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EVALUATION OF FATTY ACIDS COMPOSITION OF SOME FOOD SAMPLES BY USING GC-MS AND NMR TECHNIQUES

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Abstract

The purpose of this paper was to compare the composition (weight % of total identified FA) in saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids from 3 food matrices (sunflower oil, palm oil and lard) by 2 different techniques, gas chromatography – mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). For GC-MS technique, fatty acid methyl esters (FAMEs) identification in the samples was performed by comparison of the retention times (RT) and the mass/charge (m/z) ratio characteristic of each FAME component in the reference standards used (F.A.M.E. Mix C4 - C24 and SRM® 2377). FAMEs quantification from food samples was realized by applying correction factors calculated based on reference standards. NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus. The NMR spectra was recorded directly on the oil without any sample preparation. The difference between the mean values of the fatty acids content determined by GC-MS and NMR was not more than ± 15% for sunflower oil and lard, and ± 6% for palm oil.

Keywords: *animal fats, fatty acids, GC-MS, NMR, vegetable oils.*

INTRODUCTION

The fatty acids composition of food is very important in human health. Over the years, the legislation regarding the FAs has changed and requires the declaration of FAs, including *trans*-fatty acids content in some countries, on the nutritional labels of foods.

For fatty acids determination several methods have been used, the most known method being gas chromatography. This is a precise method in the study of the variation or determination of fatty acid profiles in vegetable oils/vegetable or animal fats, or to study the modification of the fatty acid composition in food products obtained through different technological processes, being a precise and reproducible determination.

Gas chromatography coupled with mass spectrometry (GC-MS) is a laborious method because it involves hydrolysis of triglycerides which are then subjected to GC analysis. In order to be able to analyse the lipids by gas chromatography, it must be transformed by different derivatisation methods in derivate with higher volatility, most frequently in fatty acid methyl esters (Popović et al., 2017). GC-MS is used for a better FA separation and identification of different isomers and minority FAs (Manzano et al., 2012, Zhang et al., 2015).

Usually, GC is coupled with a flame ionisation detector (FID) and uses response correction factors for FA quantification (Aued-Pimentel et al., 2010, Simionato et al., 2010, Popa et al., 2013, Firl et al., 2014).

Compared to the gas-chromatographic method, there are alternative methods of analysing the composition in total fatty acids (saturated, unsaturated) faster based on NMR spectroscopy.

In addition for being directly applied to fat (triglycerides), the NMR method also has a number of other advantages over chromategraphic methods, such as: it is fast - the samples analysed do not require pre-derivatisation before recording the actual spectra, being dissolved in a suitable deuterated solvent (most commonly used is $CDCl_3$); the procedure for recording the spectra is fast, the acquisition time of an NMR spectrum being about 2 minutes; is non-destructive (the sample can be recovered at the

end of the analysis - very useful for precious samples); is applicable to samples which cannot be analysed by chromatographic methods because of their sensitivity to heating or other factors; does not require calibration or internal standard, it requires less reagents and solvents, etc. (Li et al., 2001, Dijkstra et al., 2007, Chira N., 2011, Vicente et al., 2015, Ruiz-Aracama et al., 2017, Tan et al., 2017, Yeboah et al., 2017). This study was realized in order to compare the fatty acid composition of the studied vegetable oils/animal fats, determined by the developed method, gas chromatography coupled with mass spectrometry with the nuclear magnetic resonance technique.

MATERIALS AND METHODS

Food matrice

In this study three food matrices were used: sunflower oil and palm oil, purchased from supermarkets in Bucharest, Romania and lard obtained from the rural households in the county of Braila, Romania.

Reference standards

In the experiments two reference standards were used: SRM®2377 (NIST-certified, USA)- a mixture of 26 FAMEs, with NIST certified mass fraction values (mg/g) and F.A.M.E. Mix. C4-C24 - a mixture of 37 FAMEs with mass percent (%) for 37 FAMEs (Bellefonte, PA, USA).

All solvents and reagents used in the experiments were of chromatographic purity/ACS/residual analysis (petroleum ether 40-60° (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, isooctane (Sigma Aldrich), etc.).

The composition in fatty acids in the studied matrices was determined as relative concentration (weight p% of total identified FA) based on correction factors (CF). CF were determined from both reference standards, SRM®2377 and F.A.M.E. Mix. C4-C24 (23 FAMEs common to both standards, 3 FAMEs specific to SRM®2377 and 14 FAMEs specific to F.A.M.E. Mix C4-C24).

Preparation of FAME samples from the analysed food matrices

Samples were prepared in accordance with SR EN ISO 661:2005. FAMEs were prepared by transesterification of the sunflower oil, palm oil, and lard, with sodium methoxide solution and BF_3 methanolic solution in accordance with ISO 12966-2:2017 by applying correction factors calculated based on reference standards.

The transesterification procedure was carried out as follows: in a 50 ml long necked flask, approximately 50 mg of sample (fat) was weighted, with a precision of 0.1 mg.

Fat was dissolved in 4 mL of 0.5 M CH_3ONa solution and then was placed in a water bath at reflux and kept for about 1-2 minutes. After this step, 5 mL of a 14% methanolic solution of BF_3 was added and sample was maintained in the water bath at reflux for 3 minutes more. For FAMEs extraction, 3 mL isooctane were added into the flask and sample was kept for 1 min in the water bath. After removing the flask from the reflux, 15 mL of saturated NaCl solution was added immediately and stirred vigorously for approximately 15 s. Then another 20 mL of saturated NaCl solution was added, and stirred lightly. Sample was shaken vigorously and after layer separation, the upper isooctane layer which contains the FAME was transferred through a filter of sodium sulphate anhydrous in a 4 mL vial. The obtained extract was diluted with isooctane and it was transferred to an autosampler vial for GC analysis.

GC-MS equipment and operating conditions

FAME analysis was performed according to ISO 12966-4:2015, with changes from the reference, by using of a gas chromatograph coupled with a mass spectrometer (Trace GC Ultra/TSQ Quantum XLS, Thermo Fisher Scientific, USA).

The chromatograph is equipped with a high polarity capillary column, TR-FAME (stationary phase consisting of 70% cyanopropyl and 30% polysilphenyl-siloxane, 60 m long, 0.25 mm inner diameter and 0.25 μm stationary film thickness). Analysis of the derivatized sample extracts were performed in the positive electron impact ionization (EI +) mode, selected ion monitoring (SIM) mode, using 24 segments.

The temperature of the ion source was 250°C. The oven temperature was programmed at 100°C for 0.2 min, increased to 240°C with 20°C/min and hold for 15 min. At this temperature it was used as a mobile phase 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. Injections were performed in duplicate. Instrument control, data acquisition and processing were performed using the Xcalibur Program.

Peak identification in the food matrices was performed by comparison with the retention times of the FAME components in the used reference standards and the mass/charge (*m/z*) ratio characteristic of each component. The recording time of a GC-MS chromatogram is 85 minutes.

NMR analysis

Food samples were analyzed for total content in saturated, monounsaturated and polyunsaturated fatty acids. For fat analysis, a Bruker Ascend 400 Spectrometer, Fourier Transformed Spectrometer, equipped with field gradients on the z axis, was used operating on the 9.4 Tesla field, corresponding to the resonance frequencies of 400.13 MHz for the ^{1}H nucleus. The oil samples were dissolved in $CDCl_3$ (2:8 v/v). Samples were analysed in 5 mm NMR tubes (Wilmad 507).

The average acquisition time of the ^{1}H NMR spectra was approximately 2 minutes. Recording of NMR spectra was performed using Icon NMR software.

The composition of vegetable oils (sunflower oil, palm oil) and animal fat (lard) based on ^{1}H-NMR spectral data was calculated using a chemometric equation system which allows deduction of oils composition on four classes of FAs: mono-, di- and tri-unsaturated fatty acids, and saturated fatty acids.

RESULTS AND DISCUSSIONS

Usually the fatty acids composition of oils and fat is determined by GC-MS or by GC-FID but lately other techniques started to be used. NMR technique is used to know the classes of fatty acids presented in the oil based on the unsaturation of the fatty acids (saturated, mono-, di- or tri- unsaturated).

Figures 1, 2 and 3 show the chromatograms of sunflower oil, palm oil and lard. Based on the reference standard, 11 peaks of FAMEs were identified by GC-MS in *sunflower oil*, of which 6 SFAs (C14:0, C16:0, C18:0, C20:0, C22:0, C24:0), 3 MUFAs (C16:1n7, C18:1n9, C20:1n9) and 2 PUFAs (C18:2n6, C18:3n3); in *palm oil* were identified 8 peaks of FAMEs, of which 4 SFAs (C14:0, C16:0, C18:0, C20:0), 2 MUFAs (C18:1n9, C18:1n7) and 2 PUFAs (C18:2n6, C18:3n3); and in *lard* 14 peaks of FAMEs were identified, of which 7 SFAs (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0), 4 MUFAs (C16:1n7, C18:1n9, C18:1n7, C20:1n9) and 3 PUFAs (C18:2n6, C18:3n3, C20:4n6).

Figure 4 presents the composition of total saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids which were determined by the two techniques, GC-MS and NMR, from the matrices used in this study: sunflower oil, palm oil and lard.

In the case of sunflower oil, the difference between the mean values of saturated fatty acids content determined by the NMR technique compared to the GC-MS technique was 14.66% higher.

For MUFA, the mean values obtained when using NMR were 3.98% higher than the ones determined by GC-MS.

The difference between the mean values of PUFAs content determined by NMR compared to the mean values determined by GC-MS, was 12.44% higher.

Vicente et al. (2017) also showed a high correlation between the ^{1}H NMR and GC-FID methods used to characterize the composition in fatty acids of Sacha Inchi oil with different ratios of soybean oil and corn oil. Ionescu et al. (2015) compared the fatty acids composition of several vegetable oils from Romanian market by using FT-IR spectroscopy, GC-MS and ^{1}H NMR techniques and the results showed that there is a good correlation between the quantitative results obtained by using these methods.

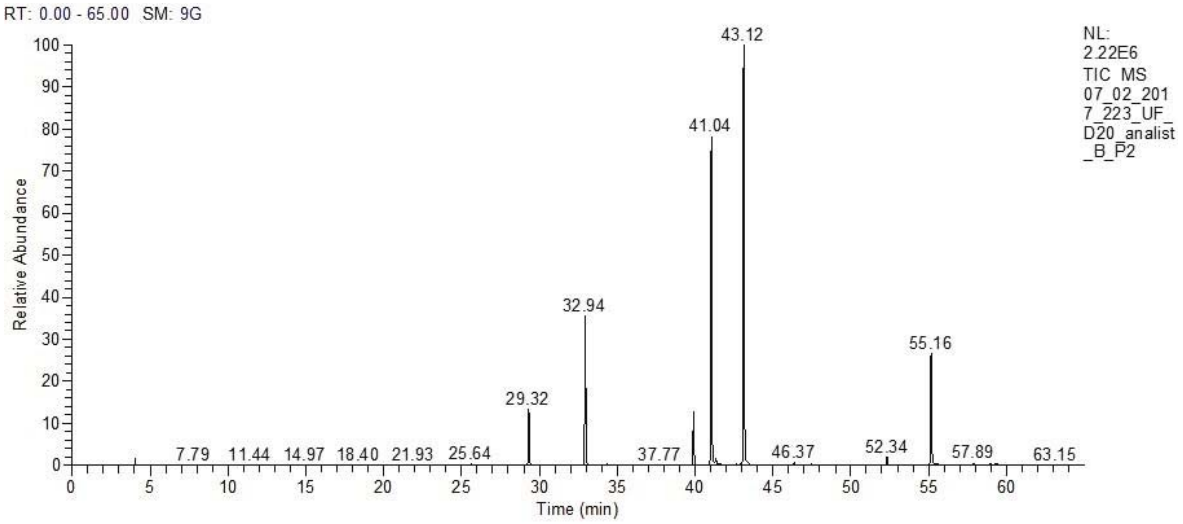

Figure 1. Chromatogram of sunflower oil FAME mixture (C14:0 (RT=25.64); C16:0 (RT=32.94); C16:1n7 (RT=34.4); C18:0 (RT=39.9); C18:1n9 (RT=41.04), C18:2n6 (RT=43.12); C18:3n3 (RT=45.62); C20:0 (RT=46.37); C20:1n9 (RT=47.47); C22:0 (RT=52.34); C24:0 (RT=57.89))

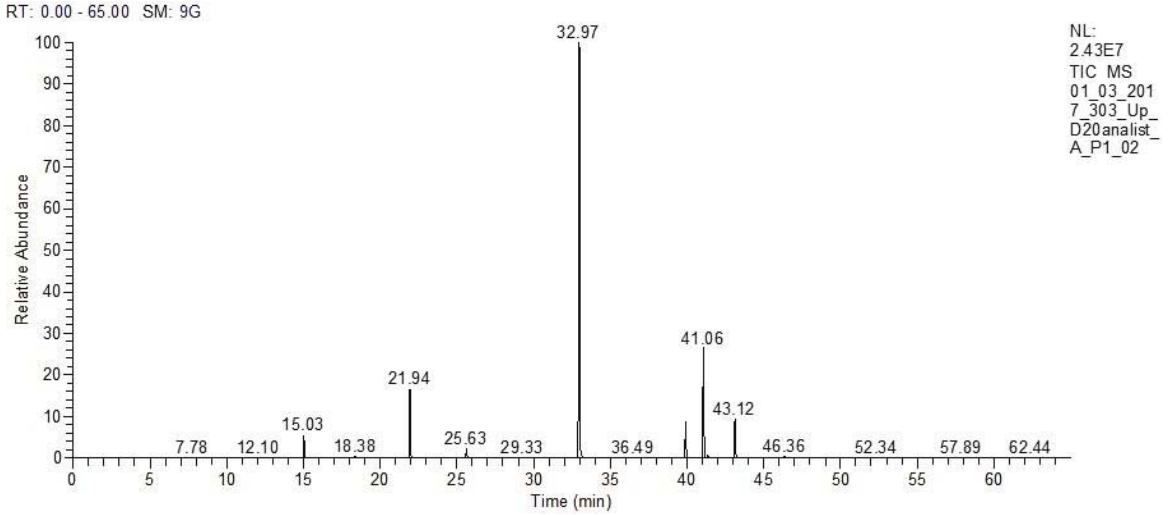

Figure 2. Chromatogram of palm oil FAME mixture (C14:0 (RT=25.63); C16:0 (RT=32.97); C18:0 (RT=39.9); C18:1n9 (RT=41.06); C18:1n7 (RT=41.34); C18:2n6 (RT=43.12); C18:3n3 (RT=45.6); C20:0 (RT=46.36))

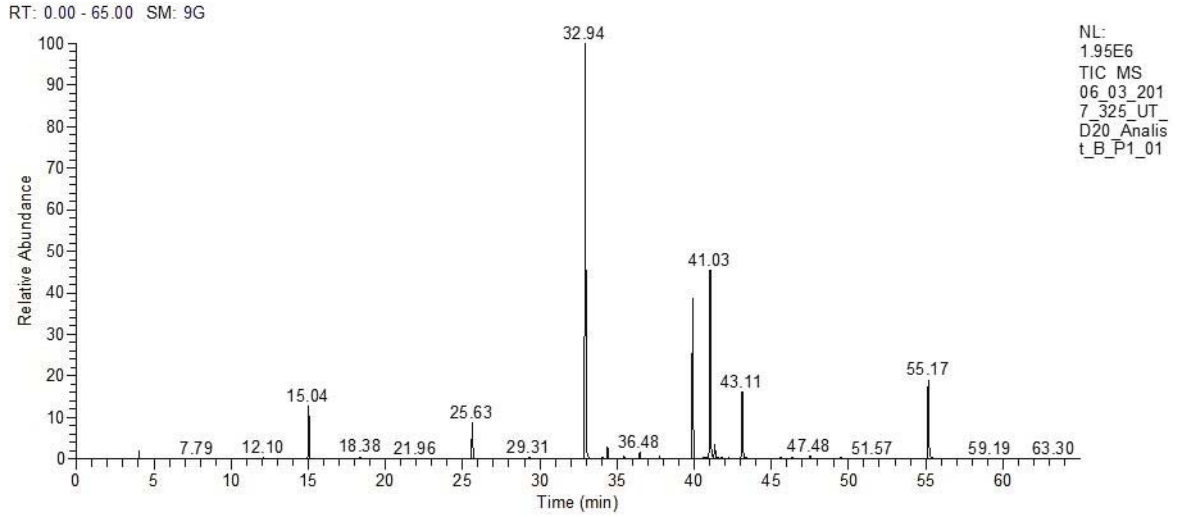

Figure 3. Chromatogram of lard FAME mixture (C8:0 (RT=7.79); C10:0 (RT=12.1); C12:0 (RT=18.38); C14:0 (RT=25.63); C16:0 (RT=32.94); C16:1n7 (RT=34.38); C18:0 (RT=39.9); C18:1n9 (RT=41.03); C18:1n7 (RT=41.33); C18:2n6 (RT=43.11); C18:3n3 (RT=45.6); C20:0 (RT=46.36); C20:1n9 (RT=47.48); C20:4n6 (RT=51.57))

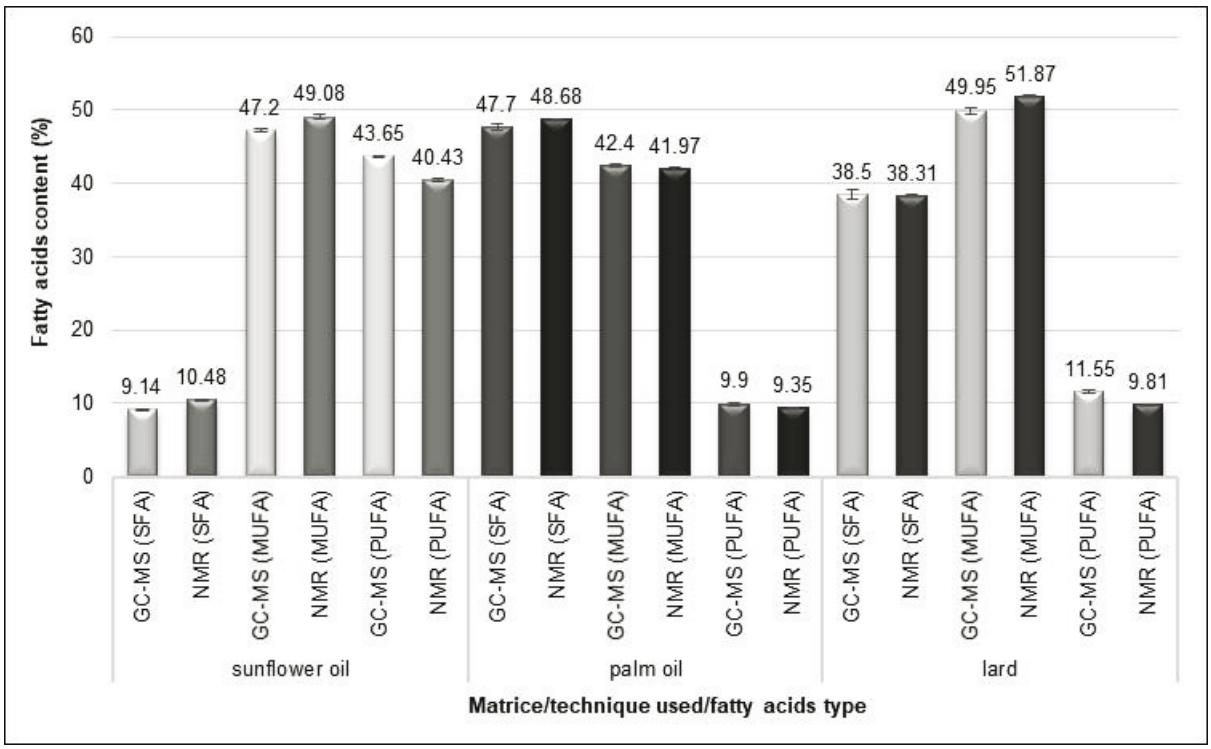

Figure 4. Comparative content (mean ± SD,%) in SFA/MUFA/PUFA determined by GC-MS and NMR from the investigated food matrices

For the palm oil, the SFA content was higher with 2.05% when using the NMR technique comparing with the GC-MS technique. In the case of MUFA, the content was lower with 1.01% when determined by NMR technique. The difference between the mean values of polyunsaturated fatty acids content determined by NMR compared to the mean values determined by GC-MS, was 5.55% lower.

For lard, the SFA content was almost similar for both used techniques, the content being lower with 0.49% for NMR technique. Comparing the content of MUFA for lard, it can be seen that by using NMR technique the value was 3.84% higher. In the PUFA case, the content was higher with 15.06% when determined based on GC-MS technique.

The composition in fatty acids determined by these two techniques was compared and the difference between the mean values determined by NMR compared to GC-MS was not more than ± 15%, for sunflower oil: SFA, + 14.66%; MUFA, + 3.98%; PUFA, + 12.44%, palm oil: SFA, + 2.05%; MUFA, - 1.01%; PUFA, - 5.55% and lard: SFA, - 0.49%; MUFA, + 3.84%; PUFA, - 15.06%.

Comparing the fatty acids composition of the studied samples, it can be seen that sunflower oil has the highest content of PUFA (43.65 ± 0.11% (GC-MS); 40.43 ± 0.21% (NMR)) and the lowest content of SFA (9.15 ± 0.09% (GC-MS); 10.48 ± 0.11% (NMR)). Similar results were obtained by Chowdhury et al. (2007) and Kostik et al. (2013) who studied the fatty acids composition of several vegetable oils and fats and showed that from all the tested matrices, sunflower oil has the highest percentage of MUFA and PUFA, making it suitable for consumption. It a study realized by Popa et al. (2013) it was also shown that sunflower oil contain a high percent of monounsaturated and polyunsaturated fatty acids, suming approximately 90% of fatty acids composition.

Regarding the fatty acids composition of palm oil, from figure 4 it can be noticed that this oil has the highest content of SFA (47.7 ± 0.40% (GC-MS); 48.68 ± 0.02% (NMR)) and a low content of PUFA (9.9 ± 0.16% (GC-MS); 9.35 ± 0.14% (NMR)). The fatty acids composition of palm oil obtained in this study appear similar with the one obtained by Montoya et al. (2014), who showed that this oil has a high content of SFA (46.6 ± 6.4%), and MUFA (43.6 ± 10.6%), but a low content of PUFA (9.8 ± 4.0%). Also Chowdhury et al. (2007) showed that palm oil has a high content of SFA (46.35 ± 0.40%) and MUFA (41.46 ± 0.56%), and a low content of PUFA (11.84 ± 0.92%).

From the food matrices investigated in this study, lard has the highest content of MUFA (49.95 ± 0.45% (GC-MS); 51.87 ± 0.13% (NMR)). Our results regarding the content of SFA, MUFA, and PUFA from lard are similar with the ones obtained by Piasentier et al. (2009) when studying the composition of the back fat of heavy pigs and obtained the SFA content between 40.3 – 41.9%, the MUFA content between 42.8 – 44.9%, and the PUFA content between 13.0 – 15.2%.

CONCLUSIONS

In this study the fatty acids composition of sunflower oil, palm oil and lard were obtained by using 2 techniques.
The results obtained by using the GC-MS method are in agreement with those obtained when using NMR technique.
The methods are suitable for fatty acids determination of samples of vegetable and animal origin, being a useful tool for the labelling of food samples.

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