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NMR FINGERPRINT TO CLASSIFY SPANISH OLIVE OIL VARIETIES

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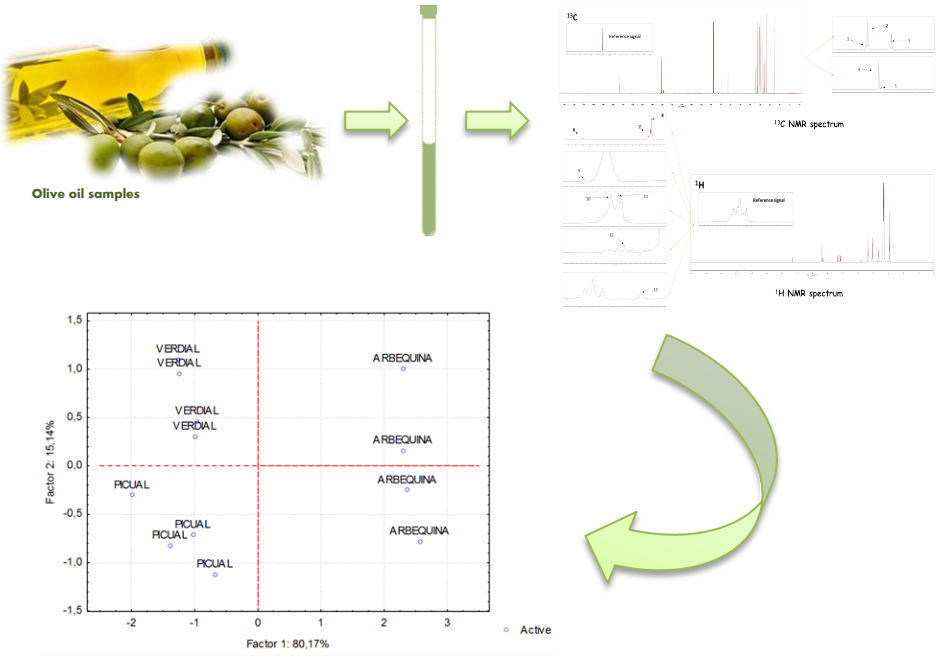
Abstract:
Traditionally the analysis of vegetable oils has been carried out by classic procedures such as gas chromatography, which requires previous derivatization of the sample. This involves the consumption of reagents and, sometimes, the composition of the sample could be altered with the corresponding error in the measurement. Therefore, it is desirable to use faster and environmental friendly techniques that do not require manipulation of the sample. In this sense, the spectroscopic techniques are an alternative. Specifically, nuclear magnetic resonance (NMR) spectroscopy can be used in the field of olive oil to measure some of its characteristics (eg fatty acids, phenols, hydrocarbons, etc.).
The aim of this work have been to study the difference between samples of extra virgin olive oil of different varieties (Picual, Arbequina and Verdial), based on the information provided by the ^{1}H and ^{13}C spectra. The chemical shifts of the spectra were referenced with respect to tetramethylsilane, using the deuterated solvent signal as internal reference.
The data obtained have been treated by multivariate statistical analysis procedures (Multiple Analysis of Variance (MANOVA) and Principal Component Analysis (PCA)) to establish relations that have been interpreted from a chemical point of view and have allowed the discrimination of samples according to their variety.

Keywords: *NMR, olive oil, variety, Multivariate analysis*

Graphical Abstract:



Introduction: Extra virgin olive oil (EVOO) represents one of the most important foodstuffs of the mediterranean diet, and is widely appreciated both for its nutritional and sensory properties (odour and taste). Such attributes depend on agronomic and climatic conditions, extraction and refining procedures, storage conditions, type of olive oil (extra virgin, virgin, refined, etc) and different genetic variety of the olives used to produce the oil. Along the last years, the consumption of EVOOs has increased considerably in relation to the consumption of virgin and refined olive oils. Owing to its distinctive and peculiar intense taste, EVOOs obtained from some pure genetic varieties are highly appreciated.

The aim of this work has been to study the differences between 12 samples of extra virgin olive oil of different varieties (picual, arbequina and verdial) from the Huelva province, based on the information provided by the ^{1}H and ^{13}C spectra.

Materials and Methods:

Samples

Twelve extra virgin olive oil samples of three different varieties, Arbequina (n=4), Picual (n=4) and Verdial (n=4) from different mills were selected in order to perform a preliminary study.

NMR methods

^{1}H NMR analyses were performed on a Varian Mercury instrument equipped with a 5 mm probe operating at the ^{1}H frequency of 500 MHz (Bo = 11.7 T). Olive oil samples (30 mg) were dissolved in $CDCl_3$ (0.5 mL) directly in the 5 mm NMR tube. The ^{1}H NMR spectra were acquired using the following conditions: number of scans 1024; relaxation delay 21 s; spectral width 18.0 ppm; the temperature of the sample in the probe was set at 300 K. ^{1}H NMR spectra were obtained by the Fourier transformation (FT) of the FID (free induction decay), applying an exponential multiplication with a line-broadening factor of 0.3 Hz and a zero filling (Size = 64 K) procedure. The time for the analysis was 120 min. The resulting ^{1}H NMR spectra were manually phased. Chemical shifts were reported with respect to the residual $CHCl_3$ signal set at 7.26 ppm. For ensuring a better quantitative comparability of the spectra, the baseline was corrected using a multi-point correction. In order to perform the statistical analyses, the intensities of the signals, see Table 1, were measured. The intensities of the selected signals were compared with that of the resonance at 2.25 ppm (reference ^{1}H signal in Fig. 1) due to methylenic protons bound to C2 normalized to 1000.

^{13}C NMR analyses were performed on a Varian Mercury instrument equipped with a 5 mm probe

operating at the ^{13}C frequency of 500 MHz. Olive oil samples (100 mg) were dissolved in $CDCl_3$ (0.5 mL) directly in the 5 mm NMR tube. The ^{13}C NMR spectra were acquired using the following conditions: number of scans 15000; relaxation delay 20 s; spectral width 230.0 ppm; the temperature of the sample in the probe was set at 300 K. 13C NMR spectra were obtained by the Fourier transformation (FT) of the FID (free induction decay), applying a Gaussian multiplication with a negative line-broadening factor of 0.1 Hz and a Gaussian position of 0.2 and using a zero filling (Size = 256 K) procedure. The time for the analysis was 18 h. The resulting ^{13}C NMR spectra were manually phased. Chemical shifts were reported with respect to the signal due to α-methylenic protons of the glycerol moiety set at 62.36 ppm. For ensuring a better quantitative comparability of the spectra, the baseline was corrected using a multi-point correction. In order to perform the statistical analyses, the intensities of the signals (Table 1) were measured. The intensities of the selected signals were compared with that of the resonance at 62.36 ppm (reference ^{13}C signal in Fig. 1) due to α-methylenic protons of glycerol moiety normalized to 100.

Results and Discussion:

After the process of sample acquisition, processing and integration of the variables, the statistical treatment was performed in order to discriminate the samples according to their variety. Only four variables (one from ^{13}C spectra and 3 from ^{1}H spectra) showed significant differences ($p < 0.05$) when a multiple analysis of variance was performed.

The Principal Component Analysis obtained using the four variables (5, 8, 9 and 11) selected from the MANOVA permit to discriminate the samples. As expected, samples tend to cluster according to the variety to which they belong. As it can be seen in Figure 2, Factor 1, which explains the 80% of the variance, allows differentiating Verdial and Picual varieties from Arbequina one, while Factor 2, which explains the 15% of the variance, establishes the differentiation between Verdial and Picual varieties.

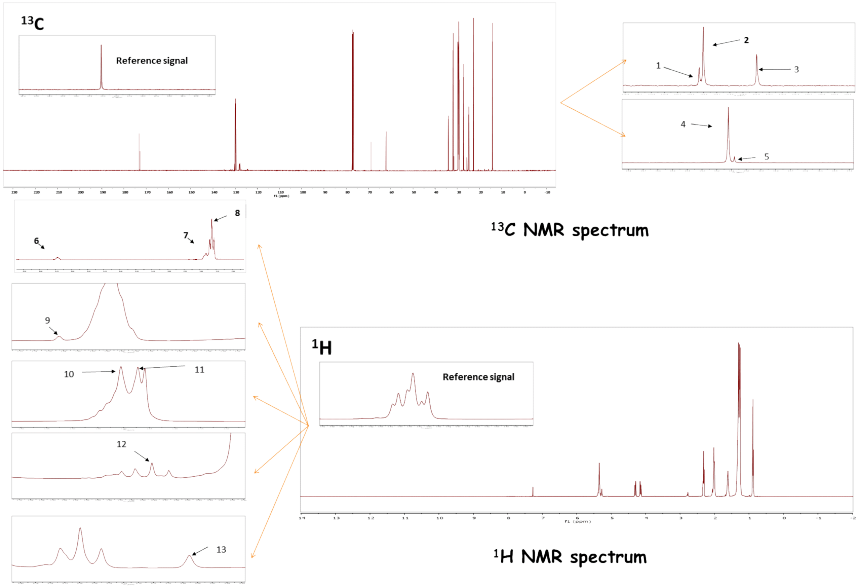


Figure 1.- ^{13}C NMR and ^{1}H NMR spectra

Table 1.- Intensity of 13 NMR resonances of olive oil samples

Peack	NMR resonances (ppm)	Signals	PICUAL OIL				VERDIAL OIL				ARBEQUINA OIL			
			A28	A29	A33	A36	A89	A93	A94	A97	A19	A20	A22	A25
1	173,27	carbonyl signal of sn 1,3 saturated fatty chain	15,54	20,13	21,68	4,64	17,53	16,01	16,12	14,25	24,34	23,49	20,24	21,46
2	173,24	carbonyl signals of sn 1,3 oleic fatty chains	40,04	34,61	34,47	51,22	37,08	40,57	36,85	39,53	31,83	31,43	33,68	31,76
3	172,83	carbonyl signals of sn 2 oleic fatty chains	28,67	27,61	26,64	27,9	25,93	27,18	27,04	26,31	27,01	26,04	26,48	26,8
4	14,12	methyl of oleic fatty chains	157,44	163,88	165,76	158,7	150,98	152,92	155,36	154,75	148,16	49,99	146,09	156,49
5	14,09	methyl of eicosenoic and vaccenic fatty chains	11,7	9,27	12,94	11,31	13,95	16,05	14,07	14,86	23,11	25,61	22,78	22,41
6	3,636	methylenic protons of glycerol moiety sn 1,2 digliceride	17,6	20,3	17,1	19,2	18,44	18,72	18,64	19,25	23,22	22,9	22,5	19,8
7	2,746	diallylic protons of linolenic fatty chains	45,7	49	46,1	47,5	52	53,88	46,51	54,9	43,25	46,6	47	39,8
8	2,71	diallylic protons of linoleic fatty chains	138,4	161,8	154,8	158,3	238,9	244,9	225,8	242,4	433,64	431,3	418	385,7
9	1,62	squalene	103,7	76	69,5	66,6	75	95,6	72,86	90,9	40,31	33,63	40,3	30,7
10	1,244	methylenic protons of all unsaturated fatty chains	11662	14321	12642	13129	13398	12916	13036	13691	12917,22	14598	12517	12196
11	1,197	methylenic protons of palmitic and stearic fatty chains	6563	6744	7067	6726	5830	6159	6264,9	6504	7232	6771	7449	7669
12	0,91	methyl of linolenic fatty chains	35,7	26,5	23,4	23,1	27,47	28,9	26,4	29	22,16	27,1	23,6	23,6
13	0,623	methyl-18 of b-sitosterol	14,6	7	7,5	9	6,89	9,38	9,1	7,8	9,14	11,2	8,2	11,4

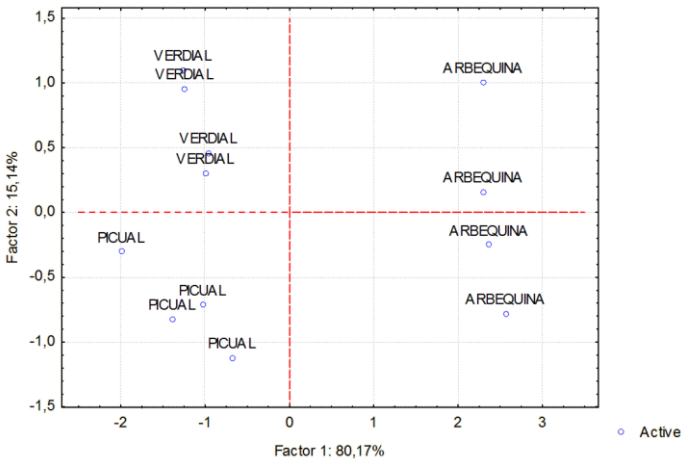


Figure 2.- Principal component analysis graph

Conclusions:

It was verified that the result was satisfactory in a high percentage with the use of a small number of variables, which means that the method used can be considered suitable for the evaluation of olive oil samples.

The acquisition times were high in the case of ^{13}C NMR, although after the statistical study it was observed that the variables that provide the ^{1}H NMR analyses are the ones that better discriminate the olive oil according to their variety, what would reduce the data acquisition time to 120 minutes.

Conflicts of Interest:

The author declares no conflict of interest.

References:

1. Binetti, G.; Del Coco, L.; Ragone, R.; Zelasco, S.; Perri, E. Cultivar classification of Apulian olive oils: Use of artificial neural networks for comparing NMR, NIR and merceological data *Food Chemistry* **2017**, *219*, 131-138.
2. Laincer, F.; Iaccarino, N.; Amato, J.; Pagano, B.; Pagano, A. et al. Characterization of monovarietal extra virgin olive oils from the province of Béjaïa (Algeria). *Food Research International* **2016**, *89*, 1123-1133
3. Girelli, C. R., Del Coco, L. and Fanizzi, F. P. ^{1}H NMR spectroscopy and multivariate analysis as possible tool to assess cultivars, from specific geographical areas, in EVOOs. *European Journal of Lipid Science and Technology* **2016**, *118*, 1380–1388.
4. Ok,S. Fast screening of turkish olive oil by NMR spectroscopy for geographical determination and discrimination purposes. *Grasas y Aceites* **2014,** *65*

5. Longobardi, F.; Ventrella, A.; Napoli, C.; Humpfer, E.; Schütz, B.; et al. Classification of olive oils according to geographical origin by using ^{1}H NMR fingerprinting combined with multivariate analysis. *Food Chemistry*, **2012**, *130*, 177-183.
6. Alonso-Salces, R.M.; Moreno-Rojas, J.M.; Holland, M.V.; Reniero, F.; Heberger, K. Virgin olive oil authentication by multivariate analyses of ^{1}H NMR fingerprints and δ^{13}C and δ^{2}H data. *Journal of Agriculture and Food Chemistry* **2010**, *58*, 5586–5596.
7. G. Vlahov Application of NMR to the study of olive oils. *Progress in Nuclear Magnetic Resonance Spectroscopy* **1999,** *35*, 341–357.