Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2018, Volume 9, Issue 2, Page 466-473

https://doi.org/10.26872/jmes.2018.9.2.50

http://www.jmaterenvironsci.com

Characterization and discrimination of three Moroccan cultivars of virgin olive oil ("Picholine","Menara" and "Hawziya")

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Received 22 Mar 2016,
Revised 25 Mar 2017,
Accepted 30 Mar 2017

Keywords

- ✓ Olive oil,
- ✓ Varieties,
- ✓ Acid value,
- ✓ Gas chromatography,
- ✓ PCA and PLS-DA.

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Abstract

The aim of this study was to characterize the olive oils from three olive varieties most cultivated in Morocco. 36 samples of olive oils representative of three cultivars (Menara, Hawziya and Picholine) were collected and stored at 4°C. A chemical characterization of these samples was carried out by determining free acidity, peroxide value and spectrophotometric index. The same samples were also analyzed by using gas chromatography. Chemometric discrimination was performed by using partial least square discriminant analysis (PLS-DA) carried out on the means of the fatty acid compositions in the samples of virgin olive oil (VOO) and fully correct classification of the three Moroccan cultivars was then obtained.

1. Introduction

Products and by olive products are rich in phenolic compounds considered natural antioxidants [1-2]. The composition of these phenolic compounds depends on the olive cultivar, weather, cultural practices, the storage period and the extraction process of olive oil [3-6].

In Morocco, there is a lack in terms of studies on olive settlements and their products and by-products, including the varietal profile. Thus, the purpose of this work is to compare the performances of three cultivars of olive established in Morocco "Hawziya", "Menara" and "Picholine". In the present study the parameters concerning the quality of the oil such as free acidity, peroxide value and the absorbance in UV spectroscopy at 232 and 270 nm are approached in relation with the content of the oil fatty acid for these three cultivars.

Various works in the world have been invested in the VOO rewarding through analytical methods coupled to chemometrics [7-14]. However, there is still a need for fast and simple routine analytical methods to control the quality of the Moroccan VOO cultivars. In the present work, PCA and PLS-DA methods are coupled to gas chromatography (GC) data to obtain a fast and robust means for this quality control. In fact, gas chromatography as a still not routine analysis technique should be developed in order that laboratories ensure easily access to such a high cost analysis technique presented as a reference method.

The goal of this work was to find a new approach for chemometric classification of three Moroccan VOO cultivars by GC data from olive oil samples. This approach could represent a real novelty in the physico-chemical characterization of the olive oil. GC data were collected from different olive oils varieties: Hawziya, Picholine and Menara, all from the Moroccan region of Benimellal. The first GC exploration, after appropriate data pre-processing procedure, was performed by PCA, allowing a rapid and simple visualisation of the cultivar samples in three classes. The supervised method PLS-DA using the GC data was then applied to achieve the chemometric classification of the unknown samples.

2. Experimental

2.1. Plant material and geographical area

We have ensured the cultivation of olive trees of the studied cultivars in three plots in the Benimellal-Khenifra geographical area in Morocco. Each plot corresponds to one of the three cultivars: "Hawziya", "Menara" and "Picholine". Benimellal-Khenifra area lies in centre of Morocco at the piedmont of Middle Atlas Mountain. The three plots of the olive plantations are in a zone presenting an irrigated soil under a continental weather.

2.2. *Collection of samples of olives*

12 olive samples have been collected from each one of the three plots at the same period of end December 2013. The picking of olives samples has been performed in order to respect the representativeness of the samples within the plot. Each olive sample of 1.5 to 2 Kg was plucked manually in stage of maturity such that the sample contains green olives, reddish and blackish.

2.3. *Extraction of the oil*

Each of the 36 samples of olives was then crushed by a mechanical crusher and then the pulp obtained is placed in a centrifuge set to a rotation of the sample 2000 t/min to separate the solid phase from the oil phase. The obtained oil is stored at 4 °C in the dark until the time of the chemical or spectroscopic analysis.

2.4. *Chemical characterization*

The determination of the acid number and peroxide of oil samples was performed according to the standards of the International Olive Council [15], acidity, expressed as oleic acid (%); peroxide value (PV), which is a measure of the amount of the hydro peroxides (meq O2/kg) due to oxidation, and the UV absorbance at 232 and 270 nm (K232, K270).

2.5. *Gas chromatography*

To determine the fatty acid composition of three varieties, methyl esters of olive oil were prepared in n-heptane (0.12g / 2ml), with a cold solution of KOH (2M) according to standard NF EN ISO 5509. These obtained esters of fatty acids have been analyzed according to NF EN ISO 5508 using a gas chromatograph Agilent Technologies 7890A (GC) equipped with a flame ionization detector (T=250 °C). The column used is a capillary column 60m x 0.25mmx 0.25 µm silica. The pressure of hydrogen, as a carrier gas, was 178 kPa, with a 1:70 ratio. The oven temperature program was as follows: 20 min at 210 °C, 210 to 245 °C at 6 °C/ min and then 10 min at 245 °C. Analyzes were performed in triple and the results are expressed as mean and standard deviations.

2.6. *Chemometric methods*

Classification and exploration among oils from three olive varieties were approached by analyzing gaz chromatographic data using the PCA and PLS-DA methods. The CG data are averages of compositions of fatty acids in the samples of VOO from the three cultivars.

Two chemometric tools were applied in this study: Principal Components Analysis (PCA), and Partial Last Square Discriminate (PLS-DA):

2.6.1 Principal component analysis (PCA)

The PCA allows determining the main features of the CG data, to compare them and to highlight links between the descriptive variables [16]. PCA projects the cloud of points in a representation space of small dimensions. It calculates new variables called principal components that are linear combinations of the starting variables. Since the objective of the analysis is simplification, choose the size of the representation space by making a compromise between two conflicting goals; take a low dimensional space and keep a maximum variance explained.

2.6.2 Partial least square discriminate PLS-DA

The partial least squares discriminate analysis method, PLS-DA, [17] was applied to find what were the variables which better discriminate between different groups of samples from their CG data (X block) according to their maximum covariance with a target class defined in a class pertinence variable (y data block). It attempts to describe whether a CG data of a sample belongs or not to a particular class, consisting of zeros and ones. According to the number of simultaneously regressed y vectors two different PLS-DA approaches are possible. In case of only one class is modelled at a time the method is the ordinary PLS1-DA. When several classes are simultaneously modelled at the same time, the PLS2-DA modified method can be used [18].

The selection of optimal number of components in PCA and of latent variables in PLSR (below) was done from the lowest prediction error in cross validation (leaving-out-one sample at a time) related to the value of PRESSk, the sum of squares prediction error for the model when k factors (components) are included, and from the number of components which give an optimal prediction for the external validation samples not included in the calibration step. The model giving the lowest relative prediction errors in external validation is finally chosen.
In the classification study of this work, PLS2-DA was preferred. All data were processed for the purposes of PLS2-DA by The Unscrambler software, version X (Camo, Norway).

3. Results and discussion

3.1. Physicochemical characterization

Table below reports the results for the acidity, peroxide value and UV absorbance at 232 and 270 nm. The results show that the percentage of acidity of olive oils studied are between 2.1350 and 2.7892 can rank in the category of common virgin olive oils as defined by international standards. Hawziya oil has a higher acid number than those of the Menara and Picholine cultivars, without exceeding the maximum values set by the international standard (International Olive Council 2015). These results are influenced by the maturity of fruit, olive varieties from different origins.

Table 1: Free acidity, peroxide value, K232 and K270 absorbance of virgin oil for the three cultivars "Hawziya", "Menara" and "Picholine"

Variety / Characteristics		Hawziya	Menara	Picholine	IOC standards
Free Acidity (% oleic acid)	Min-Max	2.22-3.21	1.65-3.00	1.54-2.90	≤ 3.3
	Average	2.7892	2.6075	2.1350	
	S.D*	0.3309	0.4587	0.4992	
IP (meq O_2/Kg)	Min-Max	14.75-19.82	11.00-17.46	14.00-18.90	≤ 20
	Average	17.5858	14.9517	16.5158	
	S.D*	1.4660	2.1873	1.6330	
K_{232}	Min-Max	1.90-2.50	2.09-2.44	1.97-2.36	-
	Average	2.2850	2.1975	2.2067	
	S.D*	0.1753	0.1037	0.1200	
K_{270}	Min-Max	0.22-0.25	0.18-0.25	0.16-0.25	≤ 0.30
	Average	0.2408	0.2300	0.1992	
	S.D*	0.0100	0.0200	0.0250	

* S.D: standard deviation

The peroxide value is slightly higher for Hawziya without exceeding the limit set by the international standard (IOC). The values of this index range for the three oils are between 14.9517 and 17.5858 millequivalents of oxygen per kilogram of oil. Some lipid degradation processes are obviously due to the different processes applied to the olives throughout the extraction of oil. Indeed, during the previous steps of oil extraction (gathering, storing olives …), two types of changes may occur, acidification and rancidity which could be the cause of the increase in acid values and peroxide. The UV absorbance K_{232} and K_{270} obtained show that the oil of the cultivar Hawziya was significantly higher than the other two cultivars; it was also the most unsaturated oil. This confirms its slight oxidability. These values indicate that olive oil studied contain only very little self-oxidation by products.

3.2. The fatty acid composition (%) (NMISO5508, NMISO5509)

Figure 1 present a type of the 36 obtained gas chromatograms. The following table 2 gives the majority fatty acids that we have detected in the 36 samples analyzed. The chromatograms of the three varieties have proven no observable difference for extracting the information about the cultivars. The following table 3 reports the results of averages and standard deviation (S.D) of the major fatty acid of each three cultivars. The results obtained are shown in Table 3 followed by Figure 2. The fatty acid composition of the studied oils meets the standards set by the International Olive Council, Indeed all three varieties are rich in oleic acid (C18:1ω9), the rate of this acid in each varieties studied is 74% for Menara, 71% for Hawziya and 70% for Picholine. Furthermore, the percentage of linoleic acid varies between 8.415 % to 11.3391% for the three varieties with a slight predominance in the Picholine oil. These percentages remain slightly above 1% on the standard set by the International Olive Oil Council. It is also interesting to note that the contents of essential fatty acids (linoleic

(18: 2ω6) and linolenic (18:3ω3) contained in the three oils, are sufficient to prevent a state carentiel fatty acid essential in people using these oils as the primary fat in their diet. It should also be noted that the fatty acid composition obtained reveals a predominance of mono-unsaturated fatty acids to the Menara variety.

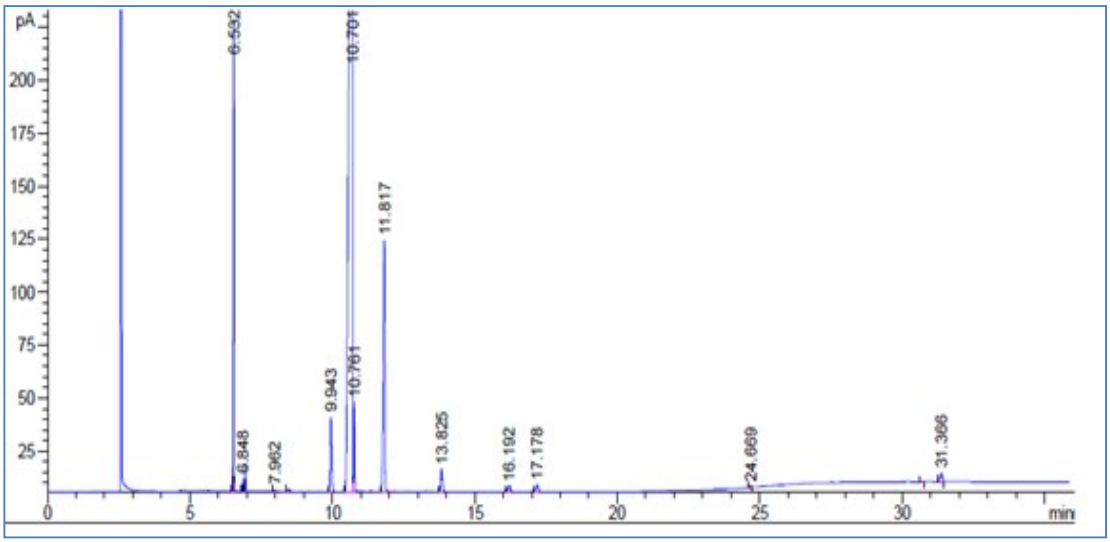


Figure 1: Gas chromatogram of virgin olive oil from the "Picholine" cultivar.

Table 2: Majority fatty acids of the three varieties.

Common name	Palmitic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic	Arachidic	Gondoic
Shorthand	16:0	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	20:0	20:1ω9
Retention time(min)	6.532	9.943	10.701	10.761	11.817	13.825	16.192	17.178

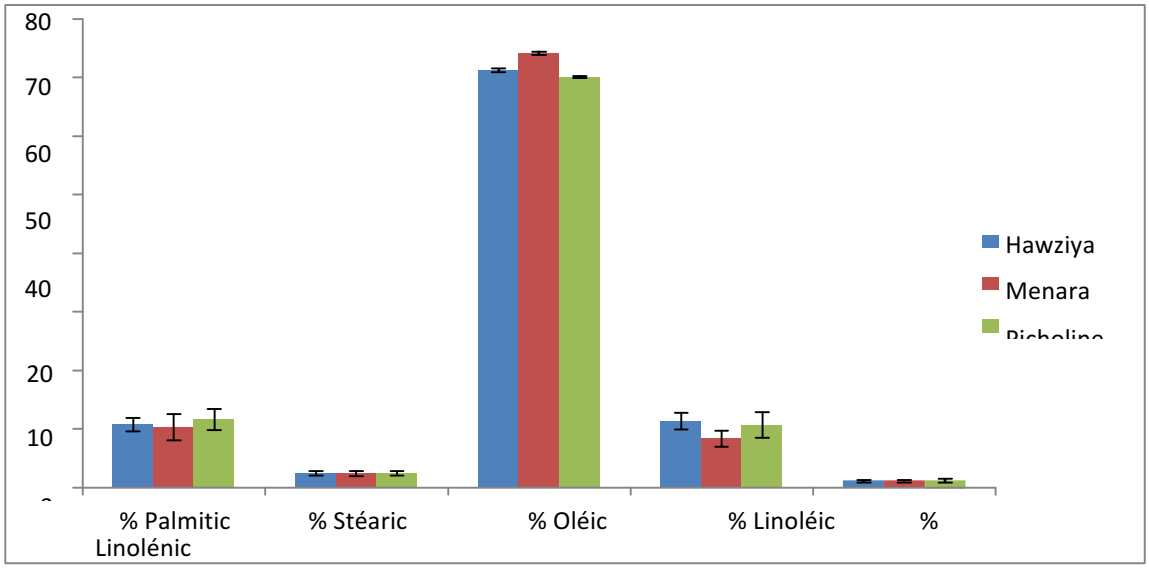


Figure 2: Average grades of the principal fatty acids of olive oil varieties studied

3.3. Chemometric methods:

3.3.1. Principal component analysis (PCA):

Actually, multivariate analysis is an essential chemometric tool to study data coming from observations made on several variables. Its aim is to resume information contained in data with a reduced number of dimensions to characterize as well as possible the differences or similarities between observations and variables.

The gas chromatographic data set of olive oils samples was subjected to the basic tool for data analysis by application of principal component analysis (PCA). This statistical method is very important especially in the preliminary steps of a multivariate analysis to perform an exploratory analysis in order to have an overview of data. It allows describing data set without a priori knowledge of the data structure.

Table 3: Weight Percent of fatty acids in virgin olive oil, for each of the three cultivars "Hawziya", "Menara" and "Picholine"

Variety / acid		Hawziya	Menara	Picholine	IOC standards
%Myristic (C14:0)	Min-Max	0.02-0.23	0.02-0.24	0.02-0.23	≤0.03
	Average	0.0825	0.0825	0.0883	
	S.D*	0.0784	0.0805	0.0767	
% Palmitic (C16:0)	Min-Max	9-12.38	6.11-13.22	9.64-15.22	7.5 - 20.0
	Average	**10.7716**	**10.3**	**11.6733**	
	S.D*	1.1889	2.2435	1.8158	
% Palmitoléic (C16 :1)	Min-Max	0.66-2.01	0.66-3.98	0.66-3.98	0.3 - 3.5
	Average	1.065	1.3683	1.5875	
	S.D*	0.5196	1.1821	1.3056	
%Heptadécanoic (C17:0)	Min-Max	0.04-3.57	0.04-3.87	0.04-3.57	≤ 0.30
	Average	0.7741	0.9108	1.0691	
	S.D*	1.0245	1.3231	1.3296	
% Heptadécénoic (C17 :1)	Min-Max	0.05-0.82	0.05-0.82	0.05-0.82	≤ 0.30
	Average	0.33	0.4241	0.3283	
	S.D*	0.3040	0.2973	0.3056	
% Stéaric (C18:0)	Min-Max	2.06-2.98	1.42-2.98	2.06-2.98	0.5 - 5.0
	Average	**2.465**	**2.4358**	**2.4283**	
	S.D*	0.3214	0.4386	0.3427	
% Oléic (C18 :1)	Min-Max	70.95-71.77	73.77-74.66	69.95-70.46	55.0 - 83.0
	Average	**71.2475**	**74.1583**	**70.0733**	
	S.D*	0.3133	0.2890	0.1570	
% Linoléic (C18 :2)	Min-Max	10.01-14.17	7.09-11.17	8.01-14.17	2.5 - 21.0
	Average	**11.3391**	**8.415**	**10.7033**	
	S.D*	1.4188	1.3794	2.2224	
%Linolénic (C18 :3)	Min-Max	1.01-1.56	0.58-1.56	0.87-1.99	≤ 1.00
	Average	1.1283	1.12	1.1941	
	S.D*	0.1700	0.2473	0.3101	
%Arachidic (C20:0)	Min-Max	0.21-0.6	0.11-0.6	0.21-0.6	≤ 0.6
	Average	0.4116	0.3858	0.4216	
	S.D*	0.1029	0.1354	0.1007	
%Gadoléic (C20 :1)	Min-Max	0.21-0.38	0.19-0.39	0.2-0.38	≤0.4
	Average	0.3083	0.3008	0.3066	
	S.D*	0.0590	0.0693	0.0663	

After analyzing of 36 samples of olive oils, Principal Component Analysis was applied to the first data set of 36 classification samples exploring the full acquired data.

PCA was used to reduce the data dimensionality in order to obtain a better visualization of the separation in groups according to the varieties. The PCA model with one component already explained 100% of the total data variance (PC1 captured 100% and PC2 captured 0% of the variance respectively). PC1 vs. PC2 scores plot of the spectra of the first data set given in Figure 3, distinguished three major clusters of samples (Picholine, Menara and Hawziya). The rapprochement between the samples of Picholine and Hawziya is probably due to geographical and climatic rapprochement of these two areas. The projection of individuals in the plane generated by the axis 1 and 2, showed the distribution of olives oils in three main groups (Picholine, Hawziya and Menara).

3.3.2 Partial least square discriminate PLS-DA:

3.3.2.1 Calibration

The set of 36 samples were divided into two subsets randomly using the Kennard–Stone algorithm (Kennard and Stone 1969). PLS2-DA was used to build a calibration model using the first subset of 24 samples. External model validation was performed using the second subset of 12 new olive oil samples (not used for the model

calibration). This PLS2-DA model has been built considering the GC data as X variables, while the Y variables have been associated with the three different cultivars (one different y variable for each cultivar, with 1 or 0 depending on whether it belongs or not to the considered data group). The model obtained in this way has been able to discriminate among the three cultivars (Picholine, Hawziya and Menara), as it can be seen from the PLS2-DA scores plot in Figure 4.

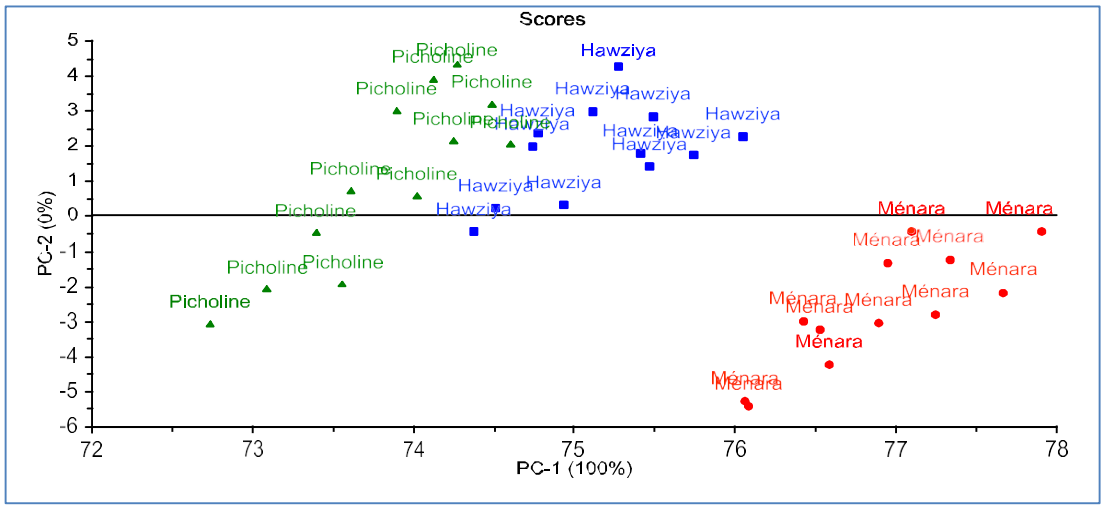
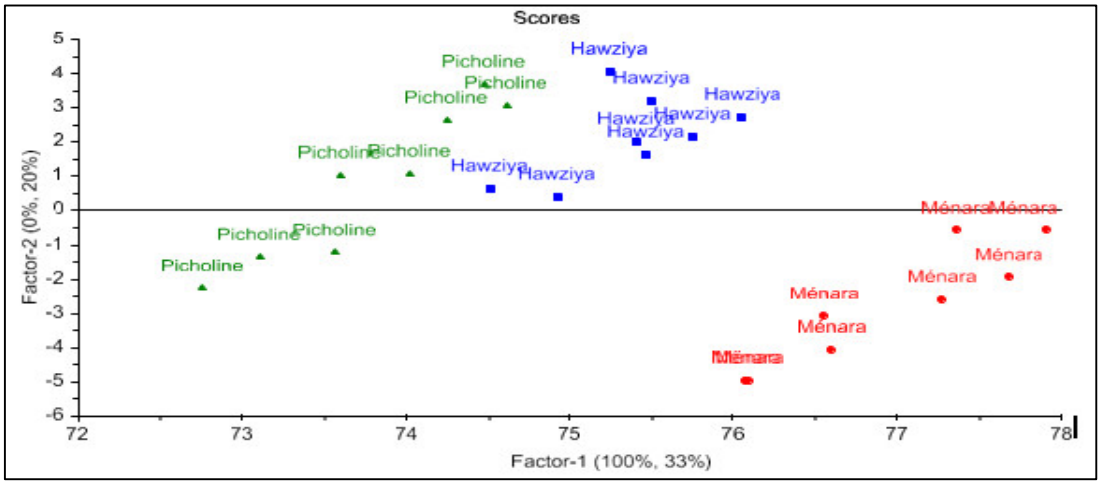
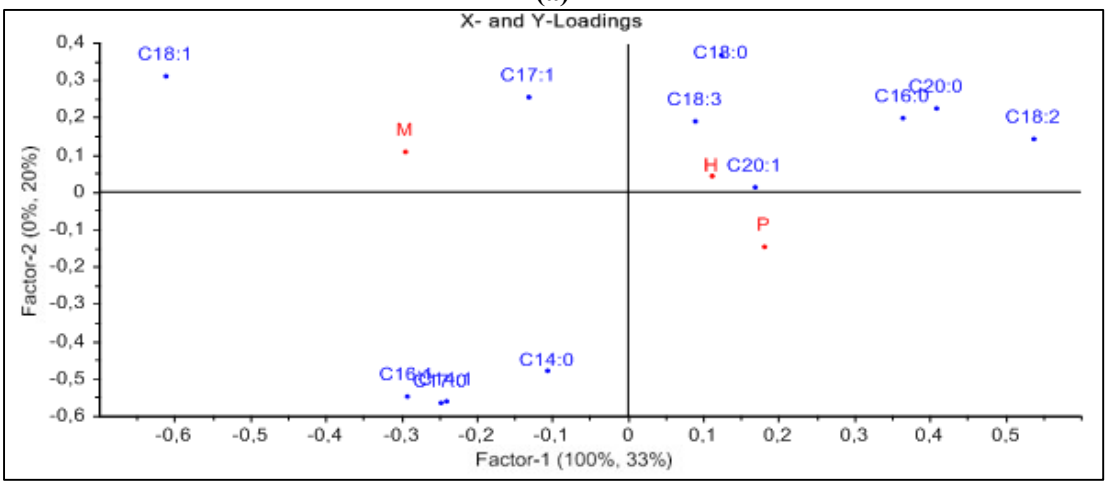


Figure 3: Scores plot of different cultivars of oils for PCA by using 2 PCs



(a)



(b)

Figure 4: PLS2-DA scores plot (LV1 versus LV2) in the analysis of the GC data.

The calibration model was first validated by internal full cross-validation. Comparison between different models was done considering some figures of merit such as R^2, root mean square error of calibration (RMSEC), and

root mean square error of cross-validation (RMSECV). Figure 4 presents the obtained PLS2-DA scores and loading plots (LV1 vs LV2) in the analysis of the GC data.
The PLS2-DA model for GC data needed two latent variables and explained 53 % of the variance in Y block with 100 % of the information GC data (X matrix). Figure 4 shows the score distribution for the three cultivars of VOO. The obtained model was able to distinguish satisfactory the three cultivars (hawziya, picholine and menara), as it can be seen from the PLS2-DA score plot (figure 4a).
Figure 4b presents the distribution of the all fatty acids parameter loadings in the space spanned by LV1 and LV2. LV1 discriminates between the C18:0, C18:2, C18:3, C20:0, C20:1and C16:0 (Positive loadings on the right side on the plot) and the rest of investigated fatty acids (on the left side on the plot with negative loadings). It was possible to conclude that Linoleic, Linolenic, Stearic and Palmitic acids are able to differentiate the VOO cultivars. Picholine and Hawziya were richer on this four fatty acids and show less acid oleic index than the variety of Menara. Figures of merit obtained by PLS2-DA model of CG data using the calibration sample subset are given in Table 4. In this case, high correlations between measured and predicted Cultivars (R^2 was around 0.962 and 0.989 in all cases) and low prediction errors (RMSEC ranging between 0.059 and 0.098) were observed.

Table 4: Figures of merit achieved by PLS2-DA discrimination of the three cultivars of virgin olive oil samples.

Classes[a]	Figures of merit[b]			
	R^2c		RMSEC	RMSECv
	Calibration	validation		
Classe 1 (Hawziya)	0.974	0.965	0.065	0.073
Classe 2 (Picholine)	0.962	0.954	0.098	0.106
Classe 3 (Menara)	0.989	0.977	0.059	0.063

[a] Investigated classes by PLS-DA.
[b] Reported model figures of merit: R^2c – R-square in calibration; RMSEC-Root Mean Squared Error in Calibration; RMSECv-Root Mean Squared Error in cross validation.

3.3.2.2 *Validation: Predicting cultivar of New Oil Samples*
The predictive ability of PLS2-DA model using CG data was tested on 12 new samples, not used in the calibration step. These include four samples from Picholine virgin olive oil, four samples from Menara virgin olive oil, and four samples with Hawziya virgin olive oil. The PLS-DA assigns an oil sample to a particular oil classes if the predicted value is comprised between 0.5 and 1.5 for that class. Table 4 shows the classification results with the comparison between known and predicted values for the three olive oil cultivars
Table 5 shows that all samples from Picholine, Menara, and Hawziya for the validation data set were correctly classified.

Table 5: Prediction of VOO cultivar by chemometric analysis of CG data in the external validation

	Classe 1:Hawziya		Classe 2:Picholine		Classe 3:Menara	
Samples	Y-Pred	Y-Ref	Y-Pred	Y-Ref	Y-Pred	Y-Ref
H09	1.023	1	0.101	0	0.096	0
H10	0.738	1	0.054	0	-0.054	0
H11	0.941	1	0.061	0	-0.067	0
H12	0.721	1	0.021	0	0.017	0
M09	0.133	0	-0.028	0	0.934	1
M10	-0.09	0	-0.157	0	0.863	1
M11	-0.109	0	0.199	0	0.927	1
M12	-0.062	0	0.127	0	1.017	1
P09	0.202	0	0.871	1	0.069	0
P10	0.048	0	0.922	1	0.042	0
P11	0.023	0	1.003	1	-0.065	0

P12	0.094	0	0.953	1	0.001	0

This means that a 100 % accurate classification was achieved, i.e., all oil olive samples of the validation data set matched correctly to the three corresponding Classes. PLS2-DA predicted values were always very close to 0 or 1. These results confirm that the predictive ability of the developed PLS2-DA model was satisfactory good. Therefore, it was concluded again that the gas chromatographic data coupled with the PLS2-DA chemometric method could be successfully used to discriminate VOO cultivars.

Conclusions

According to the results reported, it can be concluded that GC followed by chemometric treatment of the data, namely principal component analysis, partial least squares regression discriminant analysis, was an appropriate and powerful technique that can be useful in the indirect qualification of different cultivars. Discriminant analysis allows the classification of VOO made from Picholine, Menara and Hawziya cultivars using their GC data. High values of R^2 and low values of RMSEC and RMSECV were obtained for all analytical parameters studied. This study demonstrates the great potential of the application of chemometric tools in gas chromatography for the correct classification of food.

Acknowledgments-The authors wish to thank the national center of scientific and technical research for the financial support and to acknowledge University of Sultan Moulay Slimane for providing the facilities for this research.

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