# Characterization and Discrimination of Virgin Olive Oils from Different Moroccan Geographical Areas Using MIR Spectroscopy Coupled to Chemometric Methods

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**Abstract:** *A methodology based on Mid-infrared (MIR) spectroscopy, combined with multivariate analysis methods, was applied in order to Discriminate and characterize virgin olive oils from four Moroccan geographical areas. 49 Samples of virgin olive oil were collected in November 2013 from mills in the Tadla-Azilal Moroccan region then stored at 4°C. The characterization of the olive oils was made determining several variables: the free acidity, the peroxide value, spectrophotometric index, carotenoids, and chlorophylls. The same samples were also analyzed busing Infrared spectroscopy. Classification methods (PCA and PLS-DA) were applied on the spectral data to classify olive oils according to their geographical areas. The results were quite satisfactory, in spite of the similarity of cultivar compositions between four different origins. The spectroscopic methods, combined to chemometric strategies, could represent a reliable, cheap and fast classification tool.*

**Keywords:** Moroccan olive oil, spectroscopic methods, MIR, Chemometric treatment, PCA.PLS-DA, Valorization, classification.

## 1. Introduction

Extra virgin olive oil (EVOO) is a vegetable oil made from healthy and intact fruits of the olive trees (Olea europaea L.) only by mechanical means (crushing, malaxation and centrifugation) and can be directly consumed by humans without refining. As no chemicals are used in this extraction process, the EVOO keeps the original characteristics and constituents which are lost in refined oils [1]. EVOO is one of the most significant food products in Mediterranean countries, and the olive tree is among the oldest and most important oil-producing crops like the oil palm [2].The high demand for olive oil is associated with the Mediterranean culture based on dietary habits correlated with health benefits[3].This has been correlated with the presence of high content of monounsaturated fatty acids, specifically oleic acid (60–80%) and its richness in minor components, including tocopherols and phenolic compounds, that other seed oils lack [4]. These phenolic compounds have a great importance in biological systems once they act as natural antioxidants [5]. Extra virgin olive oil composition determines its intrinsic quality and could be influenced by several factors. Cultivar, environment and horticultural techniques affect the fruit physiology [6]. Other factors as latitude, climatic conditions, irrigation regime, fruit ripening, harvesting and extraction technologies influence the qualitative profile of olive [7]. The effect of geographic origin and of its interaction with the environment on the

qualitative profile and the oxidative stability of extra virgin olive oil have been studied by determining the acidity index of saturated and unsaturated fatty acids, triglycerides, diacylglycerols and triacylglycerols, sterols, phenolic compounds, hydro-carbons, pigments and volatile components. These compounds differ according to the fruit variety [8]. Traditionally, these parameters have been estimated by classical analytical methods, which are frequently based on gas chromatography (GC) and high-performance liquid chromatography (HPLC). In the last few years, attention has been focused on authentication for genetic varieties of olive oils using nuclear magnetic resonance (NMR) [9]. Nevertheless, all these methods have several draw backs, the most significant of which are low speed, high cost, and the necessity of sample pre-treatments and of highly-skilled personnel. Mid-Infrared spectroscopy, combined to multivariate data analysis, has proved to be a successful analytical method for quantitative and qualitative mode-ling of a wide variety of food and food process. These techniques facilitate real-time measurements at all stages of production, and they offer a fast, non destructive and cost effective method of food analysis [10]. Recent applications of MIR spectroscopy in edible oil analysis, reported in literature, include quality parameter determination [11] and adulteration detection [12]. These methods have been successfully applied in authentication studies on olive oil on the basis of geographical origin [13].

The aim of this paper has been to investigate the potential of MIR spectroscopy, combined to chemometric data analysis, to classify monovarietal Moroccan olive oils (*picholine*) on the basis of the geographical origin. In conjunction with this, traditional physico-chemicals characteristics were evaluated in order to verify the feasibility of MIR as a rapid and non-invasive method to classify olive oils.

## 2. Experimental

### 2.1 Sampling

49 samples of virgin olive oils were collected for this study. The different samples were collected in November 2013, specifically in four areas in the Tadla-Azilal region in the centre of Morocco (Fkih Ben saleh, Zouit Cheikh, Béni Mellal and Tagzirt). All Samples were stored in the dark at 4°C until analysis.

### 2.2 Physicochemical Olive Oil Analysis

According to official methods of the European Regulation/Commission EEC no. 1989/2003 (E.C. Reg. 1989/2003) [14], several variables were measured: the free acidity, expressed as oleic acid (%); the peroxide value (PV), which is a measure of the amount of the hydroperoxides (meqO$_2$/kg) due to oxidation; the spectrophotometric index, the UV absorbance at 232 and 270 nm (K232, K270), the carotenoid and chlorophyll determination was carried out using a spectrophotometer (Mod. 7800 with plotter PTL 396, Jasco), after the same procedure described by Tura et al., 2007 [14]. The results have been expressed in mg of β-carotene or chlorophyll per kilogram of oil.

### 2.3 MIR Spectroscopy

Spectra are obtained using vector 70 Bruker equipped with a heated ''Golden Gate'' single reflection ATR module, a DL, a TGS detector and a KBr beam spliter. The equipment is connected to computer and controlled by Win First Software – v1.1. Spectra are scanned in the absorbance mode from 4000 to 600 cm$^{-1}$. Analyses are carried out at room temperature. These spectra were recorded as absorbance values at each data point. Each sample measurement was repeated two times and the spectra averaged.

### 2.4 Chemometric Methods

a- Principal component analysis (PCA):

PCA allows determining the main features of the spectra, to compare them and to highlight links between the descriptive variables (the absorbance at different wavelengths) [15]. 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 absorbance. 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 explained variance. The selection of optimal number of components in PCA and of latent variables in PLSR is done using the lowest prediction error in cross validation (leaving-out-one sample at a time) related to the PRESSk, the sum of squares prediction error for the model which includes k factors (components), and optimal prediction of y values 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.

b- Partial least squares discrimination PLS-DA

The partial least squares discriminate analysis method, PLS-DA [16] usually is applied. This technique finds the components or latent variables which discriminate as much as possible between two different groups of samples from their FT-IR spectra (X block) according to their maximum covariance with a target class defined in the y data block. It attempts to describe whether a spectrum 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 modeled at a time the method is the ordinary PLS1-DA. When several classes are simultaneously modeled at the same time, the PLS2-DA modified method can be used [17]. For the classification study (Study A) in this work PLS2-DA, was used. All data (Physicochemical analysis and FTIR spectra) acquired were developed by the Unscrambler software version X (Camo, Norway) for the purposes of PCA and PLS2-D algorithms.

## 3. Results and Discussion

### 3.1 Chemical Analysis

As shown in Table1

**Free Acidity**: in the samples analyzed, there was a small variation of free acidity between the studied varieties. This can be explained by the good harvesting practices (choice of early maturity index, healthy fruit) and crushing, most of the oils were classed "virgin", after their acid content.

**Peroxide Values**: The low values of the peroxide recorded explain the best practices used in the crushing units such as: vibrators for harvesting olives, allowing to have healthy olives without lesions, extraction before storage period does not exceed 24 hours, the extraction conditions that meets quality requirements .

**Spectrophotometer index:** the UV absorbance K232 and K270 absorbance values are respecting the extent permitted by the COI standards for the classification of oils in the "Virgin" olive oils class (IOC, 2013).

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**Table 1:** Chemical analysis: Acidity, Peroxide Value, K232, K270, Total carotenoids and total chlorophylls

| | | *Monovarietal Oïl (N=49)* | | | |
|---|---|---|---|---|---|
| | | *BM* | *Zw* | *TZ* | *FBS* |
| *Acidity (%AO)* | Rang | 1. 14-2. 54 | 1. 06-2.46 | 1.23-2.30 | 1.46-2.12 |
| | Mean | 1.83 | 1.65 | 1.7 | 1. 82 |
| | S.D | 0.51 | 0.38 | 0.33 | 0.21 |
| *PV (mg/Kg)* | Rang | 3.22-13.72 | 3.27-13.33 | 3.27-11.94 | 4.73-11.33 |
| | Mean | 8.7 | 10.11 | 8.79 | 7.81 |
| | S.D | 3.52 | 2.62 | 2.66 | 2.38 |
| *K232* | Rang | 1.02-2.98 | 1.96-2.98 | 2.29-2.95 | 1.87-2.96 |
| | Mean | 2.63 | 2.49 | 2.67 | 2.68 |
| | S.D | 0.53 | 0.38 | 0.18 | 0.3 |
| *K270* | Rang | 0.3-1.14 | 0.13-1.14 | 0.16-0.67 | 0.17-0.62 |
| | Mean | 0.24 | 0.46 | 0.34 | 0.28 |
| | S.D | 0.12 | 0.28 | 0.2 | 0.13 |
| *Total carotenoids (mg/kg)* | Rang | 3.42-7.45 | 3.42-6.26 | 3.63-4.82 | 3.81-5.51 |
| | Mean | 4.78 | 4.16 | 4.07 | 4.6 |
| | S.D | 1.07 | 0.75 | 0.44 | 0.48 |
| *Total Chlorophylls* | Rang | 4.40-11.10 | 4.17-9.87 | 4.69-8.03 | 5.28-8.07 |
| | Mean | 7.28 | 6.09 | 5.91 | 6.84 |
| | S.D | 2.13 | 1.63 | 1.09 | 1.02 |

**Carotenoid and Chlorophylls Determination**: The observed levels of pigments for all studied samples are in the range of [0-20ppm]. The olives are characterized by a m2significant reduction in chlorophyll and to a lesser extent of the carotenoids with the evolution of the mature.

**3.2 Means Infrared (MIR) Spectra**
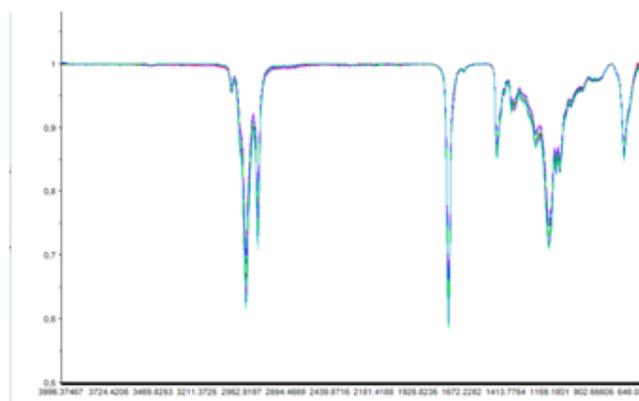


**Figure 1:** MIR spectra of the virgin olive oil samples in the 4000-600 cm$^{-1}$ spectral range

Fig. 1 shows MIR spectra of monovarietal olive oil respectively. The spectra did not evidence an obvious difference from visual inspection according to the geographic origin. The MIR spectra are dominated by some peaks at 2924, 2852, 1743, 1463, 1377, 1238, 1163, 1114, 1099 and 721 cm$^{-1}$. Absorbance at 2924 and 2852 cm$^{-1}$ are due to bands arising from $CH_2$ stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1743 cm$^{-1}$ arises from C=O stretching vibrations. In particular this peak is associated with the triglyceride ester-linkage (COOR) band and the C=O absorption of free fatty acid present in olive oil.

The band at 1460 cm$^{-1}$ arises from asymmetric stretching in methyl and methylene groups, while the peak at 1160 cm$^{-1}$ is associated with the stretching of the C–O bonds of aliphatic esters [18] [19].

**3.3 Chemometric Methods**

a- Principal component analysis (PCA):

After analyzing of 49 samples of olive oils, Principal Component Analysis with full cross validation was applied to the first data set of 20 classification samples exploring the full acquired data.

The PCA model with two components already explained 99% of the total data variance (PC1 captured 100% and PC2 captured 1% of the variance respectively). PC1 vs. PC2 scores plot of the spectra of the first data set given in Figure 2, distinguished four major clusters of samples according to conformity FBS: Fkih Ben Saleh area/ ZW: Zawit Cheikh area / TZ: Tagzirt area and BM: Béni Mellal. The rapprochement between the samples of TZ and ZW is probably due to geographical and climatic rapprochement of these two areas.

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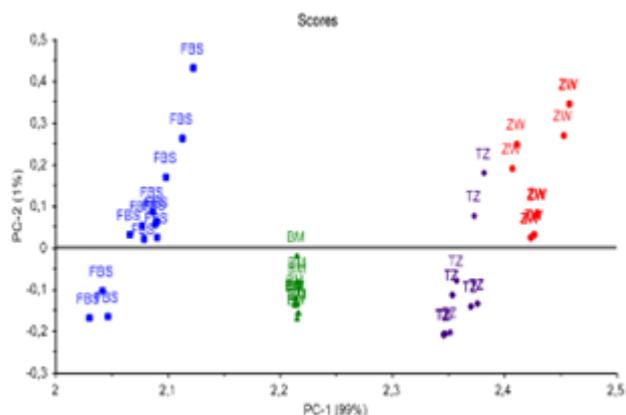
**Figure 2:** PCA scores plot (PC1 vs. PC2) in the analysis of the MIR spectra

b- Partial least squares discrimination PLS-DA

This PLS2-DA model has been built considering the MIR spectra as X variables, while the Y variables have been associated with the four different areas (one different y variable for each region, 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 four areas (BM, ZW, FBS and TZ), as it can be seen from the PLS2-DA scores plot in Figure 3.
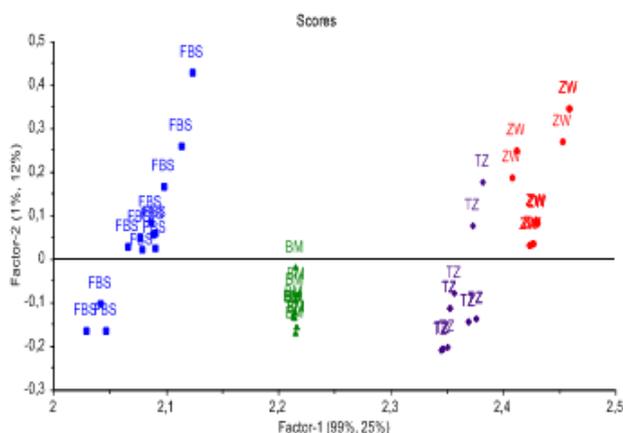


**Figure 3:** PLS2-DA scores plot (LV1 versus LV2) in the analysis of the MIR spectra.

These calibration models were first validated by internal full cross validation. Comparison between different models was done considering some figures of merit such as R2, RMSEC and RMSECV. The obtained model was able to distinguish satisfactory the four geographical origins (TG,ZW, FBS and BM), as it can be seen from the PLS2-DA scores plot in Figure 3, where the four groups of samples are more distinguishable. The first latent variable, LV1, explains 25 % of Y variance with 99 % of X variance, discriminates among the four origins. The second latent variable explains a rather small percentage of

variance of Y (12%) and also a small amount of variance in X (1%).

**Table 2:** Statistical parameters by chemometric elaboration of olive oils MIR spectra in calibration and internal validation step

| Classes [a] | Figure of merit [b] | | |
|---|---|---|---|
| | $Rc^2$ | RMEC | RMECV |
| BM | 0.904 | 0.140 | 0.193 |
| ZW | 0.974 | 0,082 | 0.147 |
| TZ | 0.945 | 0,082 | 0,147 |
| FBS | 0,984 | 0,063 | 0,115 |

[a] Investigated classes by PLS-DA.
[b] Reported model figures of merit: R2c – R-square in calibration; RMSEC-Root Mean Squared Error in Calibration; RMSECV-Root Mean Squared Error in cross validation.

**Table 2** shows the calculated figures of merit of the results obtained by the PLS2-DA model using the calibration samples. A correlation between measured and predicted classes (R2 around 0.96 and 0.98 in all cases) and low prediction errors (RMSEC between 0.049 and 0.08) were obtained.

c- Predicting geographical origin of new samples

The predictive ability of PLS2-DA model using MIR data was tested on 14 new samples, not used in the calibration step. These include four samples from TAGZIRT, three samples from Zawit Cheikh, three samples from BENI MELLAL and four samples from FEKIH BEN SALAH. 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 3 shows the classification results with the comparison between known and predicted values for the four origins.

**Table 3** shows that all samples from TZ, FBS, ZW and BM for the validation data set were correctly classified. This means that a 100% accurate classification was achieved, i.e. all oil olive spectra of the validation data set matched correctly to the four 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 has been satisfactory. Therefore, it has been concluded again that MIR spectroscopy coupled to the PLS2-DA chemometric method could be successfully used to discriminate olive oils origins.

**Table 3:** Prediction of olive oil regions by chemometric analysis of MIR spectra

| Samples | Class 1: ZW | | Class 2: BM | | Class 3: TZ | | Class 4 : FBS | |
|---|---|---|---|---|---|---|---|---|
| | y-predicted | y-reference | y-predicted | y-reference | y-predicted | y-reference | y-predicted | y-reference |
| TZ | 0.0854 | 0.000 | -0.1098 | 0.000 | **0.9621** | **1.000** | 0.0660 | 0.000 |
| TZ | 0.0792 | 0.000 | -0.1055 | 0.000 | **0.9364** | **1.000** | 0.0925 | 0.000 |
| TZ | 0.0607 | 0.000 | -0.0950 | 0.000 | **0.9727** | **1.000** | 0.0637 | 0.000 |
| TZ | -0.0320 | 0.000 | 0.0076 | 0.000 | **1.0800** | **1.000** | -0.0619 | 0.000 |
| ZW | **1.0185** | **1.000** | 0.0566 | 0.000 | -0.0138 | 0.000 | -0.0649 | 0.000 |
| ZW | **1.0448** | **1.000** | 0.0519 | 0.000 | -0.0575 | 0.000 | -0,0417 | 0.000 |
| ZW | **1.0448** | **1.000** | 0.0519 | 0.000 | -0.0575 | 0.000 | -0.0417 | 0.000 |
| FBS | 0.0676 | 0.000 | 0.2555 | 0.000 | 0.0859 | 0.000 | **0.5429** | **1.000** |
| M2FBS | 0.0934 | 0.000 | -0.0930 | 0.000 | -0.0327 | 0.000 | **1.0322** | **1.000** |
| FBS | -0.1256 | 0.000 | 0.1798 | 0.000 | 0.0567 | 0.000 | **0,8922** | **1.000** |
| FBS | -0.0383 | 0.000 | -0.0489 | 0.000 | 0.1252 | 0.000 | **0.9595** | **1.000** |
| BM | -0.0045 | 0.000 | **0.8558** | **1.000** | 0.0316 | 0.000 | 0.1263 | 0.000 |
| BM | 0.0015 | 0.000 | **0.8311** | **1.000** | 0.0246 | 0.000 | 0.1522 | 0.000 |
| BM | 0.0035 | 0.000 | **0.8672** | **1.000** | 0.0066 | 0.000 | 0.1305 | 0.000 |

## 4. Conclusion

The discrimination between olive oils from four Moroccans geographical areas was performed by a chemometric modeling procedure using the MIR data recorded by analysis of olive oils. Each area was identified on the basis of different spectral information spread along the selected 4000-600 cm$^{-1}$ spectral range. MIR analysis has proved to be rapid and simple, requiring no chemical pre-treatment of the samples. In fact, the infrared spectrum is able to give a complete called ''finger print'' of olive oil, which contains its intrinsic quality influenced by several factors such as environment, horticultural techniques and cultivar origin. The application of the PLS-DA algorithm on the samples of a prediction set has allowed a classification with an accuracy of 100%. In conclusion, the spectroscopic methods could represent a reliable, cheap and fast classification tool, able to verify the origin of olive oils and without requiring chemical analyses for discrimination.

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