Development of a Performance Method for Determination of *Cis/Trans* Isomers of Oleic, Linoleic and Linolenic Acids from Potato Chips by GC-MS

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Abstract

The aim of this study was to develop and evaluate some validation parameters of a GC-MS sensitive method for the separation, identification, and simultaneous quantification of 18 *cis*/*trans* isomers of oleic (FAME C18:1), linoleic (FAME C18:2), and linolenic acid (FAME C18:3) methyl esters. Linearity, sensitivity, and recovery of the developed method were evaluated on calibration solutions. Correlation coefficients (*R*) were higher than 0.99 in the linear domain for each isomer, the sensitivity of the method was characterized by LOD = 0.03 - 0.05 g/100 g of fat, and LOQ = 0.09 - 0.17 g/100 g of fat, and the recovery ranged between 101.32 - 102.59%. Samples of potato chips were used to demonstrate the applicability of the method and the *trans* fatty acids (TFA) levels in analyzed sample were below 0.60 g/100 g fat, complying with international recommendations of less than 2 g/100 g of total fat. The proposed GC-MS method allows simultaneous determination of 18 *cis*/*trans* isomers (13 *trans* and 5 *cis*) of FAME C18 from potato chips.

Keywords: GC-MS, TFA, validation

Introduction

Determination of fatty acid profiles (FA) is a basic requirement in food testing in response to consumer demand to improve the quality of fats in food. Moreover, the interest in dietary fats has increased in recent years due to the negative effects of *trans* fatty acids (TFA) on humans. TFA, which are fatty acids with at least one non-conjugated (namely interrupted by at least one methylene group) carbon-carbon double bond in the *trans* configuration (EU Regulation no. 1169/2011) are present mainly in partially hydrogenated oils. By using partially hydrogenated oils, food products can improve their structure, textural properties (consistency/hardness, fragility, elasticity), shelf life, oxidation stability, stability during roasting at high temperatures and storage stability at room temperature (Kuhnt et al., 2011).

From the origin point of view, there are two types of *trans* fats: natural *trans* fats or ruminant fats (r-TFA) and artificial *trans* fats or industrial fats (i-TFA). Industrial *trans* fats are obtained through industrial hydrogenation, by using partially hydrogenated edible oils in processed products, by oils deodorization or refining, or by oils heating and frying at temperatures above 220°C (EFSA, 2018).

Elaidic acid (C18:1 *trans*-9) and *trans*-vaccenic acid (C18:1 *trans*-11) are the most widespread i-TFA and r-TFA, respectively (Kuhnt et al., 2011).

Health concerns regarding the TFA began to occur in the 1990s, after the scientific studies

demonstrated that a diet rich in TFA is associated with an increased risk of coronary heart disease (CHD) (Bendsen et al., 2011; Ganguly et al., 2016), by increasing LDL cholesterol and decreasing HDL cholesterol. Other health risks such as cancer, diabetes, obesity have been associated with the intake of TFA (Dhaka et al., 2011; Islam et al., 2019).

Due to health concerns related with the consumption of *trans* fats, regulations have been implemented for reducing or labeling of the TFA content of the foods. In 2003, Denmark became the first country who banned the industrially-produced *trans* fats in food. The amount of *trans* fat was limited to 2 g/100 g fat or oil. „*Trans* fat free" oils contain less than 1 g/100 g of fat. After Denmark, other European countries began to recommend and give laws to keep the TFA intake to a level „as low as possible" (Belgium, Germany, Austria, Switzerland, the Nordic countries, Spain, Italy), with the exception of France and the UK, which required a limit of 2% of energy intake (E%). In Romania no regulation is implemented yet to require a legal limit for industrial *trans* fats in food products, but the recommendations of European Commision should be taken into consideration.

Since January 2006, American food producers are required to add the amount of *trans* fat on the Nutrition Facts label (US Food and Drug Administration- FDA, 2018). Now, FDA is trying to remove artificial *trans* fats in processed foods, and since June 18, 2018, it is forbidden for manufacturers to add partially hydrogenated oils to foods, which represent the main source of artificial *trans* fat in processed foods, but for the products already produced, the compliance date is January 1, 2020.

World Health Organization (WHO, 2018) recommends that the total *trans* fat intake to be limited to less than 1% of E% (less than 2.2 g/day in a 2000 calorie diet), and intends to implement a strategic plan to eliminate the industrially produced *trans* fatty acids from global food supply by 2023.

In 2018, The European Commision asked EFSA to publish a technical report regarding the health effect of *trans* fats and the intake of these fatty acids to maintain health (EFSA, 2018) and adviced to limit *trans* fat, other than *trans* fat naturally present in animal fat to 2 g/100 g fat, in end food products. Products which do not comply with this limit can be placed on the market only until 1 April 2021.

Several analytical methods for determination and quantification of TFA have been reported such as high-performance liquid chromatography (HPLC) (Wu et al., 2017), silver-ion-HPLC (Ag^+-HPLC) (Stolyhwo and Rutkowska, 2013), silver nitrate thin layer chromatography (Ag^+-TLC) (Ravi Kiran et al., 2013), attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Khan et al., 2017), capillary zone electrophoresis (Amorim et al., 2019; De Castro et al., 2010), but the most used technique is gas chromatography with flame ionization detector (FID) (Omar and Salimon, 2013, Petrović et al., 2010) or with mass spectrometer (MS) (Ecker et al., 2012; Zhang et al., 2015). GC-MS ensures a better separation and identification of FA isomers and less overlapping compared to GC-FID (Zhang H., 2015).

Volatile analytes are required for GC techniques so transesterification to fatty acid methyl esters is usually carried out.

For TFA identification, the separation efficiency of isomers should be taken into consideration. The effect of temperature program on the resolution of C18 *cis*/*trans* fatty acids isomers was investigated (Ravi Kiran et al., 2013, Zhang et al., 2015) and it was observed that better resolutions were obtained in the case of a time-temperature program than when an isothermal program (180°C) was used.

The aim of this study was to develop and optimize the GC operating conditions of a method for the identification and quantification of 18 *cis/trans* isomers of C18, of which 13 isomers were *trans*. Four temperature programs were proposed and the program with more ramps and lower temperature increase was considered optimal. The main advantage of the optimized method is the ability to separate the *cis/trans* isomers of C18:1, C18:2, and C18:3. This method presented the best resolutions for the *cis*/*trans* isomers and the total analysis time was the shortest from all the temperature programs used in this study. Validation of analytical method included linearity, sensitivity, recovery which were verified on calibration solutions, and repeatability which was assessed on potato chips samples. The results showed that this method is suitable for accurately

Table 1. Composition of laboratory standard mixture solution of *cis/trans* isomers of C18 fatty acid methyl esters

No.	Code of FAME components of laboratory standard mixture	FAME components of laboratory standard mixture	C.* (µg/mL)	Weight (%)
1	C18:0	Octadecanoic acid methyl ester (methyl stearate)	57.14	5.57
2	C18:1 *trans*-6	*Trans*-6-octadecenoic acid methyl ester (methyl petroselaidate)	22.86	2.23
3	C18:1 *trans*-9	*Trans*-9-octadecenoic acid methyl ester (methyl elaidate)	28.57	2.79
4	C18:1 *trans-11*	*Trans*-11-octadecenoic acid methyl ester (methyl *trans*-vaccenate)	34.29	3.34
5	C18:1 *cis-6*	*Cis*-6-octadecenoic acid methyl ester (methyl petroselinate)	22.86	2.23
6	C18:1 *cis*-9	*Cis*-9-octadecenoic acid methyl ester (methyl oleate)	28.57	2.79
7	C18:1 *cis*-11	*Cis*-11-octadecenoic acid methyl ester (methyl vaccenate)	34.29	3.34
8	C18:2 *trans-9, trans-12*	*Trans*-9, *trans*-12-octadecadienoic acid methyl ester	150.14	14.64
9	C18:2 *cis-9, trans-12*	*Cis*-9, *trans*-12-octadecadienoic acid methyl ester	60.05	5.86
10	C18:2 *trans-9, cis-12*	*Trans*-9, *cis*-12-octadecadienoic acid methyl ester	60.05	5.86
11	C18:2 *cis-9, cis-12*	*Cis*-9, *cis*-12-octadecadienoic acid methyl ester (methyl linoleate)	87.17	8.50
12	C18:3 *trans-9,trans-12, trans-15*	*Trans*-9, *trans*-12, *trans*-15-octadecatrienoic acid methyl ester	131.79	12.85
13	C18:3 *trans-9, trans-12, cis-15*	*Trans*-9, *trans*-12, *cis*-15-octadecatrienoic acid methyl ester	197.68	19.28
14	C18:3 *trans-9, cis-12, trans-15*	*Trans*-9, *cis*-12, *trans*-15-octadecatrienoic acid methyl ester		
15	C18:3 *cis-9, trans-12, trans-15*	*Cis*-9, *trans*-12, *trans*-15-octadecatrienoic acid methyl ester		
16	C18:3 *cis-9, cis-12, trans-15*	*Cis*-9, *cis*-12, *trans*-15-octadecatrienoic acid methyl ester	92.25	9.00
17	C18:3 *cis-9, trans-12, cis-15*	*Cis*-9, *trans*-12, *cis*-15-octadecatrienoic acid methyl ester		
18	C18:3 *trans-9, cis-12, cis-15*	*Trans*-9, *cis*-12, *cis*-15-octadecatrienoic acid methyl ester		
19	C18:3 *cis-9, cis-12, cis-15*	*Cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid methyl ester (methyl linolenate)	17.57	1.71

*C. - concentration

quantification of TFA for nutrition labeling and quality control of fat containing food products.

Materials and methods

Reagents and reference standards

All solvents and reagents used in experiments were of analytical grade: petroleum ether, 40-60°C (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), anhydrous sodium sulphate (LGC Standards, Germany). Methanol picograde and isooctane (2,2,4-trimethylpentane) picograde were GC grade (Sigma Aldrich, Germany).

Standard mixtures of the *cis/trans* isomers of oleic acid (cat. no. 35079) obtained from Restek Corporation (Bellefonte, PA, USA), linoleic acid (cat. no. 47791) and linolenic acid (cat. no. 47792) methyl esters purchased from Supelco Inc. (Bellefonte, PA, USA) were used for peaks identification, retention times confirmation, and to certify that the peak area reflects the actual composition of these mixtures.

A laboratory standard mixture of *cis/trans* isomers of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids was prepared by mixing

each stock solution in a proportion of 1:1:1.5 in order to simultaneously determine 18 *cis/trans* isomers of these FAME (Table 1).

Calibration solutions (M1-M5) were prepared from the laboratory standard mixture (the stock solution) by diluting this mixture with isooctane: D = 1:8 (128.16 µg/mL), D = 1:12 (85.44 µg/mL), D = 1:17 (60.31 µg/mL), D = 1:26 (39.43 µg/mL), D = 1:51 (20.10 µg/mL) so that the chromatographic peaks in the calibration solution chromatograms are appropriate and placed on the chromatogram scale. All solutions were stored at refrigeration temperature, at 4°C until analysis.

Food matrices

Six samples of potato chips were purchased from local supermarkets in Bucharest, Romania. Three brands of potato chips (A, B, C) were analyzed. The choice of brands was made based on the biggest consumption of the market. Samples were coded as follows: A – P1, P2, P3, P4; B – P5; C – P6. The P2 sample is part of the oven baked chips, and the rest of the samples are part of the category potato crisps, labeled as fried in vegetable oil (palm/rapeseed/sunflower oil).

Preparation of FAME samples

Extraction of fat from the chips samples was performed with petroleum ether, using the Büchi B811 automatic extraction unit (Switzerland), and the Soxhlet Standard working mode. Preparation of FAME was performed in accordance with SR EN ISO 12966-2:2017. Briefly, about 50 mg of fat was subjected to esterification procedure with methanolic solution of sodium hydroxide, and fatty acids were derivatized into FAME using methanolic solution of boron trifluoride. The obtained extract was diluted with isooctane in different ratios so that chromatographic peaks are appropriate and placed on the chromatogram scale. The diluted extracts were transferred to autosampler vials for GC-MS injection. The composition of *cis/trans* isomers of C18:1, C18:2, C18:3 methyl esters calculated based on correction factors was determined from the calibration solutions. Three samples from each brand were analyzed in parallel and each sample was injected 2 times at GC-MS.

GC-MS analyses

GC analyses were performed on Trace GC Ultra/TSQ Quantum XLS system (Thermo Fisher Scientific, USA) consisting of a gas chromatograph (TRACE GC ULTRA) coupled with a mass spectrometer (MS) (TSQ Quantum XLS).

The gas chromatograph is equipped with a TriPlus AS autosampler (Thermo Fisher Scientific, USA) and a PTV injector. The C18:1, C18:2 and C18:3 methyl esters separation is performed on a high polarity column (TR-FAME) with the stationary phase consisting of 70% cyanopropyl and 30% polysilphenyl-siloxane (60 m x 0.25 mm x 0.25 µm) (Thermo Fisher Scientific, USA). Helium was used as a carrier gas at a constant flow rate of 1 mL/min. A volume of 1 µL of diluted extract was injected at 250°C in split mode with a 1:10 split ratio and a 10 mL/min splitting rate. The effect of the GC oven temperature program was investigated in order to obtain the best resolution between *cis*/*trans* isomers of C18:1, C18:2, C18:3 methyl esters of the laboratory standard mixture.

The following 4 conditions of the temperature program were applied: (**V1**) initial temperature 200°C, 0.5°C/min ramp to 210°C, 5°C/min ramp to 240°C, total run time 26 min. (**V2**) initial temperature 170°C, a 2°C/min ramp to 220°C, an increase to 240°C with the ramp of 5°C/min, total run time 29 min. (**V3**) initial temperature 170°C, an increase with 1°C/min on ramp 1 to 183°C, a 0.5°C/min increase on ramp 2 to 184°C, a 10°C/min increase on ramp 3 to 204°C, then an increase of 5°C/min on ramp 4 to 240°C, total run time 25.20 min. (**V4**) initial temperature 100°C, a 2°C/min ramp to 240°C and held 15 min, total run time 85.20 min. The optimal program was established based on the best resolutions obtained between the *cis*/*trans* isomers of the laboratory standard mixture.The mass spectrometer was operated in positive electron ionization mode (EI^+) with an ionization energy of 70eV, ion source temperature 250°C, full-scan mode, in the scan range of 40 - 300 *m*/*z*, with a scanning time of 0.132 s, and solvent evaporation time of 4.40 minutes. Prior to use, the mass spectrometer was calibrated with perfluorotributylamine (PFTBA).

The *cis*/*trans* isomers of C18:1, C18:2, C18:3 methyl esters of the potato chips samples were identified by comparing the retention times with those known from the reference standards, respectively those from the laboratory standard mixture. Individual quantification of *cis*/*trans* isomers (relative content, expressed by mass %) of the potato chips samples was performed according to SR EN ISO 12966-4:2015 by applying the correction factors to the obtained areas. The total TFA content was calculated as the sum of

TFA C18:1 (*trans*-6; *trans*-9; *trans*-11), TFA C18:2 (*trans*-9, *trans*-12; *cis*-9, *trans*-12; *trans*-9, *cis*-12) and TFA C18:3 (*trans*-9, *trans*-12, *trans*-15; *trans*-9, *trans*-12, *cis*-15; *trans*-9, *cis*-12, *trans*-15; *cis*-9, *trans*-12, *trans*-15; *cis*-9, *cis*-12, *trans*-15; *cis*-9, *trans*-12, *cis*-15; *trans*-9, *cis*-12, *cis*-15). The Xcalibur Program was used to acquire and process the data.

Evaluation of some validation parameters

Validation parameters such as linearity, sensitivity, recovery were verified on calibration solutions and repeatability was assessed on potato chips samples. The calibration curves for all compounds in the laboratory standard mixture were obtained by plotting the calibration graph of area versus concentration. Five calibration solutions were prepared from laboratory stock solution (20.10; 39.43; 60.31; 85.44 and 128.16 µg/mL). Recovery was calculated as follows: Rec. (%) = Concentration read at GC-MS/ specified concentration)*100. Each calibration level had three replicates. The repeatability of the method was evaluated by analyzing three replicates from each potato chips sample and each sample was injected in duplicate.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined in accordance with the International Conference on Harmonization (ICH) guidelines (2005) and were calculated based on the standard deviation of the response and the slope of the calibration curve for each isomer of the laboratory standard mixture. Based on linear regression analysis, area (A_{FAMEi}) versus FAME concentration (C_{FAMEi}), LOD/LOQ were expressed as response of the detector given by the equations: LOD = 3.3 x SD/b and LOQ = 10 x SD/b, where: SD-residual standard deviation of the calibration curve, obtained based on equation Steyx = Sy; b-the slope of the calibration curve.

Statistical analysis

All samples were analyzed in triplicate. The results are presented as mean ± SD. The relative standard deviation (RSD%) was used to check the distribution of results. To additionally check if there are significant differences between results, the homogeneity of variance was analyzed by the Tukey test and values of $p < 0.05$ were considered significant. All analyses were performed using SPSS (IBM SPSS Statistics 24).

Results and discussions

Optimizing GC operating conditions

Studies showed that columns of high polarity having a stationary phase are suitable for the good isomers separation (Chen et al., 2014; Roach et al., 2002). A capillary column with high polarity, with the stationary phase consisting of 70% cyanopropyl and 30% polysilphenyl-siloxane was chosen for this method.

In this study, programs with different temperatures and ramps were analyzed. Overlapping of *cis/trans* isomers in the elution area of C18:1 (V1, V2, V4), C18:2 (V1) and C18:3 (V1, V2, V3, V4) were recorded.

Previous studies have shown that the most difficult separation regions are in the elution zone of *cis*/*trans* isomers of C18:1 and C18:3 (Yoshinaga et al., 2013; Zhang et al., 2015). In a study realized by Yoshinaga et al. (2013) it was also shown that some peaks of *cis*/*trans* isomers of C18:1 methyl esters overlapped.

Thus, an indicator for optimizing the conditions of the GC oven temperature program was the resolutions obtained between the isomers in these regions.

From the four experimental variants, in the V3 program the best resolutions were obtained between the *cis/trans* isomers of C18:1, C18:2, and C18:3. It was noticed that a lower temperature ramp gave better resolutions between these isomers. The conditions applied in the experimental variant V3: the initial temperature was 170°C, and temperature increase on ramps 1 and 2 was small, were considered optimal for *cis*/*trans* isomers separation. In Figure 1 is represented the chromatogram of the laboratory standard mixture of *cis*/*trans* isomers of oleic, linoleic and linolenic acid methyl esters eluted by applying the experimental variant V3.

In variant V3, in the laboratory standard mixture, isomers resolutions for C18:1 and C18:2 *cis/trans* ranged between 0.8 - 2.32 and 1.08 - 1.83, respectively, making the best separations from all the programs used. For C18:3 *cis/trans* isomers resolutions between 0.61÷3.41 were obtained, excepting the *cis*-9, *trans*-12, *trans*-15 and *cis*-9, *cis*-12, *trans*-15 C18:3 isomers which overlapped. The overlapping of these two isomers was observed in all experimental variants and it is confirmed by C18:3 quality certificate data and by other studies (Zhang et al., 2015).

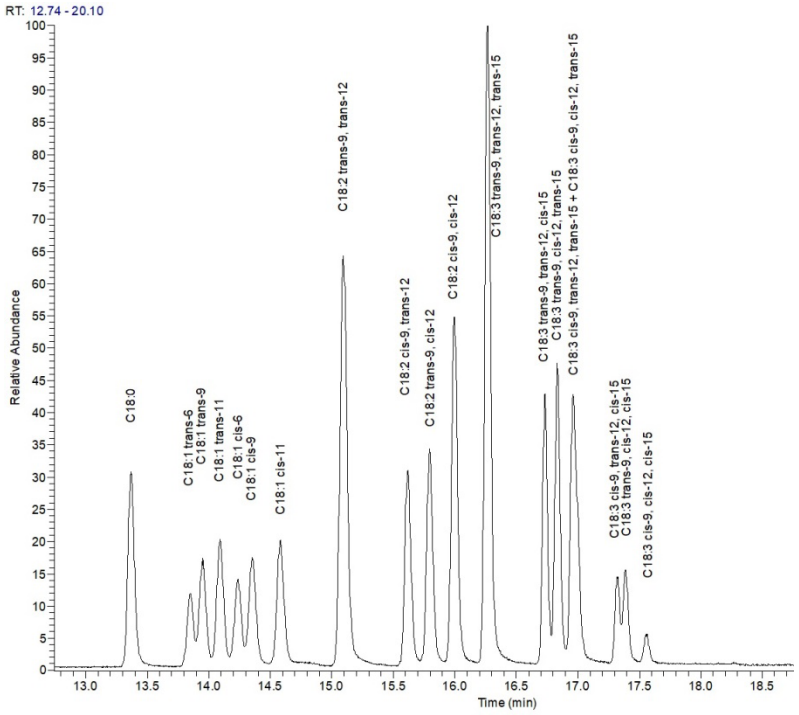

Figure 1. GC-MS chromatogram of the laboratory standard mixture of *cis*/*trans* isomers of oleic, linoleic and linolenic acid methyl esters eluted by applying the experimental variant V3

However, since overlapped C18:3 *trans* isomers will be given as sum rather than individually, the total quantification of *trans* fatty acids will not be affected.

Evaluation of some validation parameters

Linearity

The 18 individual C18 *cis*/*trans* methyl esters were quantified using five-point calibration curves obtained by using the laboratory standard mixture. The data shows a good linearity for each component, in the range of 20.10 - 128.16 µg/mL, with regression coefficient higher than 0.99. The identification parameters and performance characteristics of the method (regression equations, correlation coefficients (*R*), recoveries (Rec.)) are summarized in Table 2.

Sensitivity (LOD, LOQ)

The LOD and LOQ values obtained for the 18 individual *cis/trans* isomers of oleic, linoleic and linolenic acid methyl esters from the laboratory standard mixture, expressed in µg/mL and g/100 g fat are presented in Table 2. LOD and LOQ ranged between 0.03 - 0.05 g/100 g fat (4.95 - 9.08 µg/mL) and 0.09 - 0.17 g/100 g fat (15.00 - 27.51 µg/mL), respectively. However, our results for LOD and LOQ are higher than the ones reported by Ravi Kiran et al. (2013) and Zhang et al. (2015) due to the fact that the method was performed in the full scan mode, not in the selected ion monitoring mode. The results obtained for LOD and LOQ have shown that the method is sensitive for its purpose.

Recovery

Recovery was performed on five concentration levels of standard solutions (20.10; 39.43; 60.31; 85.44; 128.16 µg/mL) of the laboratory standard mixture. Recovery values presented in Table 2 are calculated as the mean of the recoveries obtained on the five concentration levels, each level having three replicates.

Repeatability

The values of the mean content for the six food samples are presented in Tables 3 and meet the required condition that the absolute difference between 2 independent analyses (Δ) to be less than or equal to the repeatability limit (r) ($\Delta \leq r$), as the sum of TFA, and as individual TFA according to SR EN ISO 12966-4:2015. The results are reported as mean ± standard deviation (SD). The relative standard deviations (RSD %) for all analyzed samples was below 10.5%.

Table 2. Performance characteristics of the method (linearity, sensitivity, recoveries) for the components of laboratory standard mixture

No.	FAME components of laboratory standard mixture	RT (min.)	Regression equation, (y = ax + b)	Correlation coefficient (*R*)	LOD (µg/mL)	LOQ (µg/mL)	LOD (g/100 g fat)	LOQ (g/100 g fat)	Rec. (mean ± SD) (%)
1	C18:0	13.37	y = 673230.90x – 11251801.54	0.9958	7.33	22.20	0.04	0.13	102.25 ± 12.42
2	C18:1 *trans*-6	13.85	y = 240903.08x – 4254634.15	0.9952	7.86	23.81	0.05	0.14	102.45 ± 13.34
3	C18:1 *trans*-9	13.95	y = 333888.72x – 6121458.69	0.9950	7.99	24.23	0.05	0.15	102.37 ± 13.08
4	C18:1 *trans-11*	14.09	y = 396473.39x – 7350369.20	0.9959	7.29	22.09	0.04	0.13	102.23 ± 12.27
5	C18:1 *cis-6*	14.25	y = 266720.78x – 4946220.72	0.9963	6.85	20.75	0.04	0.12	102.04 ± 11.57
6	C18:1 *cis*-9	14.35	y = 381715.91x – 7097614.45	0.9954	7.66	23.20	0.05	0.14	102.31 ± 12.85
7	C18:1 *cis*-11	14.58	y = 454742.12x – 8532345.23	0.9955	7.65	23.17	0.05	0.14	102.43 ± 13.23
8	C18:2 *trans-9, trans-12*	15.09	y = 1595222.40x – 27180366.79	0.9967	6.56	19.88	0.04	0.12	102.02 ± 11.38
9	C18:2 *cis-9, trans-12*	15.62	y = 638740.48x – 11926544.81	0.9954	7.71	23.36	0.05	0.14	102.33 ± 13.18
10	C18:2 *trans-9, cis-12*	15.80	y = 665771.09x – 11744491.11	0.9968	6.42	19.46	0.04	0.12	101.96 ± 11.21
11	C18:2 *cis-9, cis-12*	16.00	y = 1112023.55x – 18239767.07	0.9976	5.54	16.78	0.03	0.10	101.56 ± 9.58
12	C18:3 *trans-9, trans-12, trans-15*	16.27	y = 1911404.52x – 29836920.14	0.9981	4.95	15.00	0.03	0.09	101.32 ± 8.55
13	C18:3 *trans-9, trans-12, cis-15*	16.73	y = 693707.92x – 12292233.2	0.9968	6.38	19.33	0.04	0.12	101.91 ± 11.32
14	C18:3 *trans-9, cis-12, trans-15*	16.83	y = 773932.76x – 12695447.84	0.9979	5.17	15.68	0.03	0.09	101.45 ± 9.11
15	C18:3 *cis-9, trans-12, trans-15*	16.96	y = 1028291.86x – 16259257.88	0.9975	5.7	17.28	0.03	0.10	101.39 ± 9.0
16	C18:3 *cis-9, cis-12, trans-15*								
17	C18:3 *cis-9, trans-12, cis-15*	17.32	y = 220390x – 4062288.52	0.9947	8.23	24.95	0.05	0.15	102.59 ± 13.97
18	C18:3 *trans-9, cis-12, cis-15*	17.39	y = 267567x – 4671360.54	0.9951	7.95	24.09	0.05	0.14	101.90 ± 11.55
19	C18:3 *cis-9, cis-12, cis-15*	17.56	y = 82511x – 1287762.55	0.9936	9.08	27.51	0.05	0.17	102.14 ± 13.29

RT - retention time

Application on chips samples

In this study, based on the developed method, six samples of potato chips from three brands (A, B, C) from the Romanian market were analyzed. The results (Table 3) showed that the potato chips

Table 3. Content (g/100 g fat) of *cis*/*trans* isomers of C18:1, C18:2, C18:3 methyl esters from potato chips

FAME compounds from laboratory standard mixture	P1-A	P2-A	P3-A	P4-A	P5-B	P6-C
	Mean ± SD (%)					
Fat, %	28.24 ± 0.15	12.81 ± 0.07	27.05 ± 0.45	26.19 ± 0.11	25.88 ± 0.13	32.59 ± 0.04
C18:0	5.27 ± 0.26	2.03 ± 0.13	3.80 ± 0.27	3.22 ± 0.23	6.74 ± 0.34	4.33 ± 0.20
C18:1 *trans*-6	Nd*	Nd	Nd	Nd	Nd	Nd
C18:1 *trans*-9	Nd	Nd	Nd	< LOQ	Nd	Nd
C18:1 *trans-11*	Nd	< LOQ	Nd	Nd	Nd	< LOQ
C18:1 *cis-6*	Nd	Nd	Nd	Nd	Nd	Nd
C18:1 *cis*-9	81.17 ± 0.63	63.53 ± 0.75	89.78 ± 0.43	89.33 ± 0.58	70.39 ± 0.73	88.94 ± 0.63
C18:1 *cis*-11	1.08 ± 0.01	4.55 ± 0.24	0.80 ± 0.06	1.10 ± 0.07	1.28 ± 0.07	0.93 ± 0.04
C18:2 *trans-9, trans-12*	Nd	Nd	Nd	Nd	Nd	Nd
C18:2 *cis-9, trans-12*	0.15 ± 0.01	< LOQ	< LOQ	Nd	0.22 ± 0.01	< LOQ
C18:2 *trans-9, cis-12*	< LOQ	< LOQ	< LOQ	Nd	0.17 ± 0.01	< LOQ
C18:2 *cis-9, cis-12*	12.04 ± 0.34	15.35 ± 0.24	5.42 ± 0.21	6.11 ± 0.35	20.62 ± 0.95	5.64 ± 0.58
C18:3 *trans-9,trans-12, trans-15*	Nd	Nd	Nd	Nd	Nd	Nd
C18:3 *trans-9, trans-12, cis-15*	< LOQ	< LOQ	< LOQ	Nd	< LOQ	< LOQ
C18:3 *trans-9, cis-12, trans-15*	< LOQ	0.13 ± 0.01	< LOQ	Nd	< LOQ	< LOQ
C18:3 *cis-9, trans-12, trans-15*	Nd	0.26 ± 0.01	Nd	Nd	0.11 ± 0.01	< LOQ
C18:3 *cis-9, cis-12, trans-15*						
C18:3 *cis-9, trans-12, cis-15*	Nd	Nd	Nd	Nd	< LOQ	Nd
C18:3 *trans-9, cis-12, cis-15*	Nd	0.21 ± 0.01	< LOQ	Nd	< LOQ	< LOQ
C18:3 *cis-9, cis-12, cis-15*	0.29 ± 0.02	13.94 ± 0.20	0.19 ± 0.01	0.25 ± 0.02	0.47 ± 0.04	0.16 ± 0.01
Total *trans* C18:1	Nd	< LOQ	Nd	< LOQ	Nd	< LOQ
Total *trans* C18:2	0.15 ± 0.01	< LOQ	< LOQ	Nd	0.39 ± 0.01	< LOQ
Total *trans* C18:3	< LOQ	0.60 ± 0.01	< LOQ	Nd	0.11 ± 0.01	< LOQ
Total *trans* (C18:1+C18:2+C18:3)	0.15 ± 0.01	0.60 ± 0.01	< LOQ	< LOQ	0.50 ± 0.01	< LOQ
Total *cis* C18:1	82.25 ± 0.62	68.08 ± 0.52	90.58 ± 0.38	90.43 ± 0.51	71.67 ± 0.66	89.87 ± 0.59
Total *cis* C18:2	12.04 ± 0.34	15.35 ± 0.24	5.42 ± 0.21	6.11 ± 0.35	20.62 ± 0.95	5.64 ± 0.58
Total *cis* C18:3	0.29 ± 0.02	13.94 ± 0.20	0.19 ± 0.01	0.25 ± 0.02	0.47 ± 0.04	0.16 ± 0.01
Total *cis* (C18 :1+C18 :2+C18 :3)	94.58 ± 0.27	97.38 ± 0.14	96.20 ± 0.27	96.78 ± 0.23	92.76 ± 0.32	95.67 ± 0.20
Total *cis*+*trans* isomers (C18:1+C18:2+C18:3)	94.73 ± 0.26	97.97 ± 0.13	96.20 ± 0.27	96.78 ± 0.23	93.26 ± 0.34	95.67 ± 0.20

*Nd – not detected. The values are calculated as mean±standard deviation. No significant differences between results were observed.

samples were characterized by a very low content of TFA per 100 g fat.

The recorded values for total TFA C18:1 + C18:2 + C18:3 content of samples from the brand A (P3, P4), and for brand C (P6) were below the limit of quantification (LOQ = 0.09 - 0.15 g/100 g fat), and for the samples from brand A (P1, P2),

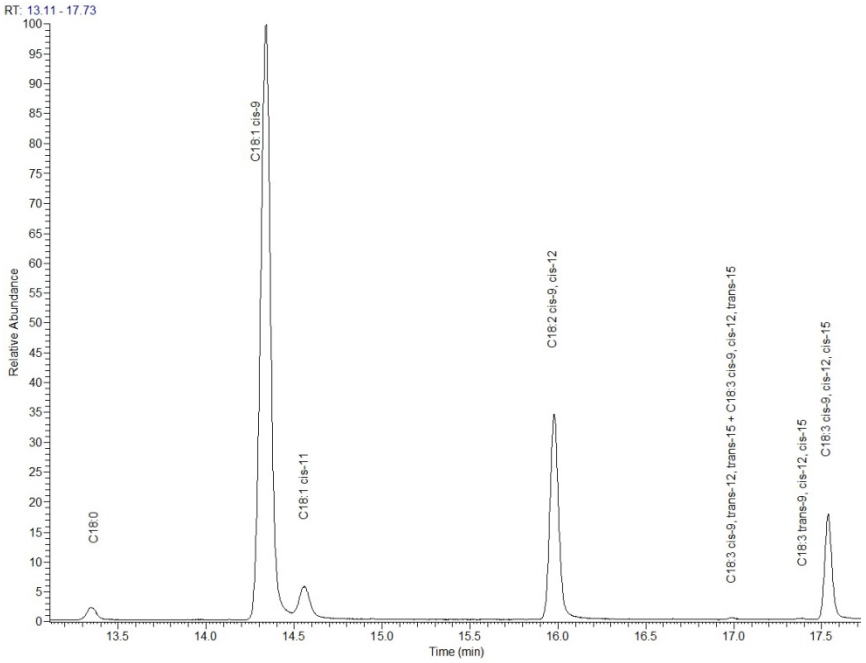

Figure 2. GC-MS chromatogram of the P2 sample of potato chips by applying the experimental variant V3

brand B (P5), the values were between LOQ - 0.60 g/100 g fat.

The samples fat content varied between 12.81 - 32.59% (w/w) (Table 3). In five out of six samples elaidic acid was not detected, and in one sample (P4-A), the level was below the limit of quantification (LOQ = 0.15 g/100 g fat). Vaccenic acid (C18:1 *trans*-11) was not detected in four samples and in two samples (P2-A, P6-C) the level was below the quantification limit (LOQ = 0.13 g/100 g fat). The *trans*-9, *trans*-12 isomer of linoleic acid (C18:2) was not detected (LOD = 0.04 g/100 g fat).

In terms of total TFA content, for three samples the sum was below LOQ (0.09 - 0.15 g/100 g fat) and for the other three, the sum was between 0.15 - 0.60 g/100 g fat. The TFA content values obtained were less than 2% of the total fat, limit recommended or imposed in some countries.

The results with regard to the total TFA content are confirmed by other studies. Roe et al. (2013) showed in their study that the level of TFA in a wide range of foods on the market in UK, including potato chips, were reduced compared to previous UK studies. The content of elaidic acid (C18:1 *trans*-9) in the chips analyzed by Roe et al. (2013) ranged from 0.02 to 0.97 g/100 g FAME, vaccenic acid (C18:1 *trans*-11) between 0.02 - 0.97 g/100 g FAME, and C18:1 *trans*-6, between 0.02 - 0.10 g/100 g FAME. In terms of total TFA, the content ranged from 0.10 to 2.05 g/100 g fat.

In a report regarding the TFA content in Portuguese foods analyzed between October and December 2013, potato chips and French fries analyzed by GC had a TFA mean content of 0.62 g/100 g fat (0.18 g/100 g food), with the values ranging between 0.17 to 1.26 g/100 g fat (0.05 ÷ 0.38 g/100 g food), the values being below the recommended limit of 2% (Costa et al., 2016).

As it can be noticed from table 3, P1, P3, P4, and P6 samples have a higher content of oleic acid (C18:1 *cis*-9), these samples being fried in sunflower oil which is known to have a high level of oleic acid. P2 sample was fried in rapeseed oil, which has a higher content of vaccenic acid (C18:1 *cis*-11, 4.55 g/100 g), and alpha linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15, 13.94 g/100 g) (Figure 2). P5 sample was fried in palm oil which has a higher content of linoleic acid (C18:2 *cis*-9, *cis*-12, 20.62 g/100 g) compared to the other samples that were fried in sunflower oil.

Pérez-Farinós et al. (2016) conducted a study on the level of TFA in some food products on the Spanish market and compared the results with the level of TFA in the same food categories analyzed in a previous study, achieved in 2010. The methodology used in both studies was similar and for chips, significant reductions were recorded:

a TFA of 0.030 g/100 g product (0.088 g/100 g fat) in 2015 and a TFA content of 0.071 g/100 g product (0.210 g/100 g fat) in 2010.

The results of our study confirm that the TFA content of the potato chips analyzed is low, below 0.6 g/100 g fat.

Conclusions

A sensitive gas chromatography coupled with mass spectrometry method was developed in full-scan mode for the simultaneous determination of 18 individual C18 *cis/trans* fatty acids isomers. The method has a good sensitivity (LOQ = 0.09 - 0.15 g/100 g fat) so that the *trans* isomers can be determined up to a content of 0.5 g TFA/100 g fat (or 0.06 ÷ 0.16 g TFA/100 g product). The applicability of the method was proven on potato chips samples. This research will continue by evaluating other validation parameters and it will be applied to other food matrices also. In the future, it is important to improve the profile of C18:3 *cis/trans* isomers by optimizing the temperature program. The method can be used for various purposes, such as for nutritional labeling, specifying TFA content, research studies, etc.

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