

## **P59: COMPARATIVE ANALYSIS OF FATTY ACIDS COMPOSITION OF VEGETABLE OILS AND ANIMAL FATS BY APPLICATION OF 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), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids from 3 food matrices (sunflower oil, palm oil and lard) by 2 different techniques, GC-MS and NMR.

FAMES analysis from food samples was performed using a gas chromatograph coupled with a Triple quadrupole mass spectrometer.

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 <sup>1</sup>H nucleus.

The composition in fatty acids determined by these two techniques was compared and the difference between the mean values determined by GC-MS and NMR was not more than ± 15% for sunflower oil, and lard, and ± 6% for palm oil.

**Keywords:** fatty acids, GC-MS, NMR

### **1. INTRODUCTION**

Gas chromatography 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.

Gas chromatography coupled with mass spectrometry is a laborious method because it involves hydrolysis of triglycerides and methylation of free fatty acids before being 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 (FAMES).

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 chromatographic methods (GC and HPLC), 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<sub>3</sub>); the procedure for recording the spectra is fast, the acquisition time of an NMR spectrum being about 2 minutes; is undestructive (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, etc. [1], [2], [3].

This study was realized in order to compare the fatty acid composition of the vegetable oils/animal fats studied, determined by the developed method, gas chromatography coupled with mass spectrometry (GC-MS) with the nuclear magnetic resonance technique (NMR).

### **2. EXPERIMENTAL**

#### **2.1. Food matrice**

In this study three food matrices were used: sunflower oil, palm oil and lard, purchased from supermarkets on the market in Bucharest, Romania.

#### **2.2. Reference standards**

In the experiments two reference standards were used: SRM 2377 - 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.

All solvents and reagents used in the experiments were of chromatographic purity/ACS/residual analysis (petroleum ether, methanolic solution of sodium hydroxide 5.4 M concentration, methanolic solution of boron trifluoride 14%, sodium chloride, methanol, isooctane, 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).

### 2.3. Fat extraction

Fat extraction from the three food matrices was performed with petroleum ether by applying the "Soxhlet Standard" extraction method using the Buchi B-811 extraction unit. The fat content was determined gravimetrically by weighting the mass of extracted substances.

### 2.4. Preparation of FAME from fat extracted from the food matrices analysed

FAMES were prepared by transesterification of the fat extracted from sunflower oil/palm oil/lard, with sodium methoxide solution and BF<sub>3</sub> methanolic solution in accordance with SR EN ISO 12966-2:2011 [4] by applying correction factors calculated based on reference standards.

### 2.5. Equipment and operating conditions of GC-MS

FAMES analysis was performed in accordance with ISO 12966-4:2015 [5], 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, SIM mode (Selected Ion Monitoring) 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 reference standards used and the mass/charge (*m/z*) ratio characteristic of each component. The recording time of a GC-MS chromatogram is 85 minutes.

### 2.6. NMR analysis

The fat obtained in section 2.3 was analyzed for total content in saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) 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 <sup>1</sup>H nucleus. The oil samples were dissolved in CDCl<sub>3</sub> (2:8 v/v). Samples were analysed in 5 mm NMR tubes (Wilmad 507). The average acquisition time of the <sup>1</sup>N-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 <sup>1</sup>H-NMR spectral data was calculated using a chemometric equation system which allows deduction of oils composition on four classes of fatty acids: triunsaturated fatty acids, diunsaturated fatty acids, monounsaturated fatty acids and saturated fatty acids.

## 3. RESULTS AND DISCUSSION

Fig. 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

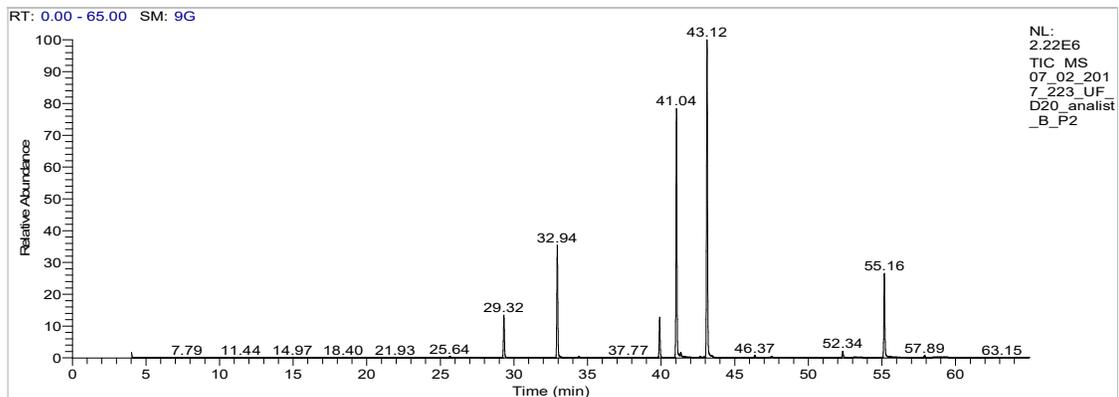


Figure 1. Chromatogram of a FAME mixture from sunflower oil (C14:0 (RT=25.64); C16:0 (RT=32.94); C16:1n7 (RT=34.4); C18:0 (RT=39.9); C18:1n9 (RT=41.04), C18:2n6 (RT=43.12); C18:3n3 (RT=45.62); C20:0 (RT=46.37); C20:1n9 (RT=47.47); C22:0 (RT=52.34); C24:0 (RT=57.89))

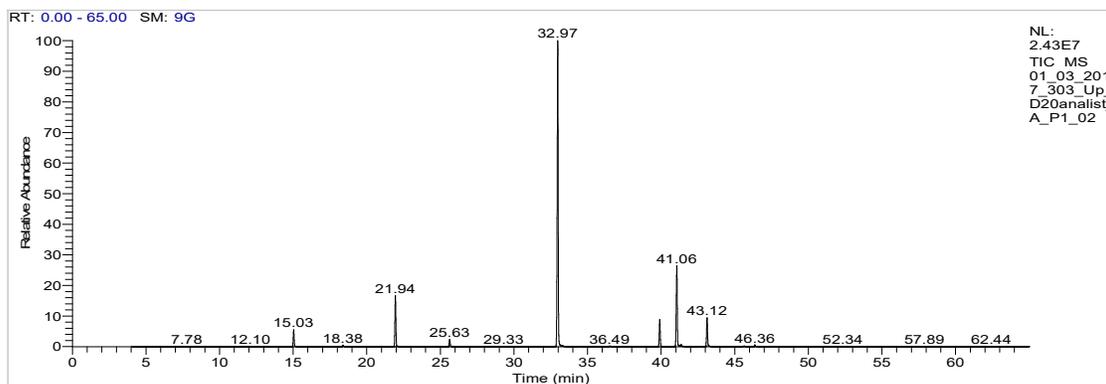


Figure 2. Chromatogram of a FAME mixture from palm oil (C14:0 (RT=25.63); C16:0 (RT=32.97); C18:0 (RT=39.9); C18:1n9 (RT=41.06); C18:1n7 (RT=41.34); C18:2n6 (RT=43.12); C18:3n3 (RT=45.6); C20:0 (RT=46.36))

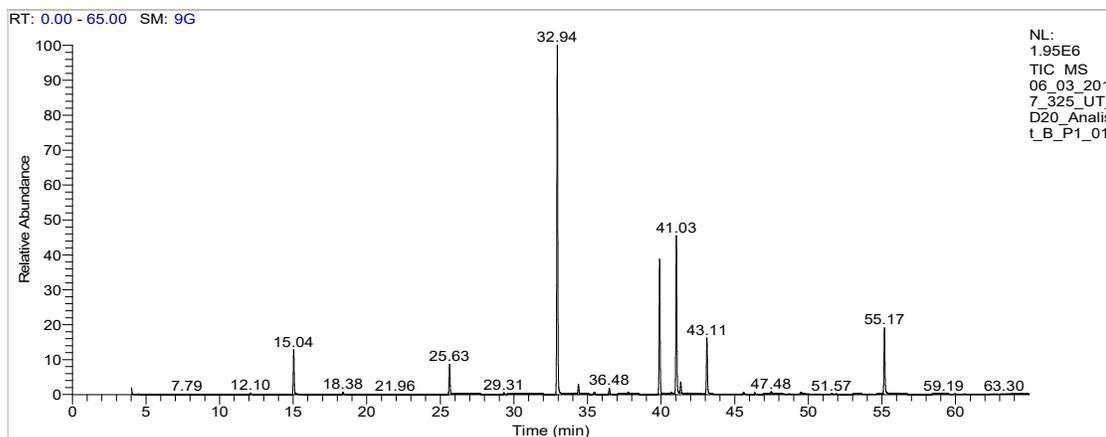


Figure 3. Chromatogram of a FAME mixture of lard (C8:0 (RT=7.79); C10:0 (RT=12.1); C12:0 (RT=18.38); C14:0 (RT=25.63); C16:0 (RT=32.94); C16:1n7 (RT=34.38); C18:0 (RT=39.9); C18:1n9 (RT=41.03); C18:1n7 (RT=41.33); C18:2n6 (RT=43.11); C18:3n3 (RT=45.6); C20:0 (RT=46.36); C20:1n9 (RT=47.48); C20:4n6 (RT=51.57))

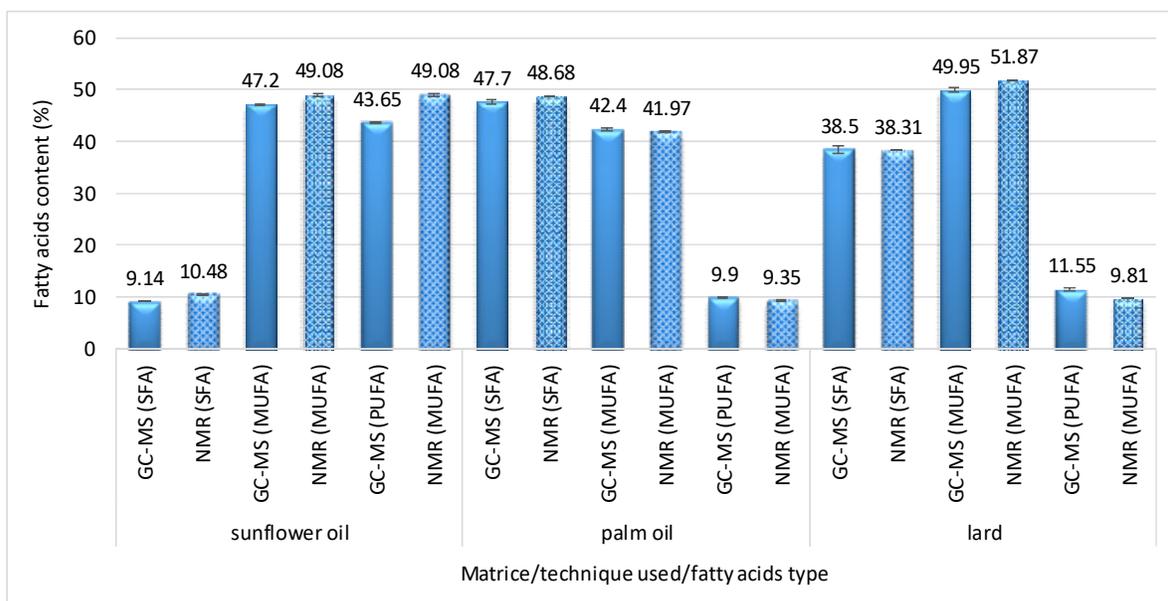


Figure 4. Comparative content (mean ± SD,%) in SFA/MUFA/PUFA determined by GC-MS and NMR from food matrices taken into study

(C16:1n7, C18:1n9, C18:1n7, C20:1n9) and 3 PUFAs (C18:2n6, C18:3n3, C20:4n6).

In Fig. 4 there are presented the compositions 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 (SFA) content determined by the NMR technique compared to the GC-MS technique was 14.66% higher. For MUFA, by comparing the mean values obtained by using NMR with the ones determined by GC-MS, the MUFA content was higher with 3.98% for NMR. 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.

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 it were 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 techniques used, the content being lower with 0.49% for NMR technique. By comparing the content of MUFA for lard, it can be seen that by using RMN technique the value was higher, the difference between RMN and GC-MS technique being of 3.84%. In the PUFA case, the content was higher with 15.06% when it were 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%.

#### ACKNOWLEDGMENT

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## REFERENCES

- [1] Y. Li, B.A. Watkins, *Current Protocols in Food Analytical Chemistry*, Ed. John Wiley and Sons Inc., New York, 2001, p. D1.2.1-D1.2.15
- [2] N. Chira, *Food Fat: Methods of Analysis, Authentication (Grăsimile alimentare: Metode de analiză, autentificare)*, Ed. Printech, Bucuresti, 2011, ISBN 978-606-521-696-9
- [3] A.J. Dijkstra, W.W. Christie, G. Knothe, „Nuclear Magnetic Resonance Spectroscopy”, in “*The Lipid Handbook*”, 3rd Edition, Ed. CRC Press, New York, 2007, ISBN 9780849396885, pp. 455-467
- [4] SR EN ISO 12966-2:2011: "Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids
- [5] ISO 12966-4:2015: "Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters– Part 4: Determination by capillary gas chromatography, 2015.