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CLIMATE IMPACT ON FATTY ACID CONTENT OF GRAPE SEED OIL

Mihaela TOCIU[1], Maria-Cristina TODASCA[2], Michaela DINA STANESCU[3*], Victoria ARTEM[4], Valentin IONESCU[5]

Climatic conditions have a significant impact on the chemical composition of grapes and grape seeds. This fact is also relevant for the proposed "Mamaia" grape variety, which was developed by RCVE Murfatlar. This variety has higher production potential in comparison with other traditional varieties and produces a high quality red wine. The paper aims to correlate the climatic conditions with variation in fatty acid profile (FAP) of the oil extracted from grape seeds of this "Mamaia" variety. The samples were collected and analyzed over a 4 years-period. To establish the FAP, NMR method was used, results being provided in short time and without prior sample processing. NMR obtained results were confirmed by GC-MS standard method. The ratio unsaturated/saturated fatty acid was discussed in connection with climatic conditions.

Keywords: FAP, grape seed oil, climate, ^{1}H-NMR spectrum

1. Introduction

Grapes (*Vitis vinifera*) are mainly used for wine production. Waste resulting from the winemaking industry can be exploited for new food production [1]. The valorization of wine waste is compulsory for a cost-effective and sustainable production [2].

Geographical origin and authenticity of grapes are factors influencing the quality of wine. The origin of the grapes can be established through a series of wet analytical methods, or by means of spectroscopic methods [3].

There are other factors of impact like: grape variety [4], processing conditions [5], harvest process [6], and agrotechnical treatments applied to plants

[1] PhD student, Faculty of Applied Chemistry and Materials Science University POLITEHNICA of Bucharest, and Department of Organic Chemistry „C. Nenitescu", Bucharest, Romania

[2] PhD, Faculty of Applied Chemistry and Materials Science University POLITEHNICA of Bucharest, and Department of Organic Chemistry „C. Nenitescu", Bucharest, Romania

[3] Prof., Faculty of Applied Chemistry and Materials Science University POLITEHNICA of Bucharest, and Department of Organic Chemistry „C. Nenitescu", Bucharest, Romania, e-mail: smichaeladina@yahoo.com, michaela.stanescu@yahoo.com

[4] PhD student, Research Centre for Viticulture and Enology Murfatlar (RCVE), Murfatlar, Constanta, Romania

[5] PhD, National Research & Development Institute for Food Bioresources - IBA, Bucharest, Romania

[7]. The fermentation conditions and type of yeast used may also generate changes in the chemical compositions of wines [8].

A way of valorizing the wine industry residue consists in grape seed oil extraction. The fatty acid profile (FAP) of the resulted oil is established by standard chromatographic methods. A modern method, rapid and nondestructive, is NMR [9, 10], representing a good alternative to standard chromatographic method [11]. Some Romanian grape seed oils have been accurately characterized by our group, using spectroscopic methods such as: ^{1}H-NMR [12] and ^{13}C-NMR [13].

Literature studies have shown that oil FAP is influenced in the period before harvest by a number of factors, climate being among them [14, 15].

The aim of this study is to establish the influence of climatic conditions on the FAP of the grape seed oil obtained from *Mamaia* variety, in order to improve the oil quality. Samples were obtained from grape seed harvested in 4 different years (2010, 2011, 2014 and 2015) under distinctive climatic conditions of ripeness (temperature, precipitation).

2. Experimental part

The grape seed oil was extracted according to the Soxhlet protocol [16] from the harvested *Mamaia* variety.

The **gas chromatograms** of the fatty acid methyl esters mixtures were recorded on an Agilent Technologies model 7890A instrument, coupled with an Agilent Technologies model 5975 C VL MSD mass detector with Triple Axis Detector and Agilent auto-sampler. The separation into components was made on a capillary column especially designed for the fatty acids methyl esters (FAMEs) analysis (Supelco SP^{TM} 2560, with the following characteristics: 100 m length, 0.25 mm inner diameter, 0.2 μm film thickness), as standard procedure [17]. The ready for injection solutions were prepared in CH_2Cl_2 of HPLC purity grade. Fatty acids identification was made by comparing the retention time for each peak with those of a standard mixture of 37 fatty acid methyl esters (Supelco^{TM} 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 grape seed oils were chromatographically separated under the same conditions, according to the Supelco specifications, using the same temperature program (oven initial temperature 140 ºC to final temperature 240 ºC, heating rate 4 ºC/min.), injection volume 1 μL, split rate 100:1, carrier gas He. The calibration of the signals was made by taking into account the concentration of each component in the standard mixture, correlated with the detector’s response.

Fatty acid methyl esters (FAMEs) were prepared by trans-esterification of oils with methanol, using BF_3-MeOH complex as catalyst, according to the known method [18].

The ^{1}H-NMR spectra of the grape seed oils extracted were recorded on a Bruker Avance III 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 probe head and field gradients on *z* axis. Samples were analyzed in 5 mm NMR tubes (Norell 507). The chemical shifts are reported in ppm. Typical parameters for ^{1}H-NMR spectra were: 45° pulse, 2.05 s acquisition times, 6.4 KHz spectral window, 32 scans, 26 K data points. The FID was not processed prior to Fourier transform. The average acquisition time of ^{1}H-NMR spectra was approximately 2 minutes. The sample preparation was simply reduced to the dilution of 200 μL of grape seed oil in 800 μL of $CDCl_3$.

3. Results and discussions

In order to correlate the compositional changes in *Mamaia* grape seed oil with the climatic conditions, climate factsheets for the studied 4 years are given. These sheets contain information on the active vegetation period (April-September) and the grape maturation period (July-September). Table 1 presents the mean air temperature and precipitation content recorded each month.

Table 1

The conditions of ripeness for grape seed, Murfatlar - Romania

Month/ period	Air temperature (T med, °C)				Precipitations (mm)			
	2010	2011	2014	2015	2010	2011	2014	2015
April	14.5	10.2	13.9	13.5	19.2	36.0	49.6	68.6
May	18.7	18.4	20.0	21.1	61.1	42.5	54.8	10.6
June	24.0	24.0	24.0	25.5	43.6	22.7	153.2	10.2
July	25.4	26.6	26.6	28.3	211.5	85.7	98.8	44.0
August	29.6	25.0	27.1	27.3	1.2	8.0	32.3	59.2
September	23.2	22.5	21.0	23.1	49.3	5.0	31.2	10.0
Vegetation	22.6	21.1	22.1	23.1	64.3	33.3	70.0	33.8
Maturation	26.1	24.7	24.9	26.2	87.3	32.9	54.1	37.7

Grape seed samples were collected at different times. The sugar content of grapes was the determining factor for grape harvest, therefore the harvesting period were in some way different, as presented in Table 2.

Table 2

Samples *Mamaia* grape harvest time

Year	Harvest date	Δ days
2010	16 September	± 20
2011	26 August	-
2014	11 September	± 15
2015	8 September	± 7

Grape seed samples from *Mamaia* variety, annually harvested, have undergone extraction with petroleum ether using Soxhlet method, as previously described [16].

The cultivar and ripening stage are determining factors for oil content and the FAP [19]. The amounts of oil differ, being influenced by climatic conditions and the harvesting time. Thus, average values of the corresponding amount of oil extracted from 100 g ground grape seeds, resulting after three extractions, are presented in Table 3. It may be noticed that, in case of 2011 samples, the amount of oil is the smallest, most probably due to the shortest ripening period.

Table 3

Oil content from *Mamaia* grape seed samples

Amount of oil (g) / 100 g grape seeds			
2010	2011	2014	2015
7.96 ± 0.09	7.13 ± 0.08	9.97 ± 0.04	13.01 ± 0.02

Determination of fatty acids profile of grape seeds oil samples using ^{1}H-NMR spectroscopy

Nuclear magnetic resonance spectroscopy method is a fast and modern alternative for the determination of FAP classes of grape seed oils [20]. For this purpose, the ^{1}H-NMR spectra of grape seed oils from *Mamaia* variety were recorded in triplicate. The relevant spectral area for ^{1}H-NMR analysis of grape seed oils is 0 - 5.5 ppm. ^{1}H-NMR spectra were converted into a series of values using the MestReNova program. The integral values of signals are used in the chemometrical computations, in agreement with their spectral attribution. Each integral is assigned to a type of compound, as is shown in Fig. 1.

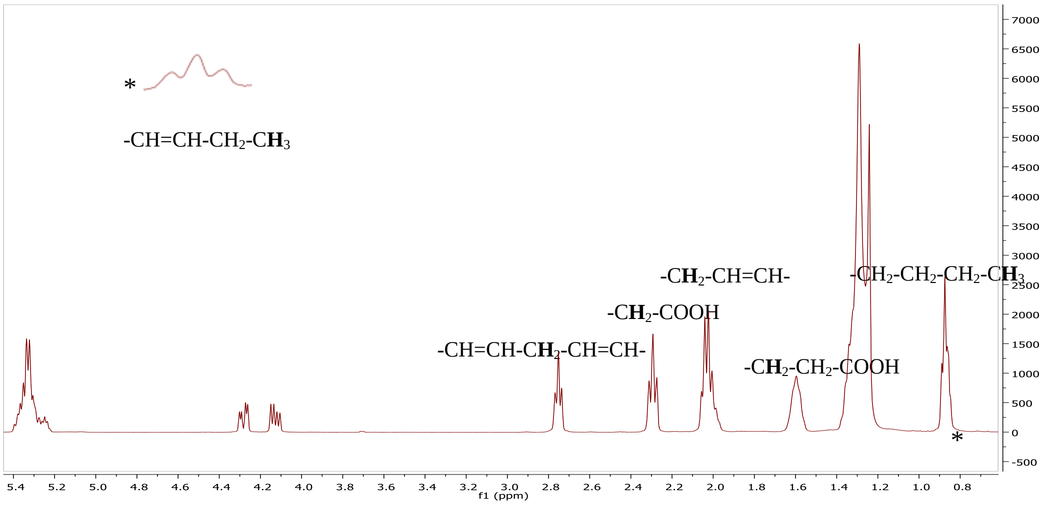

Fig. 1. ^{1}H-NMR spectrum of *Mamaia* grape seed oil

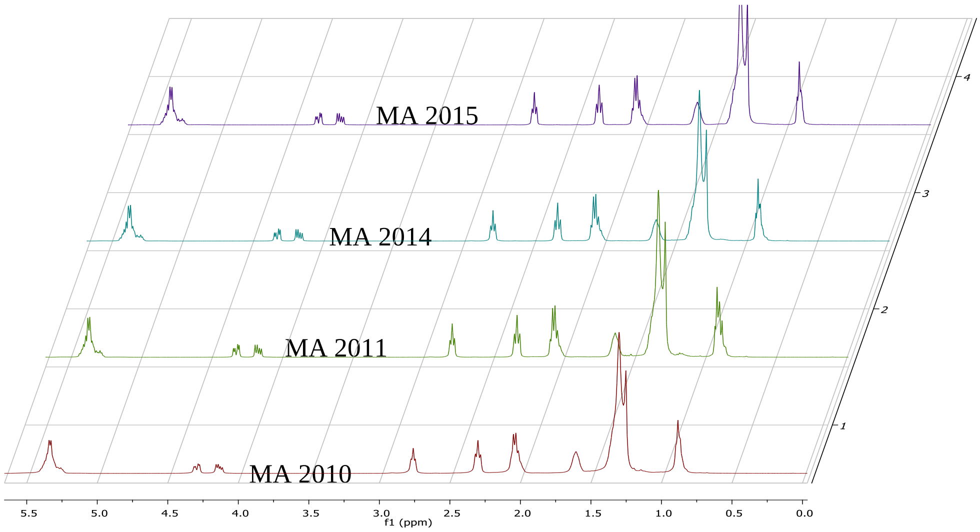

Fig. 2. Comparative ^{1}H-NMR spectra of *Mamaia* grape seed oils

Fig. 2 presents the ^{1}H-NMR profile of the *Mamaia* variety samples. As displayed, the signals are similar in all 4 cases but their integrals are distinctive, therefore the ratio between signals is different. This indicates a similar content in terms of fatty acids, but a different amount of each fatty acid.

In order to calculate the molar composition, the average values of the relevant integrals were worked up. Based on chemometric equations developed by our research group and previously described [11, 12], the FAP of the analyzed oil samples was obtained (see Table 4).

Determination of fatty acids composition of grape seeds oil samples using GC-MS spectrometry

The method of gas chromatography coupled with mass spectrometry was used to confirm the amount of fatty acids present in *Mamaia* variety grape seed oil obtained by NMR analysis. Identification of the FAP of samples was possible by comparing the retention times of peaks in each chromatogram (Fig. 3a) with those of the standard mixture of 37 FAMEs (Fig. 3b).

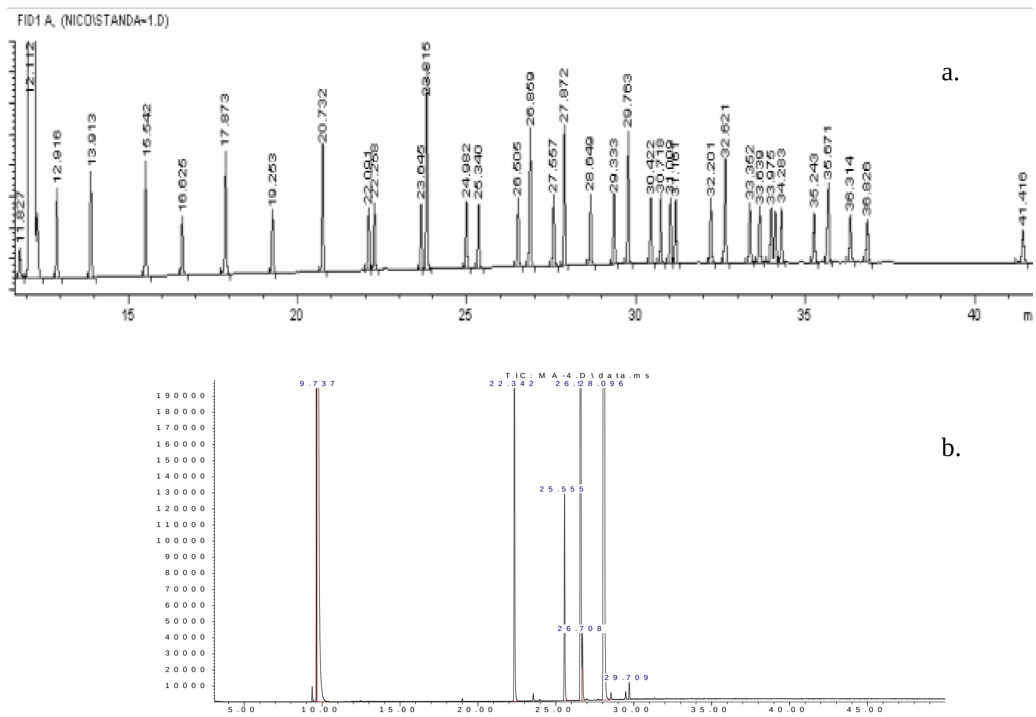

Fig. 3. Chromatogram of a. standard Supelco 37 FAME-Mix and
b. *Mamaia* variety grape seeds oil

The fatty acids composition of grape seed oils analyzed is presented in Table 4. These results represent average values obtained from the three successive determinations. The results presented in Table 4 were obtained either by NMR method, or the standard GC-MS, being comparable as values. The fatty acids are classified in three groups: saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), and poly-unsaturated fatty acids (PUFAs). As shown in Table 4, climatic conditions directly influence the types of fatty acids identified.

Table 4

Fatty acid profile of grape seed oils, *Mamaia* variety

FAP (% mol.)	2010		2011		2014		2015	
	GC-MS	NMR	GC-MS	NMR	GC-MS	NMR	GC-MS	NMR
SFA	9.75	9.93	7.59	7.72	8.08	8.13	9.73	9.95
MUFA	17.25	17.65	13.61	13.75	15.29	15.66	13.34	13.59
PUFA	73.00	72.42	78.80	78.53	76.63	76.21	76.94	76.46

Thus, a significant quantity of MUFAs is produced in grape seeds grown in years with high temperature and high precipitation during ripening period. While low temperatures correlated with low amounts of precipitations favored the formation of PUFAs and also a decrease in SFAs. Such changes enhance the healthy effect of the grape seed oil [13, 21]. These assertions are confirmed by other studies from literature [14, 15].

6. Conclusions

The harvesting time is an important factor when a higher content of oil is desired. The amount of oil content in *Mamaia* grape seeds increases when the harvesting moment is delayed (second part of September). The results obtained through this study could be applied also to other grape varieties.

Even though the total amount of fatty acids identified and quantitatively measured in *Mamaia* grape seed oil samples are similar, their ratio is different according with the climatic conditions during ripening. In the years with high temperature and high precipitation during ripening period, an increasing of MUFA (mono-unsaturated fatty acid) content was observed. In the dry years, the PUFA (poly-unsaturated fatty acid) content is higher and the SFA (saturated fatty acid) content is lower making the oil better due to the enhancement of the antioxidant effect.

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