Evaluation of the Computational Methods for Determining Vegetable Oils Composition using ^{1}H-NMR Spectroscopy

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The ^{1}H-NMR spectroscopy represents a possible alternative to conventional chromatographic methods for oil composition determination. In this study, 25 samples of edible vegetable oils have been analyzed by ^{1}H-NMR spectroscopy. Based on data provided by the ^{1}H-NMR spectra, a system of chemometric equations for the calculation of the oil composition has been established.

Keywords: vegetable oils, composition, ^{1}H-NMR, chemometry

The fatty acid composition of vegetable oils is the main factor influencing their properties, concerning both nutrition [1, 2] and exploitation as raw materials for oleochemistry [3-6]. In recent years, interest of scientist to develop new and efficient methods for the determination of the fatty acid profile of different kind of samples (edible oils [7], lipid extracts from different human [8] or animal tissues [9], etc.), has increased, being partially fed by the general trend in the chemical literature concerning the food authenticity assessment. Among the most envisaged physical methods of analysis, gas-chromatography is by far the most popular [10], but ^{1}H-NMR spectroscopy is considered an interesting alternative [11, 12].

The present paper aims to exploit the potential of the ^{1}H-NMR spectroscopy regarding structural and compositional information applied to vegetable oils field, as well as its non-destructive nature and mild analysis conditions which does not require chemical transformations prior to/during analysis, as compared to other methods (chromatographic techniques) [13]. In addition, ^{1}H-NMR method is fast and allows simultaneous identification of several compounds, thus being suitable for the analysis of complex mixtures, such as oils and fats.

Experimental part

Vegetable oils were extracted by standard Soxhlet protocol [14] from oilseeds obtained from different Romanian agricultural research stations.

The standard mixture of 37 fatty acids methyl esters (Supelco™ 37 Component FAME Mix) used for the gas-chromatographic analyses was purchased from Supelco.

Fatty acid methyl esters (FAME) were prepared by transesterification of oils with methanol, using BF_3-MeOH complex as catalyst, according to the standard method [15].

The gas-chromatograms of the fatty acid methyl esters mixtures were recorded on an *Agilent Technologies* 6890 N instrument with FID detection. Separation into components was made on a capillary column especially designed for the FAME analysis (Supelco SP™ 2560, with the following characteristics: 100 m length, 0.25 mm inner diameter, 0.2 μm film thickness). The ready for injection solutions were prepared in CH_2Cl_2 of HPLC purity grade. Fatty acids identification was made by comparing for each peak the retention time with those of a standard mixture of 37 fatty acid methyl esters (Supelco™ 37 Component FAME Mix). In the standard mixture the exact concentration of each component is known. Both standard mixture and each of the fatty acid methyl esters of the analyzed oils were chromatographically separated under the same conditions, using the same temperature program, according to the Supelco specifications. The calibration of the signals was made by taking into account the concentration of each component of the standard mixture, correlated with the detector response.

^{1}H-NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer, operating at 9.4 Tesla, corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus, equipped with a direct detection four nuclei probehead and field gradients on *z* axis. Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving 0.5 mL oil in 0.5 mL $CDCl_3$. The chemical shifts are reported in ppm, using the TMS as internal standard.

Typical parameters for ^{1}H-NMR spectra were: 30° pulse, 4s aquisition time, 6.4 KHz spectral window, 8 scans, 52 K data points. The FID was not processed prior to Fourier transformation.

Results and discussions

^{1}H-NMR spectra of transesterified vegetable oils analyzed have the same shape, they differ only by integrals values and signal intensities. Figure 1 presents for exemplification the ^{1}H-NMR spectrum of transesterified soybean oil and table 1 lists the chemical shifts and the peak assignment of the resulted fatty acid methyl esters (FAME).

Based on ^{1}H-NMR spectral data of FAME, it was developed a system of chemometric equations leading to the determination of oils composition on four classes of fatty acids: linolenic acid, linolic acid, mono-unsaturated fatty acids (oleic acid, erucic acid, 11-eicosenoic acid) and saturated fatty acids (palmitic acid, stearic acid, behenic acid).

The following notations were adopted for the next chemometric equations:

- *x, y, z* and *t* represent the molar ratios of linolenic acid, linolic acid, mono-unsaturated fatty acids (oleic acid), and saturated fatty acids respectively;

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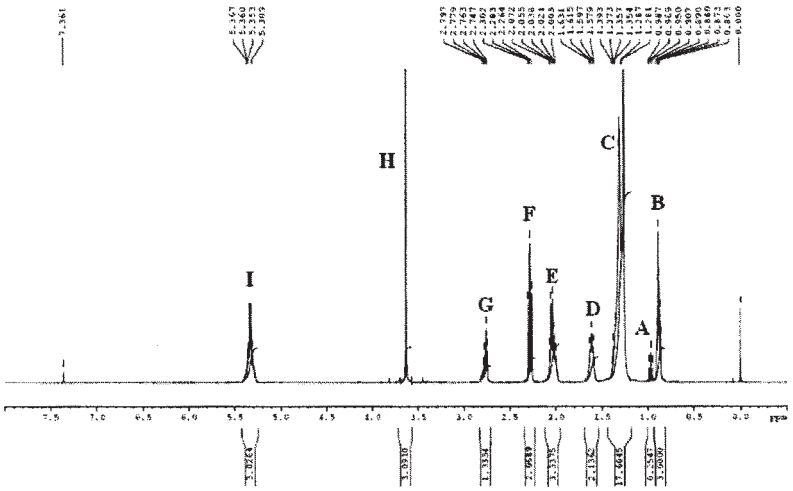

Fig. 1. ^{1}H-NMR spectrum of the fatty acid methyl esters of soybean oil

Signal	δ (ppm)	Proton	Compound
A	0.95	-CH=CH-CH$_2$-C***H***$_3$	linolenic acid
B	0.85	-CH$_2$-CH$_2$-CH$_2$-C***H***$_3$	all acids except linolenic acid
C	1.2	-(C***H***$_2$)$_n$-	all fatty acids
D	1.6	-C***H***$_2$-CH$_2$-COOH	all fatty acids
E	2.02	-C***H***$_2$-CH=CH-	all unsaturated fatty acids
F	2.2	-C***H***$_2$-COOH	all fatty acids
G	2.76	-CH=CH-C***H***$_2$-CH=CH-	linolic acid and linolenic acid
H	3.61	-COO-C***H***$_3$	all fatty acids
I	5.36	-C***H***=C***H***-	all unsaturated fatty acids

Table 1
CHEMICAL SHIFTS AND PEAK ASSIGNMENT OF ^{1}H-NMR SPECTRA OF FAME

-I_A, I_B, I_C, I_D, I_E, I_F, I_G, etc. represent the integral values of the corresponding signals.

-*k* is a spectrometer constant; is a computed coefficient which correlates the signal integral with the number of protons that signal is due to. In consequence, *k* value can be determined on the basis of different signals.

For a good precision, spectra were integrated in triplicate, the mean integral being used in further computations.

Computation of the constant k

First chemometric equation is obtained taking into account that the sum of the molar ratios must be equal to 1:

$$x + y + z + t = 1 \quad (1)$$

FAME composition from ^{1}H-RMN data was determined on the basis of the proton balances accounting for different signals in ^{1}H-NMRspectra. The determination of molar percentage of linolenic acid, x, is based on the fact that the triplet A at 0.95 ppm, generated by the three protons from the terminal methyl group (-CH=CH-CH$_2$-C**H**$_3$) of linolenic acid, is a marker of this fatty acid. So, the molar percentage of linolenic acid will be calculated as ratio of the integral value of the marker signal A and the integrals sum of the signals of all terminal methyl groups from all fatty acids ($I_A + I_B$):

$$x = \frac{I_A}{I_A + I_B} \quad (2)$$

The chemomeric equations on the basis of the protons balances for different signals in the spectra are:

- balance on signal **A**: $I_A = k \cdot 3 \cdot x$ (3)
- balance on signal **B**: $I_B = k \cdot 3 \cdot (y + z + t)$ (4)
- balance on signal **D**: $I_D = k \cdot 2 \cdot (x + y + z + t) = 2k$ (5)
- balance on signal **E**: $I_E = k \cdot 4 \cdot (x + y + z)$ (6)
- balance on signal **F**: $I_F = k \cdot 2 \cdot (x + y + z + t) = 2k$ (7)
- balance on signal **G**: $I_G = k \cdot 2 \cdot (2x + y)$ (8)

Thus we can obtain 8 equations with 5 unknowns (*k*, *x*, *y*, *z* şi *t*), the values I_A, I_B, I_C, I_D, I_E, I_F and I_G being read from the spectra. It is then evident that the 5 unknowns can be computed by different methods.

Thus, constant *k* can result:

- from equation (5): $k = \frac{I_D}{2}$ (9)
- from equation (7): $k = \frac{I_F}{2}$ (10)
- from equations (3)+(4):

$$I_A + I_B = k \cdot 3 \cdot (x + y + z + t) = 3k$$

$$k = \frac{I_A + I_B}{3} \quad (11)$$

- or as a mean of the values determined from equations (9) – (11):

$$k = \frac{\frac{I_D}{2} + \frac{I_F}{2} + \frac{I_A + I_B}{3}}{3} \quad (12)$$

Chemometric equations for the composition determination

The molar ratio of linolenic acid (*x*) can be obtained from equation (3):

$$x = \frac{I_A}{3k} \quad (13)$$

The molar ratio of linolic acid (*y*) is obtained by replacing (13) in equation (8):

$$y = \frac{I_G - 4kx}{2k} \quad (14)$$

The molar ratio of monounsaturated fatty acids (*z*) can be determined from equation (6):

$$z = \frac{I_E}{4k} - x - y \quad (15)$$

Finally, the molar ratio of saturated fatty acids (*t*) results as difference to 1:

$$t = 1 - x - y - z \quad (16)$$

By properly combining equations (9)-(16), 5 chemometric methods for the oils composition computation of composition can be imagined (table 2):

Method	k	x	y	z	t
1.	$k = \frac{I_D}{2}$	$x = \frac{I_A}{3k}$	$y = \frac{I_G - 4kx}{2k}$	$z = \frac{I_E}{4k} - x - y$	$t = 1 - x - y - z$
2.	$k = \frac{I_F}{2}$	$x = \frac{I_A}{3k}$	$y = \frac{I_G - 4kx}{2k}$	$z = \frac{I_E}{4k} - x - y$	$t = 1 - x - y - z$
3.	$k = \frac{I_A + I_B}{3}$	$x = \frac{I_A}{3k}$	$y = \frac{I_G - 4kx}{2k}$	$z = \frac{I_E}{4k} - x - y$	$t = 1 - x - y - z$
4.	$k = \frac{\frac{I_D}{2} + \frac{I_F}{2} + \frac{I_A + I_B}{3}}{3}$	$x = \frac{I_A}{3k}$	$y = \frac{I_G - 4kx}{2k}$	$z = \frac{I_E}{4k} - x - y$	$t = 1 - x - y - z$
5.	$k = \frac{I_F}{2}$	$x = \frac{I_A}{I_A + I_B}$	$y = \frac{I_G - 4kx}{2k}$	$z = \frac{I_E}{4k} - x - y$	$t = 1 - x - y - z$

Table 2
CHEMOMETRIC METHODS FOR THE DETERMINATION OF OIL COMPOSITION

Based on the chemometric equations in table 2, it was calculated the composition of 25 oil samples in terms of linolenic, linoleic, mono-unsaturated and saturated fatty acids. The results (table 3) were compared with the values determined by GC method, considered as reference values.

Nr. crt.	Sample	GC	^{1}H-RMN				
			Method 1	Method 2	Method 3	Method 4	Method 5
Linolenic acid (%)							
1.	Sunflower 1	0.44	0	0	0	0	0
2.	Linseed 1	42.71	44.86	44.69	43.35	44.53	43.35
3.	Sunflower 2	0	0	0	0	0	0
4.	Rapeseed 1	6.23	7.34	9.76	7.07	7.89	7.07
5.	Sunflower 3	0	0	0	0	0	0
6.	Sunflower 4	0	0	0	0	0	0
7.	Rapeseed 2	7.85	8.14	9.47	7.71	8.38	7.71
8.	Sunflower 5	0	0	0	0	0	0
9.	Sunflower 6	0	0	0	0	0	0
10.	Soybean 1	6.20	6.52	7.72	6.39	6.83	6.39
11.	Sunflower 7	0	0	0	0	0	0
12.	Rapeseed 3	6.84	6.51	7.99	6.89	7.08	6.89
13.	Sunflower 8	0	0	0	0	0	0
14.	Soybean 2	6.44	7.38	8.37	7.03	7.55	7.03
15.	Rapeseed 4	9.29	8.99	9.22	8.82	9.01	8.82
16.	Linseed 2	59.15	56.85	61.52	57.74	58.63	57.74
17.	Rapeseed 5	8.58	9.02	9.36	8.81	9.06	8.81
18.	Rapeseed 6	8.69	9.00	10.21	8.78	9.29	8.78
19.	Rapeseed 7	9.16	8.44	10.65	8.51	9.09	8.51
20.	Sunflower 9	0	0	0	0	0	0
21.	Rapeseed 8	8.82	8.77	10.22	8.67	9.17	8.67
22.	Rapeseed 9	8.48	8.65	9.94	8.71	9.06	8.71
23.	Soybean 3	7.11	7.01	8.19	7.34	7.48	7.34
24.	Rapeseed 10	9.24	8.11	9.08	8.70	8.62	8.70
25.	Rapeseed 11	8.56	9.08	9.77	8.53	9.10	8.53
Linoleic acid (%)							
1.	Sunflower 1	62.50	62.78	62.52	63.04	62.45	62.52
2.	Linseed 1	19.49	15.92	17.04	17.03	18.31	19.73
3.	Sunflower 2	66.49	63.42	64.90	63.01	63.76	64.90
4.	Rapeseed 1	19.07	17.47	16.27	20.98	17.11	16.65
5.	Sunflower 3	66.62	63.41	65.12	63.32	63.94	65.12
6.	Sunflower 4	66.13	63.18	65.00	62.94	63.69	65.00
7.	Rapeseed 2	21.62	20.64	19.03	19.86	20.06	20.55
8.	Sunflower 5	66.23	63.52	65.12	62.90	63.83	65.12
9.	Sunflower 6	65.43	62.60	64.39	63.10	63.36	64.39
10.	Soybean 1	50.51	46.87	47.84	48.10	47.65	48.50
11.	Sunflower 7	65.76	63.76	65.78	63.46	63.23	65.78
12.	Rapeseed 3	17.23	14.71	14.36	14.39	14.73	16.56
13.	Sunflower 8	66.35	62.88	64.79	62.70	63.44	64.79
14.	Soybean 2	52.70	49.36	49.14	49.29	49.36	51.81
15.	Rapeseed 4	18.97	16.51	16.87	16.23	16.53	15.65
16.	Linseed 2	14.96	12.93	11.25	11.81	12.00	13.81
17.	Rapeseed 5	19.01	18.10	18.79	17.69	18.18	19.88
18.	Rapeseed 6	19.09	17.10	16.00	17.75	17.55	18.87
19.	Rapeseed 7	18.13	19.74	14.81	18.83	16.64	19.11
20.	Sunflower 9	61.67	59.48	61.60	59.16	60.18	61.60
21.	Rapeseed 8	19.38	17.72	16.00	17.57	17.35	19.09
22.	Rapeseed 9	19.03	17.13	15.09	16.23	16.76	17.54
23.	Soybean 3	55.45	54.04	51.42	53.08	52.98	53.11
24.	Rapeseed 10	18.30	21.20	19.26	18.45	19.27	20.02
25.	Rapeseed 11	19.04	13.40	14.41	16.59	16.42	16.89

Table 3
OIL COMPOSITION RESULTS (GC VERSUS ^{1}H-NMR VALUES)

Table 3 (continued)

Mono-unsaturated fatty acids (%)							
1.	Sunflower 1	**21.39**	24.03	23.94	23.75	23.91	22.94
2.	Linseed 1	**24.18**	26.25	23.27	25.53	26.23	23.75
3.	Sunflower 2	**22.07**	22.92	23.45	22.77	23.04	23.45
4.	Rapeseed 1	**66.30**	63.31	67.93	63.37	63.34	68.24
5.	Sunflower 3	**21.98**	22.56	23.17	22.53	22.75	23.17
6.	Sunflower 4	**22.00**	22.09	22.72	22.00	22.27	22.72
7.	Rapeseed 2	**63.35**	61.94	65.21	61.89	62.57	64.45
8.	Sunflower 5	**21.02**	21.25	21.79	21.05	21.36	21.79
9.	Sunflower 6	**21.39**	21.79	22.41	21.97	22.06	22.41
10.	Soybean 1	**23.37**	21.72	22.72	21.28	21.74	24.38
11.	Sunflower 7	**21.67**	20.63	22.72	21.26	21.83	22.22
12.	Rapeseed 3	**71.61**	69.59	73.12	69.07	68.78	72.02
13.	Sunflower 8	**22.84**	22.73	23.42	22.66	22.93	23.42
14.	Soybean 2	**22.14**	20.90	23.68	19.90	21.38	22.35
15.	Rapeseed 4	**63.19**	64.68	64.27	64.44	64.78	65.87
16.	Linseed 2	**14.59**	16.20	17.42	17.84	16.46	15.64
17.	Rapeseed 5	**62.97**	61.85	64.20	60.45	62.13	63.66
18.	Rapeseed 6	**64.43**	66.52	66.18	60.89	62.21	64.75
19.	Rapeseed 7	**64.16**	61.02	66.93	61.45	62.13	64.79
20.	Sunflower 9	**26.32**	24.88	26.36	25.17	24.04	26.36
21.	Rapeseed 8	**64.30**	62.30	65.64	62.70	61.88	64.09
22.	Rapeseed 9	**60.22**	58.73	64.04	58.15	58.40	61.82
23.	Soybean 3	**22.34**	19.66	22.95	20.57	20.98	22.11
24.	Rapeseed 10	**65.92**	62.51	65.52	62.77	64.13	65.14
25.	Rapeseed 11	**60.29**	62.23	66.96	58.48	59.36	62.72
Saturated fatty acids (%)							
1.	Sunflower 1	**15.67**	13.18	13.54	13.21	13.65	14.54
2.	Linseed 1	**13.62**	16.97	13.18	14.09	14.93	13.18
3.	Sunflower 2	**11.44**	13.67	11.65	14.22	13.19	11.65
4.	Rapeseed 1	**8.40**	11.89	8.04	11.40	11.67	8.04
5.	Sunflower 3	**11.40**	14.03	11.71	14.16	13.31	11.71
6.	Sunflower 4	**11.87**	14.73	12.28	15.05	14.04	12.28
7.	Rapeseed 2	**7.18**	9.27	6.29	9.54	8.99	7.29
8.	Sunflower 5	**12.75**	15.23	13.09	16.05	14.81	13.09
9.	Sunflower 6	**13.18**	15.61	13.20	14.93	14.59	13.20
10.	Soybean 1	**19.92**	23.90	21.73	24.24	22.79	20.73
11.	Sunflower 7	**12.57**	15.61	11.50	15.28	14.94	12.00
12.	Rapeseed 3	**4.29**	9.19	4.52	8.65	8.42	4.52
13.	Sunflower 8	**10.81**	14.40	11.79	14.64	13.63	11.79
14.	Soybean 2	**18.72**	22.36	18.82	23.78	21.71	18.82
15.	Rapeseed 4	**8.55**	9.81	9.65	10.50	9.68	9.65
16.	Linseed 2	**11.31**	14.02	9.81	12.59	12.91	12.81
17.	Rapeseed 5	**8.03**	11.03	7.65	13.05	10.63	7.65
18.	Rapeseed 6	**7.79**	11.55	7.60	12.57	10.94	7.60
19.	Rapeseed 7	**7.30**	10.80	7.60	11.21	12.13	7.60
20.	Sunflower 9	**12.01**	15.64	12.04	15.67	15.78	12.04
21.	Rapeseed 8	**7.86**	11.22	8.14	11.05	11.60	8.14
22.	Rapeseed 9	**12.99**	15.50	10.93	15.91	15.78	11.93
23.	Soybean 3	**16.97**	19.28	17.44	19.01	18.56	17.44
24.	Rapeseed 10	**5.80**	8.17	6.14	10.07	7.99	6.14
25.	Rapeseed 11	**13.02**	15.29	8.86	16.40	15.11	11.86

Method	Standard deviations				
	linolenic acid	linoleic acid	Mono-unsaturated fatty acids	Saturated fatty acids	TOTAL
1.	0.79	2.72	1.87	3.11	**8.49**
2.	1.36	2.61	2.12	1.24	**7.33**
3.	0.45	2.56	2.02	3.42	**8.45**
4.	0.66	2.27	1.70	2.71	**7.34**
5.	0.45	1.44	1.18	0.67	**3.74**

Table 4
STANDARD DEVIATIONS OF THE INVESTIGATED METHODS

The evaluation of the 5 chemometric methods was made on the basis of the standard deviations for each class of fatty acids. Standard deviations were calculated taking the GC results as reference values. The resulting standard deviations on each class of fatty acids are shown in table 4.

The accuracy of the investigated 5 methods was evaluated by summing – for each method – the computed standard deviations of the corresponding fatty acid classes. The most precise chemometric method will be assigned, of course, on the basis of the minimum value of the total standard deviation.

It is evident from table 4 that the method that best fits the GC results is method 5 (the combined method, in which the reference signal is **F** and the molar ratio of linolenic acid is determined as fraction between integral **A** and the sum of integrals **A** and **B**).

In conclusion, it was examined the potential of five different chemometric methods of determining the composition of vegetable oils based of ^{1}H-NMRspectra. The method which best fitted the GC results is the combined method where the reference signal is the signal of methylene protons next to the carbonyl groups, **F**, and

the molar ratio of linolenic acid is determined from the balance of the terminal methyl groups of the fatty acids.

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