-	-	(0.39)	(0.21)	-	-
C18:3n3 α-linolenic acid (ALA)	(0.05), [0.05]	(0.02), [0.01]	(0.17), [0.20]	(0.03), [0.02]	(1.22)	(0.63)	(0.36), [0.39]	(0.05), [0.07]
C20:0 arachidic acid	(0.18), [0.17]	(0.01), [0.02]	(0.27), [0.34]	(0.03), [0.05]	(0.25)	(0.13)	(0.15), [0.16]	(0.01), [0.03]
C20:1n9 gondolic acid	(0.16), [0.15]	(0.02), [0.02]	-	-	(1.40)	(0.70)	(0.71), [0.80]	(0.07), [0.18]
C21:0 heneicosanoic acid	-	-	-	-	(0.05)	(0.03)	-	-
C20:2n6 eicosadienoic acid	-	-	-	-	(0.27)	(0.14)	-	-
C20:3n6 dihomo-γ-linolenic (DGLA)	-	-	-	-	(0.48)	(0.25)	-	-
C20:4n6 arachidonic acid (ARA)	-	-	-	-	(2.18)	(1.12)	(0.12), [0.14]	(0.01), [0.02]
C20:3n3 eicosatrienoic acid (ETE)	-	-	-	-	(0.13)	(0.07)	-	-
C22:0 behenic acid	(0.56), [0.52]	(0.03), [0.06]	-	-	(0.14)	(0.07)	-	-
C22:1n9 erucic acid	-	-	-	-	(0.22)	(0.11)	-	-
C20:5n3 eicosapentaenoic acid (EPA)	-	-	-	-	(28.38)	(1.92)	-	-
C23:0 tricosanoic acid	-	-	-	-	(0.03)	(0.02)	-	-
C22:2n6 docosadienoic acid	-	-	-	-	(0.03)	(0.01)	-	-
C24:0 lignoceric acid	(0.23), [0.22]	(0.02), [0.03]	-	-	(0.06)	(0.03)	-	-
C24:1n9 nervonic acid	-	-	-	-	(0.86)	(0.43)	-	-
C22:5n3 docosapentaenoic acid (DPA)	-	-	-	-	(1.93)	(0.18)	-	-
C22:6n3 docosahexaenoic acid (DHA)	-	-	-	-	(23.32)	(2.10)	-	-
SFA	(9.2), [8.51]	(0.18), [0.47]	(47.8), [57.21]	(2.31), [6.89]	(22.1)	(1.05)	(38.3), [40,74]	(1.19), [6.77]
MUFA	(47.4), [45.33]	(0.20), [2.78]	(42.3), [53.00]	(1.46), [7.05]	(18.5)	(0.75)	(50.0), [55.28]	(0.43), [8.91]
PUFA	(43.4), [39.27]	(0.07), [2.46]	(9.9), [11.79]	(0.87), [1.71]	(59.4)	(1.79)	(11.7), [12.28]	(0.80), [2.20]

(*) – relative concentration (% FAi from total FAs); [**] – absolute concentration (g FAi/100 g); *R* - limit of reproducibility; *R* = 2.8 x SD(R)

Accuracy

The accuracy of the method was achieved by using a NIST certified reference material, peanut butter (SRM®2387). The fatty acids composition was evaluated by applying the developed method and comparing the results with the certified values of SRM®2387. The determined fat content of SRM®2387 was compared to the certified value. This consists in comparing the absolute difference between the certified value and the measured value (Δ_m), with the expanded uncertainty (U_Δ) (LINSINGER, 2010).

As shown in Table 4, the experimentally measured values did not show significant differences ($p > 0.05$) from the NIST-certified values except for the C16:1n7 acid, where the value found was below LOQ (21.92 µg/mL = 0.13 g/100 g) and higher than LOD (7.23 µg/mL = 0.04 g/100 g). Results are presented as mean of repeated measurements (n= 6).

Table 4. Accuracy test results of SRM®2387 (peanut butter)

Fat and FAi profile	Concentration (g FAi/100 g)	
	Certified values	Measured values (n= 6)
Fat	51.60 ± 1.4	51.72 ± 0.49
Saturated fatty acids (SFA)	10.4 ± 0.2	10.6 ± 0.56
C14:0 myristic acid	0.024 ± 0.002	0.022 ± 0.00
C16:0 palmitic acid	4.94 ± 0.15	5.09 ± 0.24
C18:0 stearic acid	2.13 ± 0.008	2.18 ± 0.12
C20:0 arachidic acid	0.710 ± 0.029	0.685 ± 0.05
C22:0 behenic acid	1.81 ± 0.08	1.89 ± 0.18
C24:0 lignoceric acid	0.781 ± 0.044	0.765 ± 0.08
Monounsaturated fatty acids (MUFA)	24.4 ± 0.9	26.5 ± 1.3
C16:1n7 palmitoleic acid	0.044 ± 0.010	< LOQ
C18:1n9 oleic acid	23.38 ± 0.90	25.44 ± 1.26
C18:1n7 vaccenic acid	0.255 ± 0.016	0.277 ± 0.01
C20:1n9 gondolic acid	0.643 ± 0.031	0.622 ± 0.044
Polyunsaturated fatty acids (PUFA)	13.2 ± 0.4	14.0 ± 0.7
C18:2n6 linoleic acid (LA)	13.15 ± 0.41	13.93 ± 0.69
C18:3n3 α-linolenic acid (ALA)	0.030 ± 0.001	0.033 ± 0.004

Recovery

Recovery was calculated for sunflower oil, peanut butter and lard. The obtained mean recoveries for the $FAME_i/FA_i$ determined in the food matrices studied is shown in Table 5. The addition of an internal standard has been largely used in the analysis of fatty acids, as it allows the expression of results as weight (g/100 g). This method is less susceptible to errors, because the internal standard and the sample are injected together. Recovery determined using the two internal standards, TAG-IS and FAME-IS, complies with the required conditions (100% ± 20%). When recovery is not between these limits, the origin of the problem could be due to shorter reaction time, lower reaction temperature, incomplete transesterification of internal standard, partial degradation or loss due to evaporations of and other derivatives. Recovery is only an informative title, it does not apply to correct the results.

Robustness

Robustness testing was performed by varying the operational parameters, one at a time, such as sample weight (± 10% in relation to the fat weight taken under work, according to the procedure) and detector temperature (± 5°C in relation to the method conditions) for the sunflower oil, and fat extraction method (3 different extraction approaches), in the case of peanut butter. Three different ways of peanut butter (SRM®2387) fat extraction were studied on the FAi composition: fat extraction according to ISO 17189 (2003); fat extraction using the Soxhlet extractor; and fat extraction using the Büchi automatic unit by applying the Soxhlet hot extraction method.

The composition in FA_i was performed by both quantification approaches, based on CF_i and RRF_i. The results obtained by both quantification approaches (CF_i/RRF_i), did not show significant differences ($p > 0.05$) in FA_i composition in the test samples when varying the sample weight taken into work, detector temperature (Table 6) and fat extraction method (Table 7).

Table 5. Mean recoveries for the food samples

Food sample	Internal standards used		Number of samples, n	Recovery ± SD, %
	TAG-IS	FAME-IS		
Sunflower oil	C15:0	C23:0	15	89.86 ± 6.21
Peanut butter	C15:0	C23:0	14	94.30 ± 13.66
Lard	C11:0	C23:0	14	96.16 ± 14.37

Table 6. Robustness test results for weight and detector temperature variation for sunflower oil

Compound name	Concentration (*), [**] (n= 4)				
	Procedural parameters (0.050 g; 250°C)	Sample weight (± 0.005 g)		Detector temperature (± 5°C)	
		0.045 g	0.055 g	245ºC	255ºC
C14:0 myristic acid	(0.05), [0.05]	(0.06), [0.06]	(0.05), [0.06]	(0.05), [0.05]	(0.05), [0.05]
C16:0 palmitic acid	(5.60), [5.92]	(5.65), [5.74]	(5.58), [6.00]	(5.66), [5.95]	(5.65), [5.92]
C16:1n7 palmitoleic acid	(0.11), [0.12]	(0.12), [0.13]	(0.12), [0.13]	(0.11), [0.12]	(0.12), [0.13]
C18:0 stearic acid	(2.24), [2.40]	(2.25), [2.32]	(2.30), [2.45]	(2.25), [2.41]	(2.25), [2.41]
C18:1n9 oleic acid	(47.63), [52.37]	(47.44), [50.30]	(47.42), [52.35]	(47.49), [52.21]	(47.62), [51.96]
C18:2n6 linoleic acid (LA)	(43.35), [44.94]	(43.42), [43.42]	(43.42), [45.28]	(43.45), [45.14]	(43.37), [44.70]
C18:3n3 α-linolenic acid (ALA)	(0.05), [0.05]	(0.05), [0.05]	(0.05), [0.05]	(0.05), [0.05]	(0.05), [0.05]
C20:0 arachidic acid	(0.16), [0.18]	(0.17), [0.18]	(0.18), [0.18]	(0.16), [0.18]	(0.16), [0.17]
C20:1n9 gondolic acid	(0.15), [0.16]	(0.14), [0.16]	(0.16), [0.17]	(0.15), [0.16]	(0.13), [0.15]
C22:0 behenic acid	(0.49), [0.53]	(0.52), [0.53]	(0.55), [0.56]	(0.47), [0.52]	(0.46), [0.51]
C24:0 lignoceric acid	(0.16), [0.18]	(0.19), [0.20]	(0.19), [0.20]	(0.16), [0.18]	(0.15), [0.16]
SFA	(8.7), [9.25]	(8.8), [9.03]	(8.8), [9.44]	(8.8), [9.29]	(8.7), [9.21]
MUFA	(47.9), [52.64]	(47.7), [50.59]	(47.7), [52.65]	(47.7), [52.49]	(47.9), [52.23]
PUFA	(43.4), [44.99]	(43.5), [43.47]	(43.5), [45.33]	(43.5), [45.19]	(43.4), [44.75]

(*) – relative concentration (% FAi from total FAs); [**] – absolute concentration (g FAi/100 g)

Table 7. Robustness test results for the fat extraction approaches from SRM®2387 (peanut butter)

Compound name	Concentration [**] (n= 4)			
	Certified values	Measured values		
		ISO 17189 (2003)	Soxhlet extractor	Soxhlet hot extraction
C14:0 myristic acid	0.024 ± 0.002	0.022 ± 0.002	0.024 ± 0.002	0.022 ± 0.01
C16:0 palmitic acid	4.94 ± 0.15	4.94 ± 0.07	5.12 ± 0.05	5.04 ± 0.20
C16:1n7 palmitoleic acid	0.044 ± 0.010	< LOQ	< LOQ	< LOQ
C18:0 stearic acid	2.13 ± 0.08	2.10 ± 0.09	2.15 ± 0.11	2.15 ± 0.19
C18:1n9 oleic acid	23.38 ± 0.9	24.65 ± 0.66	25.23 ± 0.55	24.92 ± 1.23
C18:1n7 vaccenic acid	0.255 ± 0.016	0.267 ± 0.011	0.257 ± 0.009	0.273 ± 0.023
C18:2n6 linoleic acid (LA)	13.15 ± 0.41	13.52 ± 0.51	14.07 ± 0.41	14.09 ± 0.90
C18:3n3 α-linolenic acid (ALA)	0.030 ± 0.001	0.031 ± 0.003	0.028 ± 0.002	0.029 ± 0.001
C20:0 arachidic acid	0.710 ± 0.029	0.663 ± 0.054	0.678 ± 0.050	0.696 ± 0.091
C20:1n9 gondolic acid	0.643 ± 0.031	0.602 ± 0.047	0.604 ± 0.054	0.628 ± 0.071
C22:0 behenic acid	1.81 ± 0.08	1.84 ± 0.18	1.90 ± 0.16	1.97 ± 0.30
C24:0 lignoceric acid	0.781 ± 0.044	0.741 ± 0.082	0.789 ± 0.068	0.85 ± 0.164
SFA (g/100 g)	10.4 ± 0.2	10.3 ± 0.5	10.7 ± 0.4	10.7 ± 0.9
MUFA (g/100 g)	24.4 ± 0.9	25.5 ± 0.7	26.1 ± 0.6	25.8 ± 1.3
PUFA (g/100 g)	13.2 ± 0.4	13.5 ± 0.5	14.1 ± 0.4	14.1 ± 0.9

[**] – absolute concentration (g FAi/100 g)

Similar results with the ones presented in table 6 were obtained by Petrovic (2010) who varied the sample weight of pumpkin oil and showed that there is no significant change when varying this parameter.

The FA_i composition of sunflower oil was analyzed by both CF_i and RRF_i quantification approaches. The results obtained (Table 6) by the temperature variation of the detector (245°C, 255°C) did not show significant

differences ($p > 0.05$) in FAi composition in the test sample compared to the working procedure variant (250°C).

The composition in FA_i of peanut butter in the three fat extraction variants was compared with the certified values of the certified reference material (LINSINGER, 2010). The obtained results (Table 7) showed that there are no significant differences ($p > 0.05$) between the measurement results and the certified value ($\Delta m \leq U_\Delta$) and demonstrated that the method is robust.

Conclusions

A precise and robust method for the simultaneous determination of $FAME_i/FA_i$ from oils/fats of vegetable and/or animal origin has been developed and validated. The proposed method allows $FAME_i/FA_i$ analysis by two quantification approaches. The first is based on correction factors, by simultaneous determination of max. 40 FAME (relative concentration expressed as weight % of total identified FAME) and/or as the sum of SFA, MUFA, PUFA from oils/fats of vegetable and/or animal origin. The second one is based on the relative response factors, by simultaneous determination of max. 26 FAME (absolute concentration expressed in g/100 g), as individual $FAME_i/FA_i$ and/or as the sum of SFA, MUFA, PUFA from oils/fats of vegetable and/or animal origin, using GC-MS. The quality of the calibration curves (R^2), detection limit, quantification limit, precision, accuracy, recovery tests, robustness of the developed method have provided sufficient evidence suggesting that the proposed method is an appropriate alternative for determination of $FAME_i/FA_i$ composition of oils/fats of vegetable and/or animal origin, with a view to nutritional characterization of foods.

Abbreviations

CF, correction factors; FA, fatty acids; FAME, fatty acid methyl esters; IS, internal standard; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Rec., recovery; RRF, relative response factors; RT, retention time; SFA, saturated fatty acids.

Acknowledgments

This work was performed through the Core Program, carried out with the support of the Ministry of Research and Innovation, project no. PN 16 46 01 01.

References

1. ADJEPONG M, VALENTINI K, PICKENS CA, LI W et al. Quantification of fatty acid and mineral levels of selected seeds, nuts and oils in Ghana. *J. Food Compos. Anal.* 2017; 59: 43-49. http://dx.doi.org/10.1016/j.jfca.2017.02.007.
2. AL-KHUSAIBI M, GORDON MH, LOVEGROVE JA, NIRANJAN K. Frying of potato chips in a blend of canola oil and palm oilein: changes in levels of individual fatty acids and tocols. *Int J Food Sci Technol,* 2012; 47: 1701-1709. https://doi.org/10.1111/j.1365-2621.2012.03024.x
3. CASTEJÓN D, MATEOS-APARICIO I, MOLERO MD, CAMBERO MI et al. Evaluation and optimization of the analysis of fatty acid types in edible oils by ^{1}H-NMR. *Food Anal. Methods.* 2014; 7: 1285-1297. doi 10.1007/s12161-013-9747-9.
4. CHEN Y, YANG Y, NIE S, YANG X. et al. The analysis of *trans* fatty acid profiles in deep frying palm oil and chicken fillets with an improved gas chromatography method. *Food Control.* 2014; 44: 191-197. http://dx.doi.org/10.1016/j.foodcont.2014.04.010
5. CHOWDHURY K, BANU LA, KHAN S, LATIF A. Studies on the fatty acid composition of edible oil. *Bangladesh J. Sci. Res.* 2007; 42(3): 311-316. https://doi.org/10.3329/bjsir.v42i3.669
6. Commission Decision of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, (2002/657/EC), L 221/8-36. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002D0657&from=EN Accessed on January 2018.
7. CRUZ-HERNANDEZ C, GOEURIOT S, GIUFFRIDA F, THAKKAR SK et al. Direct quantification of fatty acids in human milk by gas chromatography. *J. Chromatogr. A.* 2013; 1284: 174-179. http://dx.doi.org/10.1016/j.chroma.2013.01.094
8. DA COSTA FILHO PA. Developing a rapid and sensitive method for determination of *trans*-fatty acids in edible oils using middle-infrared spectroscopy. *Food Chem.* 2014; 158: 1-7. http://dx.doi.org/10.1016/j.foodchem.2014.02.084
9. DE CASTRO BARRA PM, BARRA MM, AZEVEDO MS, FETT R, et al. A rapid method for monitoring total *trans* fatty acids (TTFA) during industrial manufacturing of Brazilian spreadable processed cheese by capillary zone electrophoresis. *Food Control.* 2012; 23: 456-461. https://doi.org/10.1016/j.foodcont.2011.08.014
10. De la MATA-ESPINOSA P, BOSQUE-SENDRA JM, CUADROS-RODRÍGUEZ L. Quantification of triacylglycerols in olive oils using HPLC-CAD. *Food Anal Methods* 2011; 4: 574-581. doi 10.1007/s12161-011-9207-3
11. DELMONTE P. Evaluation of poly (90% biscyanopropyl/10% cyanopropylphenylsiloxane) capillary columns for the gas chromatographic quantification

of *trans* fatty acids in non-hydrogenated vegetable oils. *J. Chromatogr. A.* 2016; 1460: 160-172. doi 10.1016/j.chroma.2016.07.019
12. DEMIREL Z. Identification and fatty acid composition of Coccolithophore and diatom species isolated from Aegean sea. *Rom Biotech Lett.* 2016; 21(4): 11746-11753
13. DIKBAS N., KARAGÖZ K., DADAŞOĞLU F., KOTAN R. Determination of relationship between *Satureja hortensis* L. essential oil susceptibility of *Bacillus cereus* strains and their fatty acid methyl ester profiles. *Rom Biotech Lett*. 2012; 17(5): 7564-7569.
14. FIRL N, KIENBERGER H, RYCHLIK M. Validation of the sensitive and accurate quantitation of the fatty acid distribution in bovine milk, *Int. Dairy J*. 2014; 35: 139-144. http://dx.doi.org/10.1016/j.idairyj.2013.11.007
15. ICH (2005). Validation of Analytical Procedures: Text and Methodology Q2(R1).: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf Accessed Januarie 2018.
16. ISO 12966-2 (2017). Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids.
17. ISO 12966-4 (2015). Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 4: Determination by capillary gas chromatography.
18. ISO 17189 (2003). Butter, edible oil emulsions and spreadable fats – Determination of fat content.
19. ISO 5725-6 (1994). Accuracy (trueness and precision) of measurement methods and results – Part 6: Use in practice of accuracy values.
20. ISO 661 (2003). Animal and vegetable fats and oils – Preparation of test sample.
21. KAMATOU GPP, Viljoen AM. Comparison of fatty acid methyl esters of palm and palmist oils determined by GCxGC–ToF–MS and GC–MS/FID. *S. Afr. J. Bot.* 2017; 112: 483-488. http://dx.doi.org/10.1016/j.sajb.2017.06.032
22. KARUNATHILAKA SR, CHOI SH, MOSSOBA MM, YAKES BJ et al. Rapid classification and quantification of marine oil omega-3 supplements using ATR-FTIR, FT-NIR and chemometrics. *J. Food Compos. Anal.* 2019; 77: 9-19. https://doi.org/10.1016/j.jfca.2018.12.009
23. KOSTIK V, MEMETI S, BAUER B. Fatty acid composition of edible oils and fats. *JHED.* 2013; 4: 112-116. UDC 664.3:577.115.3
24. LINSINGER T. (2010). Application Note 1, Comparison of a measurement result with certified value. https://ec.europa.eu/jrc/sites/jrcsh/files/erm_application_note_1_en.pdf Accessed January 2018.
25. LUCARINI M, DURAZZO A, SANCHEZ DEL PULGAR J, GABRIELLI P. et al. Determination of fatty acid content in meat and meat products: The FTIR-ATR approach. *Food Chem.* 2018; 267: 223-230. https://doi.org/10.1016/j.foodchem.2017.11.042
26. LUNA P, JUÁREZ M, de la FUENTE MA. Gas chromatography and silver-ion high-performance liquid chromatography analysis of conjugated linoleic acid isomers in free fatty acid form using sulphuric acid in methanol as catalyst. *J. Chromatogr. A.* 2008; 1204: 110-113. https://doi.org/10.1016/j.chroma.2008.07.050
27. MANZANO P, DIEGO JC, NOZAL MJ, BERNAL JL et al. Gas chromatography-mass spectrometry approach to study fatty acid profiles in fried potato crisps. *J. Food Compos. Anal.* 2012; 28: 31-39. http://dx.doi.org/10.1016/j.jfca.2012.07.003
28. MAZUREK B, CHMIEL M, GÓRECKA B. Fatty acids analysis using gas chromatography-mass spectrometer detector (GC/MSD) – Method validation based on berry seed extract samples. *Food Anal. Methods*, 2017; 10: 2868-2880. doi 10.1007/s12161-017-0834-1
29. MIHAI AL, NEGOIȚĂ M, ADASCĂLULUI AC, IONESCU V, et al. Evaluation of fatty acids composition of some food samples by using GC-MS and NMR techniques. "*Agriculture for Life, Life for Agriculture*" *Conference Proceedings*, 2018; 1(1): 548-554. DOI: 10.2478/alife-2018-0086
30. MONTOYA C, COCHARD B, FLORI A, CROS D, et al. Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis* Jacq.) compared to its wild relative *E. oleifera* (H.B.K) Cortés. *PLoS ONE*, 2014; 9(5): 1-13. https://doi.org/10.1371/journal.pone.0095412
31. PETROVIĆ M, KEZIĆ N, BOLANČA V. Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. *Food Chem.* 2010; 122: 285-291. https://doi.org/10.1016/j.foodchem.2010.02.018
32. PIASENTIER E, Di BERNARDO N, MORGANTE M, SEPULCRI A. et al. Fatty acid composition of heavy pig back fat in relationship to some animal factors. *Ital. J. Anim. Sci.* 2009; 8(2): 531-533. https://doi.org/10.4081/ijas.2009.s2.531
33. PINTILIE L, PARASCHIV IC, HLEVCA C, RADULESCU G, PATRUT E, PAVALOIU RD. Studies on two-step acid-base catalyzed transesterification of refined ostrich oil. *Rom Biotech Lett.* 2014; 19(2): 9222-9231.
34. SALIMON J, OMAR TA, SALIH N. Comparison of two derivatization methods for the analysis of fatty acids and *trans* fatty acids in bakery products using gas chromatography. *The Scientific World Journal*, 2014; 1-10. http://dx.doi.org/10.1155/2014/906407

35. SIMIONATO JI, GARCIA JC, DOS SANTOS GT, OLIVEIRA CC, et al. Validation of the determination of fatty acids in milk by gas chromatography. *J. Braz. Chem. Soc*. 2010; 21(3): 520-524. http://dx.doi.org/10.1590/S0103-50532010000300018
36. SOBRADO LA, FREIJE-CARRELO L, MOLDOVAN M, ENCINAR JR et al. Comparison of gas chromatography-combustion-mass spectrometry and gas chromatography-flame ionization detector for the determination of fatty acid methyl esters in biodiesel without specific standard. *J. Chromatogr. A.* 2016; 1457: 134-143. http://dx.doi.org/10.1016/j.chroma.2016.06.033
37. TAVERNIERS I, DE LOOSE M, VAN BOCKSTAELE E. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry*, 2004; 23: 535–552. https://doi.org/10.1016/j.trac.2004.04.001
38. VONGSVIVUT J, HERAUD P, ZHANG W, KRALOVEC J.A. et al. Quantitative determination of fatty acid compositions in micro-encapsulated fish-oil supplements using Fourier transform infrared (FTIR) spectroscopy. *Food Chem*. 2012; 135: 603-609. https://doi.org/10.1016/j.foodchem.2012.05.012
39. ZHANG H, WANG Z, LIU O. (2015). Development and validation of a GC–FID method for quantitative analysis of oleic acid and related fatty acids. *J. Pharm. Anal.* 2015; *5*: 223-230. http://dx.doi.org/10.1016/j.jpha.2015.01.005
40. ZHANG M, YANG X, ZHAO HT, DONG AJ et al. A quick method for routine analysis of C18 *trans* fatty acids in nonhydrogenated edible vegetable oils by gas chromatography-mass spectrometry. *Food Control*. 2015; 57: 293-301. http://dx.doi.org/10.1016/j.foodcont.2015.04.027