DETERMINATION OF THE TECHNICAL QUALITY INDICES OF VEGETABLE OILS BY MODERN PHYSICAL TECHNIQUES

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Această lucrare prezintă utilizarea spectroscopiei de rezonanţă magnetică nucleară de proton şi cromatografia de gaze pentru determinarea indicilor de calitate ai uleiurilor vegetale. Pe baza datelor ^{1}H-RMN, s-au dedus ecuaţii chemometrice care permit calcularea masei moleculare medii şi indicilor de iod şi de saponificare ai uleiurilor vegetale. Aceste rezultate au fost comparate cu valorile obţinute prin metodele standard de analiză şi s-au dovedit a fi în concordanţă cu acestea.

This work presents the use of proton nuclear magnetic resonance spectroscopy and gas-chromatography for the determination of quality indices of vegetable oils. Based on the ^{1}H-NMR data, chemometric equations were developed, leading to the computation of the average molecular weight, iodine and saponification indices of vegetable oils. The results were compared with those obtained by standard methods and proved to be in agreement.

Keywords: vegetable oils, iodine index, saponification index, ^{1}H-NMR, gas-chromatography, chemometry

1. Introduction

Vegetable oils are important both from the point of view of their nutritional value [1-3] and as valuable renewable raw materials for the chemical [4-6] and energetic industry [7, 8]. From this point of view, the quality assessment of oils has become an important issue. Two intensively used factors for the evaluation of oils are their iodine and saponification indices. The iodine index quantifies the degree of unsaturation of oils, being determined, according to the standard protocol [9], by reacting oils with iodine and titrating the excess of

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iodine with $Na_2S_2O_3$. The saponification index is a useful tool for the evaluation of the chain length of fatty acids occurring in the triacylglycerols in oil and is determined in the standard protocol [10] by saponification of the sample with excess alcoholic KOH solution under reflux, followed by titration of the excess KOH solution with a HCl solution of known concentration. Beside the fact that these are time consuming methods, they are susceptible to errors. Physical methods instead are fast, accurate, and require small amounts of sample. Nevertheless, they provide detailed information and a global profile of the sample [11, 12]. That is why physical methods are suitable for the analysis of food complex mixtures [13], such as vegetable oils. Among them, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gas-chromatography (GC) are two of the most promising.

The present paper deals with the development of new methods for the computation of the iodine and saponification indices of oils using ^{1}H-NMR and gas-chromatographic data.

2. Experimental

Different types of vegetable oils were purchased from S.C. Manicos S.R.L. (white sesame oil, sweet almond oil, grape seeds oil, and walnut oil), TIS Farmaceutic S.A. (wheat germ oil), S.C. Hofigal S.A. (sea-buckthorn - *Hippophae rhamnoides* - oil), S.C. Argus S.A. (sunflower oil, rapeseed oil, and soybean oil), S.C. Arpis S.A. (corn oil) and S.C. Parapharm S.R.L (pumpkin oil).

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

Iodine index was experimentally determined by treatment with Wijs reagent followed by titration of the iodine excess with $Na_2S_2O_3$, according to the standard protocol [9].

Saponification index was experimentally determined by treatment with alcoholic KOH solution, followed by titration of the KOH excess with HCl, according to the standard protocol [10].

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 [14].

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^{TM} 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 each peak the retention time with those of a standard mixture of 37 fatty acid methyl esters (Supelco^{TM} 37 Component FAME Mix). The exact concentration of each component is known in the standard mixture. 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's 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.

3. Results and discussions

The ^{1}H-NMR spectra of vegetable oils have similar shape (Fig. 1), and the peak assignment is shown in *Table 1* [15].

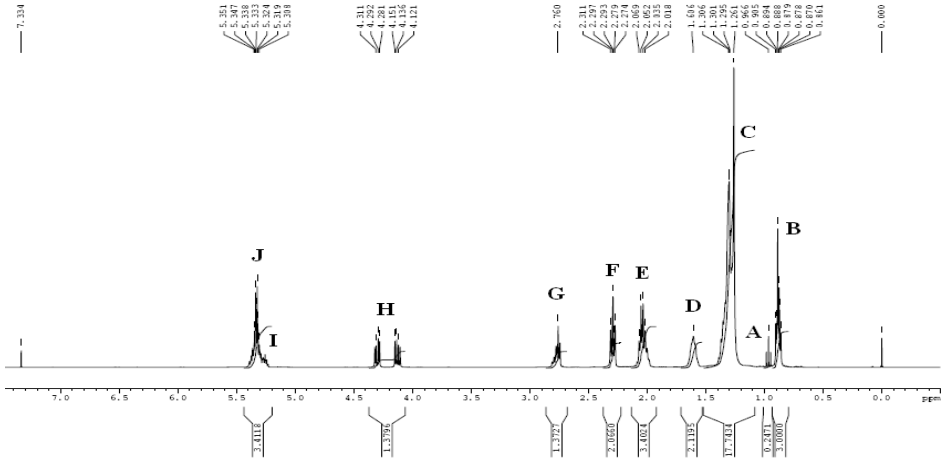


Fig. 1. ^{1}H-NMR spectrum of soybean oil.

Table 1

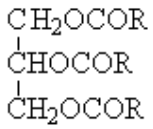
Peak assignment of the ^{1}H-NMR spectra of vegetable oils

Peak	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 acyl chains, except for linolenic
C	1.2	-(C***H***$_2$)$_n$-	All acyl chains
D	1.6	-C***H***$_2$-CH_2-COOH	All acyl chains
E	2.02	-C***H***$_2$-CH=CH-	Allylic protons (all unsaturated fatty acids)
F	2.2	-C***H***$_2$-COOH	All acyl chains
G	2.76	-CH=CH-C***H***$_2$-CH=CH-	*bis*-allylic protons (linolenic and linoleic acid)
H	4.19	-C***H***$_2$-O-COR	Glycerol (α position)
I	5.15	-C***H***-O-COR	Glycerol (β position)
J	5.29	-C***H***=C***H***-	All unsaturated fatty acids

The following notations were adopted for the next chemometric equations: I_A, I_B, I_C, I_D, I_E, I_F, I_G, I_H, and I_{I+J} for the integral values of the corresponding signals in the ^{1}H-RMN spectra of triacylglycerols.

a. Average molecular weight computation of triacylglycerols.

The average molecular formula of triacylglycerols was determined prior to their average molecular weight. In order to do that, we assumed that oils have a unitary composition, consisting of a single type of triacylglycerols with the following structure:



This represents a hypothetical triacylglycerol, meaning the weighted average of all triacylglycerols present in oils.

The integral balances of the signals generated by the protons of the methylene groups and of those in the double bonds of the R chain lead to the computation of:

- α (average number of -CH_2- groups in the R chain);
- β (average number of -HC=CH- groups in the R chain).

$$\alpha = \frac{3}{2} \cdot \frac{I_C + I_D + I_E + I_F + I_G}{I_A + I_B} \tag{1}$$

$$\beta = \frac{3}{2} \cdot \frac{I_{I+J} - I_H / 4}{I_A + I_B} \tag{2}$$

In Equations (1) and (2), the cumulated values of the **A** and **B** signal integrals were considered as reference. The number of protons in each group generating a signal was taken into account (three for the terminal methyl groups and two for the methylene groups), thus appearing the normalization factor 3/2. A difficulty in the case of the ^{1}H-NMR spectra of triacylglycerols is that the signals **I** (the proton in the β position in glycerol) and **J** (the protons in the *-HC=CH-* groups) are overlapped, which makes them impossible to be integrated separately. Nevertheless, the integral value of signal **I** is $I_H/4$ (**H** being generated by 4 protons, while **I** by a single one), so the integral of the **J** signal will be computed as difference from I_{I+J}:

$$I_I = \frac{I_H}{4} \tag{3}$$

$$I_J = I_{I+J} - \frac{I_H}{4} \tag{4}$$

From the spectra there were also computed:

- the average number of carbon atoms in the R chain (n_C) :

$$n_C = \alpha + 2\beta + 1 \tag{5}$$

- the average number of hydrogen atoms in the R chain (n_H):

$$n_H = 2\alpha + 2\beta + 3 \tag{6}$$

Thus, the average formula of R can be determined ($C_{\alpha + 2\beta + 1}H_{2\alpha + 2\beta + 3}$), as well as that of the average triacylglycerol ($C_{6 + 3(\alpha + 2\beta + 1)}H_{5 + 3(2\alpha + 2\beta + 3)}O_6$), which leads to the computation of the average molecular weight:

$$M_{TG} = 12 \cdot [6 + 3(\alpha + 2\beta + 1)] + 1 \cdot [5 + 3(2\alpha + 2\beta + 3)] + 16 \cdot 6 \tag{7}$$

b. Iodine index computation based on the ^{1}H-NMR data

According to the Romanian standard [9], the iodine index represents the amount of I_2 (in g) necessary for 100g of oil in the addition reaction.

The chemometric approach for the iodine index computation stands on the number of moles of triacylglycerols *per* gram of oil:

$$n = \frac{1}{M_{TG}} \tag{8}$$

The number of moles of double bonds *per* gram of oil:

$$n_{-CH=CH-} = 3 \cdot \beta \cdot n \tag{9}$$

The iodine index (g I_2/100 g oil) was chemometrically determined taking into account that each double bond reacts with two iodine atoms:

$$I_{iodine} = 3 \cdot \beta \cdot n \cdot 2 \cdot 127 \cdot 100 \quad (10)$$

The iodine index was computed based on the ^{1}H-NMR data for a series of oils from different plant species and it was compared with the values determined by the standard method [9], which were taken as reference values. The results are shown in *Table 2*.

Table 2

Iodine index (computed values according to the ^{1}H-RMN method and experimentally determined by the standard method) for a series of different vegetable oils (g I_2/100 g oil)

No.	Sample	I_{iodine} (^{1}H-RMN method) (A)	I_{iodine} (standard method) (B)	Deviation (A)-(B)
1.	Sea-buckthorn oil	63.4	65.6	-2.2
2.	Pumpkin oil	124.6	123.9	0.7
3.	Sunflower oil	121.6	122.0	-0.4
4.	Wheat germ oil	130.7	128.9	1.8
5.	White sesame oil	110.6	113.7	-3.1
6.	Soybean oil	128.0	128.7	-0.7
7.	Grape seeds oil	128.1	129.5	-1.4
8.	Rapeseed oil	111.2	113.2	-2.0
9.	Corn oil	120.1	119.6	0.5
10.	Walnut oil	148.4	149.7	-1.3
11.	Sweet almond oil	98.9	99.6	-0.7

The iodine index was also computed for a series of oil mixtures of different compositions, in order to cover a large range of values. The composition of the mixtures is given in *Table 3*.

Table 3

Composition of the studied mixtures (weight %)

Sample	Soybean oil	Linseed oil	Rapeseed oil
1	-	-	100
2	-	100	-
3	50	50	-
4	50	-	50
5	25	-	75
6	75	-	25
7	12.5	-	87.5
8	87.5	-	12.5
9	75	25	-
10	25	75	-
11	87.5	12.5	-
12	12.5	87.5	-
13	100	-	-

The values of the iodine indices computed based on the ^{1}H-NMR data were compared with those experimentally determined by the standard method, the results being given in *Table 4*.

Table 4

Iodine index (computed values according to the ^{1}H-RMN method and experimentally determined by the standard method) for a series of oil mixtures of different composition (g I_2/100 g oil)

Sample	I_{iodine} (^{1}H-RMN method) (A)	I_{iodine} (standard method) (B)	Deviation (A)-(B)
1	111.2	113.2	-2.0
2	186.3	185.2	1.1
3	156.3	159.4	-3.1
4	120.7	122.8	-2.1
5	117.3	119.0	-1.7
6	127.4	126.3	1.1
7	116.1	119.4	-3.3
8	125.6	124.2	1.4
9	142.1	144.2	-2.1
10	171.3	174.3	-3.0
11	135.8	136.6	-0.8
12	172.7	168.7	4.0
13	128.0	128.7	-0.7

For the cumulated data from *Table 2* and *Table 4*, a standard deviation of 2.0 was calculated, corresponding to an accuracy of 1.5%. This shows a good correlation between the iodine index values computed from the ^{1}H-NMR spectra and the reference values determined by the standard method. This makes the ^{1}H-NMR method efficient for the determination of the iodine index of oils.

b. Iodine index computation based on the GC data

The fatty acids composition of oils was chromatographically determined in molar percentages, as shown in the Experimental section. In order to calculate the iodine index, the fatty acids composition must be converted into mass concentrations (weight %), by multiplying – for each methyl ester – its molar concentration with the corresponding molecular weight, summing the values and expressing them as percentages. Then, the number of moles of each methyl ester was determined, by dividing its mass concentration with the corresponding molar weight.

For the determination of the iodine index (g I_2/100 g oil), the number of double bonds in the chain of each identified methyl ester was taken into account.

The iodine index was computed from the GC data for the series of oil mixtures of different compositions presented in *Table 3*. The computed values

were compared with those determined by the standard method, which were taken as reference values. The results are presented in *Table 5*.

Table 5

Iodine index (computed values according to the GC method and experimentally determined by the standard method) for a series of oil mixtures of different composition (g I_2/100 g oil)

Sample	I_{iodine} (GC method) (A)	I_{iodine} (standard method) (B)	Deviation (A)-(B)
1	114.7	113.2	1.5
2	187.9	185.2	2.7
3	157.2	159.4	-2.2
4	122.1	122.8	-0.7
5	118.2	119.0	-0.8
6	127.2	126.3	0.9
7	118.1	119.4	-1.3
8	125.1	124.2	0.9
9	142.3	144.2	-1.9
10	172.1	174.3	-2.2
11	138.1	136.6	1.5
12	171.0	168.7	2.3
13	131.0	128.7	2.3

For the evaluation of the GC method of iodine index determination, a standard deviation of 1.8 was calculated, based on the data in *Table 5*, corresponding to an accuracy of 1.3%. This value indicates that the GC method is suitable for the determination of the iodine index of oils. Comparing the GC and the ^{1}H-NMR methods, it was concluded that they have the same precision, but the ^{1}H-NMR method is more rapid and less laborious than GC.

c. The saponification index computation based on the ^{1}H-NMR data

According to the Romanian standard [10], the saponification index of oils represents the necessary amount (in mg) of KOH for the saponification of 1 g of oil. In order to calculate it, it is necessary to find out the moles of ester groups *per* gram of oil:

$$n_{-CO-O-} = 3 \cdot n \tag{11}$$

where n (moles of triacylglycerols *per* gram of oil) was previously determined (8).

The saponification index (mg KOH/g oil) will be determined with the following chemometric equation:

$$I_{saponification} = 3 \cdot n \cdot 56 \cdot 10^3 \tag{12}$$

The saponification index was computed based on the ^{1}H-NMR spectra of a series of different vegetable oils. The computed values were compared with the

values determined by the standard titration method [10], taken as reference values. The results are shown in *Table 6*:

Table 6

Saponification index (computed values according to the ^{1}H-RMN method and experimentally determined by the standard method) for a series of different vegetable oils (mg KOH/g oil)

No.	Sample	$I_{saponificaton}$ (^{1}H-RMN method) (A)	$I_{saponification}$ (standard method) (B)	Deviation (A)-(B)
1.	Sea-buckthorn oil	211.9	198.1	13.8
2.	Pumpkin oil	197.6	185.8	11.8
3.	Sunflower oil	215.9	204.8	11.1
4.	Wheat germ oil	197.2	183.6	13.6
5.	White sesame oil	197.2	185.4	11.8
6.	Soybean oil	195.5	185.0	10.5
7.	Grape seeds oil	195.7	184.0	11.7
8.	Rapeseed oil	196.8	186.2	10.6
9.	Corn oil	199.9	187.4	12.5
10.	Walnut oil	195.0	188.2	6.8
11.	Sweet almond oil	192.4	199.1	-6.7

To evaluate the ^{1}H-NMR method of the saponification index computation, a standard deviation of 11.8, corresponding to an accuracy of 6.2% was calculated from the data in *Table 6*. Thus, the ^{1}H-NMR method has a relatively low accuracy but, in some cases it can be efficient due to the fact that it is faster comparatively to the standard method.

4. Conclusions

In conclusion, the ^{1}H-NMR spectroscopy and the gas chromatography prove to be efficient for the determination of the iodine index of vegetable oils. In addition to this, ^{1}H-NMR spectroscopy can be used for the rapid determination of the saponification index. In comparison with the standard methods, ^{1}H-NMR spectroscopy is faster, it does not require any sample preparation prior to the analysis. It requires a small quantity of sample but, most importantly, it can offer a global profile of the sample (average molecular weight, structural and compositional information). Moreover, the computations can be computer-assisted and thus, the method becomes even faster. The gas-chromatographic method, in spite of having practically the same precision as the ^{1}H-NMR method, is more laborious and time consuming but, in comparison with the standard method of iodine index determination, it offers (as well as the ^{1}H-NMR) a global profile of the fatty acids composition of the sample. Thus, the ^{1}H-NMR method proves to be a good alternative for the rapid determination of the iodine and saponification indices of vegetable oils.

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