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Front-Face Fluorescence Using UV-LED Coupled to USB Spectrometer to Discriminate between Virgin Olive Oil from Two Cultivars

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Abstract

A simple setup using a 365 nm LED coupled to a USB spectrometer through an optical fibre, in a front-face fluorescence configuration, was used to investigate the ability of fluorescence spectroscopy technique to discriminate between varieties of olive oil. To achieve this task, Virgin Olive Oils (VOO) from two major Tunisian olive cultivars known as Chetoui and Chemlali were used. Spectral analysis showed a clear separation between these two VOO varieties. A one-way ANOVA attests that this discrimination is significant. The Principal Components Analyses (PCA) showed that the normalized fluorescence intensities are the good parameters for this discrimination. This observation strengthens the potential of our spectral parameters to perform reliable analysis.

Keywords

Chemometrics, Fluorometry, Oils, Olive Oil, PCA, UV/Visible

1. Introduction

The qualitative and quantitative difference between olive oils are mainly due to the specific characteristics of cultivars used [1]. Rightness of analyses which lead

to accurate authentication of olive oil constitutes one of the major challenges in the industry of olive oil since the demand of olive oils is increasing and there is a growing commercial interest in high quality products.

Tunisia is well known for the high quality of its olive oil. This industry depends mainly on two cultivars namely the "Chetoui" which grows in the mountainous regions and the "Chemlali" which is prevalent in the central semi-arid regions and southern arid regions of the country [2] [3]. These two cultivars represent 95% of the plantations and contribute to more than 80% of the national production of olive oil [2].

The Chemlali cultivars cover 2/3 of the plantations and represent approximately 60% of national production of olive oil [2] [3] [4]. The lack of water in arid zones has a negative impact on their quality. However, despite these shortcomings, most of the Chemlali cultivars have ordinary organoleptic composition and a very characteristic taste with high levels of palmitic acids [4] [5]. The Chetoui cultivar represents about 20% of national production [6]. Several studies have shown that this cultivar has a great capacity to adapt to various climatic conditions [5] [6]. The Chetoui cultivar is also known for the quality of its oil that is characterized by a high antioxidants rate and a high proportion of oleic acids [5] [6]. In general, and regardless to the region and to the climatic conditions, the olive oil produced from the Chetoui cultivar has a high oxidative stability and high anti-radical activity compared to the Chemlali cultivar [7].

Indeed, several analytical techniques have been proposed and are used for qualitative and quantitative analyses of olive oils [8]. Most of these techniques are often very expensive and rather laborious to achieve.

Recently, fluorescence spectroscopy has become an emerging analytical technique, due to its high sensitivity and simplicity and requires little or no sample preparation. Many works have been successfully applied for authentication of olive oil [9], for the detection of adulteration of olive oil [10] [11], for the characterization of olive oil [12] and for the analysis of oxidation of olive oil [13] [14] [15].

The aim of this work is to use the high sensitivity of fluorescence spectroscopy coupled to chemometric analyses to discriminate olive oil according to the cultivars with a very simple setup. This is done through its application to the two Tunisian Virgin Olive Oil (VOO) from cultivars Chetoui and Chemlali. The confrontations of these measures with another proven technique, such as Peroxide Value (PV) measures, strengthen and promote this technique for widest diffusion.

2. Material and Methods

The study was carried out on seventeen VOOs from two main Tunisian cultivars, namely Chetoui and Chemlali. VOO samples were collected in several regions during the same harvest. All samples were placed in amber glass bottles and stored in the dark.

2.1. Analytical Indices

Analytical indices such as free acidity, peroxide value (PV) and UV absorbance at 232 nm and 270 nm (K_{232} and K_{270}) were determined according to the European Official Methods of Analysis [8].

2.2. Fluorescence Spectroscopy

We used the experimental setup described by Mbesse *et al.* [15]. A light emitting diode (LED) with an emission wavelength at 365 nm, is used as the excitation source. The excitation light from the source is channelled to the sample through a Y-type optical fibre bundle. The fluorescence emission of the sample was captured by the same optical fibre bundle and goes to the Ocean Optics USB2000 spectrometer which was connected to a computer. The signal acquisition was controlled by the OOIBase32 software. The emission wavelength range was set between 400 nm to 800 nm. For the analysis, a quartz vat was filled by each sample and placed in front of the optical fibre.

To ensure the validity of the comparison between the different samples, each spectrum represented the average of 10 scans recorded after 100s of excitation. This lapse of time corresponds to the delay after which the intensity reaches a steady state. We used these parameters for all the samples in order to reproduce the measures in the same experimental conditions. Deconvolution of the fluorescence spectra was used to identify fluorophores. The data processing was made with the software Igor Pro 6.0.

2.3. Chemometric Methods

Analysis of variance (ANOVA) is applied to the data. The one-way ANOVA is used to show if there is significant difference between these two VOOs cultivar. In this analyze, the factor is the cultivar and the variables are the normalize intensities (R_1, R_2 and R_3 defined further in the subsection 3.2.) and the analytical indices (PV, K_{232}, K_{270} and Acidity). A Principal Components Analysis (PCA) is also applied to both spectral data and physico-chemical parameters. The aim of PCA is to reduce the large number of parameters influencing the measures to a much smaller number of principal components (PCs) that capture the vast majority of variance in the data. This approach considerably reduces the dimensionality of the original data, enabling effective visualization, regression and classification of multivariate data [16]. For the PCA, samples were considered as individuals while the variables were the normalized fluorescence intensities (R_1, R_2, R_3), PV, K_{232}, K_{270} and Acidity. PCA is used in this study to find the correlation that may exist between the set of variables. The statistical analysis is done using "R" software.

3. Results and Discussion

3.1. Analysis of Analytical Indices

According to several analytical indices (Acidity, Peroxide Value, K_{232}, K_{270}) es-

tablished by the European standards VOOs are classified with respect to the quality of oil [8]. Table 1 shows the average on measurements of analytical indices for Chetoui samples and Chemlali samples.

For our samples, we noted that Acidity's values are less than 0.8% and then peroxide value (PV) measured is significantly lower than the threshold of 20 meqO_2/Kg fixed for Extra Virgin Olive Oil (EVOO). We also observed that the absorbance at 232 nm and 270 nm (K_{232} and K_{270}) slightly exceeds the threshold established for EVOO. However, all the determined analytical indices values show that our samples involve standard characteristics of VOO and these values do not show significant difference between Chetoui and Chemlali cultivars of olive oil.

3.2. Spectral Analysis

Fluorescence spectra of all VOO samples are shown in Figure 1. We can clearly see a grouping of oil spectra according to their cultivars in the emission band between 400 nm and 600 nm.

Vegetable oils are a complex mixture of several fluorescent molecules. Consequently, VOO spectrum shows several broad emission peaks. To highlight the main emission peaks, a deconvolution is performed for each fluorescence spectrum of VOO according to the method described in our previous works [17].

Table 1. Average of analytical indices for our sample.

Analytical indices	Chetoui	Chemlali
Acidity (%)	0.4 ± 0.2	0.5 ± 0.2
PV (meq O^2/kg)	6.5 ± 2.1	5.1 ± 4
K_{232}	2.25 ± 0.38	2.42 ± 0.25
K_{270}	0.26 ± 0.04	0.28 ± 0.04

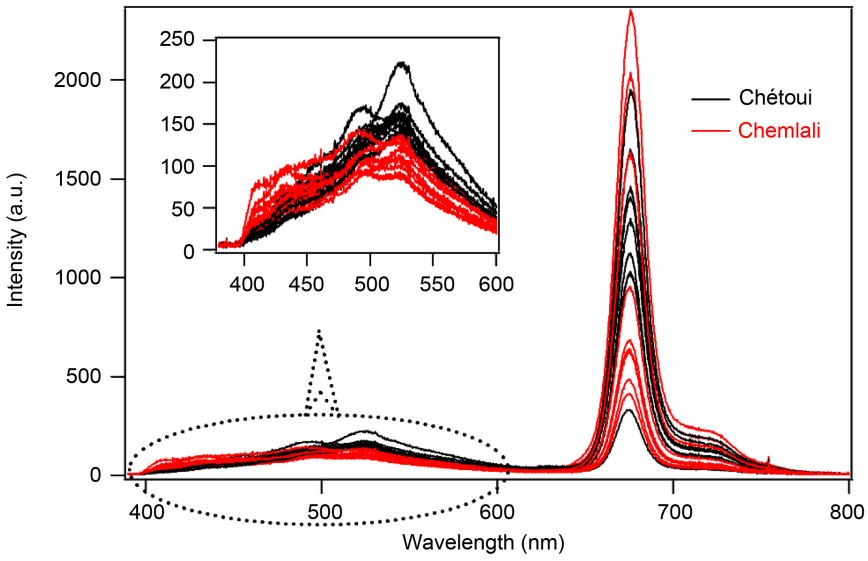

Figure 1. Superposition of oils spectra for all samples.

Seven peaks were obtained at the emitting wavelength around 432 nm, 455 nm, 489 nm, 524 nm, 565 nm, 680 nm and 730 nm. The emission at 432 nm, 455 nm and 489 nm are assigned to oxidation products and the one around 524 nm is assigned to the vitamin E [11] [18]. The two intense peaks at 680 nm and 730 nm correspond to the emission of the chlorophylls [11] [18]. In the following we focus the analysis in the band located between 400 nm and 600 nm.

From the deconvolution operation we determine the position and the intensities of the emission peaks [17]. The intensity of these peaks is characteristic of proportion of emitting compound. In order to compare the different spectra, we proceeded to the normalization of each spectrum according to the method used by Mbesse *et al.* [17]. In this method the intensity of the peaks around 432 nm, 455 nm and 489 nm are divided by the intensity of the peaks of vitamin E at 524 nm. We define at the same time three variables, namely R_1, R_2 and R_3 corresponding to the normalized intensities of the peaks at 432 nm, 455 nm and 489 nm respectively. **Figure 2** shows the normalized intensities obtained by using method of deconvolution of spectrum for each sample. We can see clearly the grouping of the curves according to the variety of the oil.

The low normalized intensity means a low proportion of oxidation products compared to vitamin E. We can note that the VOO's Chetoui group has relatively a low normalized intensity compared to the VOO's Chemlali group. This observation is in agreement with the works of Issaoui *et al.* [7] who emphasizes that the Chetoui has high levels of antioxidant and high oxidative stability compared to the Chemlali.

3.3. Chemometric Analysis

It is important to know that each cultivar includes several samples and each sample is represented by the three normalized intensities (R_1, R_2 and R_3) and the four analytical indices (PV, K_{232}, K_{270} and Acidity). The results of the

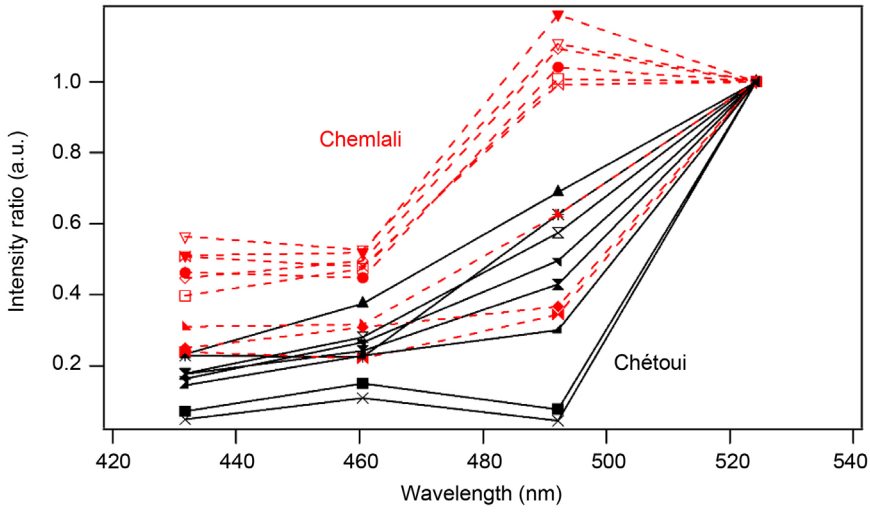

Figure 2. Intensity ratio of peaks around 432 nm, 455 nm and 489 nm to the peak of vitamin E (at 524 nm).

ANOVA performed on each variable are summarized in Table 2. We can see that the results of ANOVA for the PV, K_{232}, K_{270} and Acidity show clearly no difference between the cultivars groups. But the p-values obtained in the cases of spectral data are below the threshold ($\alpha = 0.05$). This means that the difference between the groups formed according to the VOO cultivar is significant. Several studies have shown the difference between these two VOO cultivars in terms of acidic composition and minor compound concentrations (phenolic compounds, vitamin E, etc.) [7] [19]. Here, this difference is observed directly from fluorescence spectrum analysis.

The peaks used to show the difference between the two cultivars are the peaks of oxidation products and the peak of antioxidants. A PCA is performed on all data (spectral data and analytical indices data). The goal is to find potential correlations between variables and to show which variables that most contribute in the principal components. Like this we can find the good parameters for this discrimination. Figure 3 presents PCA results and shows that all the variables are well represented by the two first principal components (PCs) that account for 73%. We obtain on the individuals factor map (Figure 3(a)) a clear separation between Chetoui and Chemlali as that observed on the Figure 1 and Figure 2.

The variables factor map (Figure 3(b)) shows that all the variables, except variable "Acidity", are well represented in the correlation circle. The discrimination between Chetoui and Chemlali observed in individual factor map (Figure 3(a)) is mostly due to PC1. Regarding to the high correlation of R_1, R_2 and R_3 with PC1 in Figure 3(b), we can confirm that the spectral variables are the good parameters of this discrimination. We observe a correlation (inversely correlated) between all spectral variables and peroxide value (PV). It is worthy to recall here that peroxide value measures the quantity of hydroperoxides present in VOO. So, the weak normalized intensities of the peaks of oxidation products of Chetoui in Figure 2 and the correlation between R_1, R_2, R_3 and PV shows the high antioxidant content and the high oxidative stability of the Chetoui cultivar compared to the Chemlali cultivar. This correlation encourages us to use these spectral parameters to make more complete analysis of VOOs.

Table 2. Results of ANOVA test ($\alpha = 0.05$) performed for each variable.

	p-value	Interpretation
Acidity	0.47500	No significant difference
PV	0.13450	No significant difference
K_{232}	0.53060	No significant difference
K_{270}	0.65770	No significant difference
R1	0.00004	Significant difference
R2	0.00100	Significant difference
R3	0.01700	Significant difference

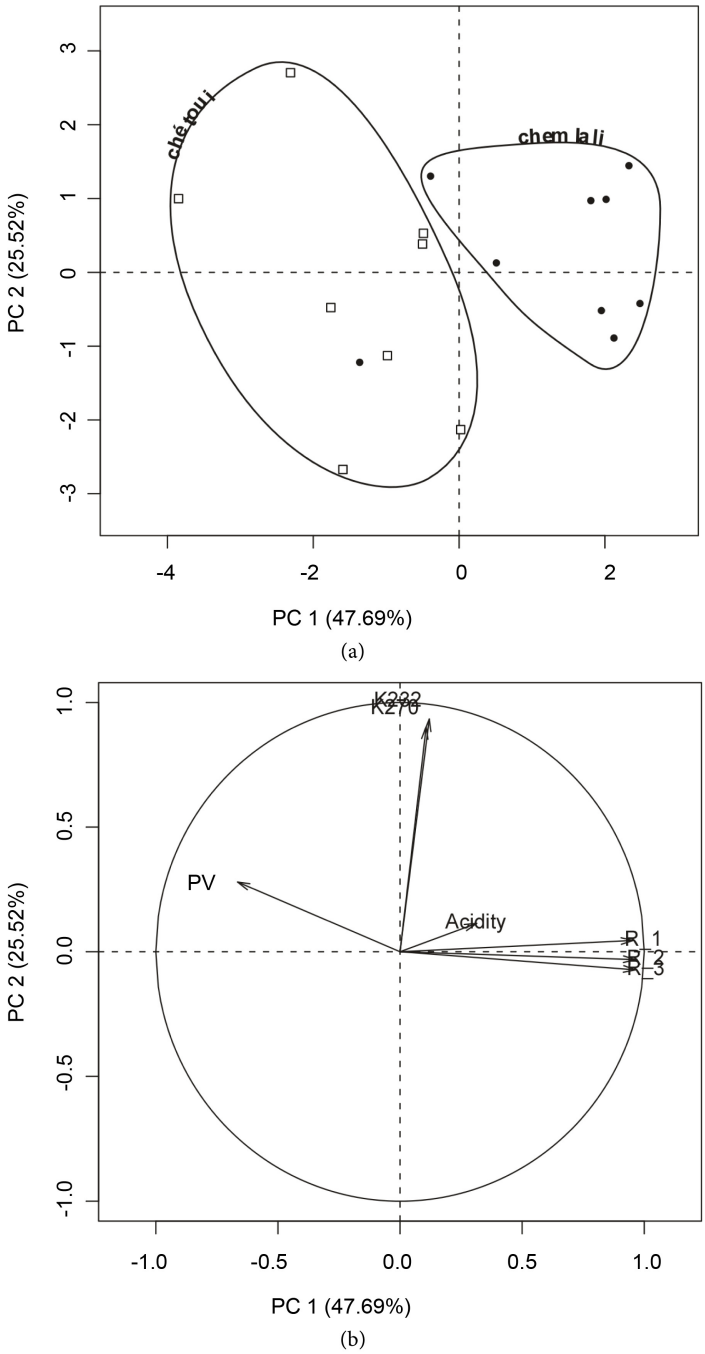

Figure 3. PCA results. (a) Individuals factor map (PCA) showing discrimination between chétoui and chemlali; (b) Variables factor map showing an inverse correlation between the Peroxide Value (PV) and the oxidation products (R_1, R_2, R_3).

4. Conclusion

Due to the high sensibility and ease of use (very simple setup), fluorescence spectroscopy presents itself as a suitable technique for the analysis of olive oils and quality control. We have, based on the fluorescence spectra, and observed a small difference between the two cultivars Chetoui and Chemlali. The analysis of

variance (ANOVA) shows that this difference is significant ($p < 0.05$). This first result proves the ability of the fluorescence technique to quickly identify special variety of oil among others with the help of simple established spectral parameters (R_1, R_2 and R_3). Secondly, the Principal Components Analysis (PCA) emphasized the existence of correlation between Peroxide Value (PV) and spectral variables. This correlation points out the high oxidative stability of Chetoui compare to Chemlali. All of our results can lead to a new kind of analysis because it gives the possibility to use this technique to investigate oil's stability and quality control.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hannachi, H., Breton, C., Msallem, M., Ben El Hadj, S., El Gazzah, M. and Bervillé, A. (2008) Differences between Native and Introduced Olive Cultivars as Revealed by Morphology of Drupes, Oil Composition and SSR Polymorphisms: A Case Study in Tunisia. *Scientia Horticulturae*, **116**, 280-290. https://doi.org/10.1016/j.scienta.2008.01.004

[2] Issaoui, M., Flamini, G., Chehab, H., Luigi Cioni, P., Braham, M. and Hammami, M. (2011) Irrigation Regimes and Growing Area Effects on Chetoui Olive Oil Quality. *Journal of Food Biochemistry*, **37**, 185-192. https://doi.org/10.1111/j.1745-4514.2011.00623.x

[3] Taamalli, A., Gomez-Caravaca, A.M., Zarrouk, M., Segura-Carretero, A. and Fernandez-Gutiérrez, A. (2010) Determination of Apolar and Polar Compounds and Other Chemical Parameters for the Discrimination of Six Different Varieties of Tunisian Extra-Virgin Olive Oil Cultivated in Their Traditional Area. *European Food Research and Technology*, **231**, 965-975. https://doi.org/10.1007/s00217-010-1350-3

[4] Dabbou, S., Rjiba, I., Nakbi, A., Gazzah, N., Issoui, M. and Hammami, M. (2010) Composition Quality of Olive Oils from Cultivars Introduced in Tunisian Arid Zones in Comparison to Chemlali Cultivars. *Scientia Horticulturae*, **124**, 122-127. https://doi.org/10.1016/j.scienta.2009.12.017

[5] Dabbou, S., Issoui, M., Servili, M., Taticchi, A., Sifi, S., Montedoro, G.F. and Hammami, M. (2009) Characterisation of Virgin Olive Oils from European Olive Cultivars Introduced in Tunisia. *European Journal of Lipid Science and Technology*, **111**, 392-401. https://doi.org/10.1002/ejlt.200800032

[6] Ben Temine, S., Compeol, E., Cioni, P.L., Daoud, D. and Zarouk, M. (2006) Volatile Compounds from Chetoui Olive Oil and Variations Induced by Growing Area. *Food Chemistry*, **99**, 315-325. https://doi.org/10.1016/j.foodchem.2005.07.046

[7] Issaoui, M., Flamini, G., Brahmi, F., Dabbou, S., Ben Hassine, K., Taamali, A., Chehab, H., Ellouz, M. and Zarrouk, M. (2010) Effect of the Growing Area Conditions on Differentiation between Chemlali and Chetoui Olive Oils. *Food Chemistry*, **119**, 220-225. https://doi.org/10.1016/j.foodchem.2009.06.012

[8] IOOC/T.15/NC No. 3/Rev.2 (2006) International Olive Council, Trade Standard Applying to Olive Oils and Olive-Pomace Oils.

[9] Guimet, F., Boqué, R. and Ferré, J. (2005) Study of Oils from the Protected Denomination of Origin "Siurana" Using Excitation-Emission Fluorescence Spectroscopy and Three-Way Methods of Analysis. *Grasas y Aceites*, **4**, 292-297.

[10] Guimet, F., Boqué, R. and Ferré, J. (2004) Rapid Detection of Olive-Pomace Oil Adulteration in Extra Virgin Olive Oils from the Protected Denomination of Origin Siurana Using Excitation-Emission Fluorescence Spectroscopy and Three-Way Methods of Analysis. *Analytica Chimica Acta*, **52**, 6673-6679.

[11] Sayago, A., Morales, M.T. and Aparicio, R. (2004) Detection of Hazelnut Oil in Virgin Olive Oil by a Spectrofluorimetric Method. *European Food Research and Technology*, **218**, 480-483. https://doi.org/10.1007/s00217-004-0874-9

[12] Sikorska, E., Romaniuk, A., Khmelinskii, I.V., Herance, R., Bourdelande, J.L., Sikorski, M. and Koziol, J. (2004) Characterization of Edible Oils Using Total Luminescence Spectroscopy. *Journal of Fluorescence*, **14**, 25-35. https://doi.org/10.1023/B:JOFL.0000014656.75245.62

[13] Engelsen, S.B. (1997) Explorative Spectrometric Evaluations of Oil Deterioration. *Journal of the American Oil Chemists Society*, **74**, 1495-1508. https://doi.org/10.1007/s11746-997-0068-2

[14] Cheikhousman, R., Zude, M., Bouve-Resse, D.J.-R., Léger, C.L., Rutledge D.N. and Birlouez-Aragon, I. (2005) Fluorescence Spectroscopy for Monitoring Deterioration of Extra Virgin Olive Oil during Heating. *Analytical and Bioanalytical Chemistry*, **382**, 1438-1443. https://doi.org/10.1007/s00216-005-3286-1

[15] Mbesse Kongbonga, Y.G., Ghalila, H., Majdi, Y., Mbogning Feudjio, W. and Ben Lakhdar, Z. (2015) Investigation of Heat-Induced Degradation of Virgin Olive Oil Using Front Face Fluorescence Spectroscopy and Chemometric Analysis. *Journal of the American Oil Chemists' Society*. https://doi.org/10.1007/s11746-015-2704-6

[16] Brereton, R.G. (1992) Multivariate Pattern Recognition in Chemometrics, Illustrated by Case Studies. Elsevier Science, Amsterdam. https://doi.org/10.4236/fns.2011.27095

[17] Mbesse Kongbonga, Y.G., Ghalila, H., Boyomo Onana, M., Majdi, Y., Ben Lakhdar, Z., Mezlini, O. and Sevestre-Ghalila, S. (2011) Characterization of Vegetable Oils by Fluorescence Spectroscopy. *Food and Nutrition Sciences*, **2**, 692-699.

[18] Kyriakidis, N.B. and Skarkalis, P. (2000) Fluorescence Spectra Measurement of Olive Oil and Other Vegetable Oils. *Journal of AOAC International*, **83**, 1435-1439.

[19] Aouidi, F., Dupuy, N., Artaud, J., Roussos, S., Msallem, M., Perraud-Gaime, I. and Hamdi, M. (2012) Discrimination of Five Tunisian Cultivars by Mid InfraRed Spectroscopy Combined with Chemometric Analyses of Olive *Olea europaea* Leaves. *Food Chemistry*, **131**, 360-366. https://doi.org/10.1016/j.foodchem.2011.08.041