# Euclidean distance and HAC methods coupled to FT-MIR spectroscopy as chemometric tool for the authentication of the virgin olive oil

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**ABSTRACT:** Therapeutic efficiency of virgin olive oil components, like antioxidants, has been proved. Moreover, the adulteration of virgin olive oil by the refined olive oil is a known fraudulent practice in non formal markets in some developing countries. In Morocco, there is a need of non expensive and fast tool to quantify the adulteration of virgin olive oil by the refined one. That is why we used a coupling between Fourier transform middle infrared spectroscopy, as a non expensive analysis technique, and Euclidean distance and hierarchical ascending cluster methods.

Virgin olive oil was extracted from "Picholine" cultivar olives in Tadla Azilal area, in Morocco. Fourier transform middle infrared spectroscopic parameters of prepared mixtures of virgin and refined olive oils have been used to determine the adulteration. The result of the Euclidean Distance concerning such an adulterated virgin olive oil has allowed the quantification of the adulteration percentage. The results of the hierarchical ascending cluster could provide a fast classification of virgin oil oils. Thanks to its rapidity and relatively low cost, coupling between middle infrared spectroscopy and chemometric methods would be an efficient tool to ensure authentication and traceability of virgin olive oil.

**Keywords:** Virgin olive oil, adulteration, authentication, FT-MIRS, Chemometrics.

# 1 INTRODUCTION

Virgin olive oil (VOO), known for its health properties as a Mediterranean food is still interesting by its scientific and dietetic communities. It contents antioxidant substances that the therapeutic efficiency has been proved [1], [2], [3], [4], [5],

[6], [7], [8], [9]. Moreover, the adulteration of olive oil by the used olive oil is a known fraudulent practice in non formal markets is some developing countries. This adulteration is made in order a material benefit without respecting the security of the consumers. Many works have been carried out until now are, namely, to find markers of adulteration in blends of extra virgin olive oil with refined olive oil (ROO). These anterior studies have contributed to increase the knowledge about this problematique of the adulteration. Several physical and chemical methods are used to detect and quantify the adulteration in virgin olive oil [10], [11], [12], [13], [14], [15], [16]. Moreover chemometric methods are used to solve the problematique of the adulteration. In fact, to discriminate French virgin olive oils (VOOs) that are registered as designations of origin (RDOS) and thanks to chemometrics, Ollivier D. and al. have in 2006 [17] used sensory and chromatography characteristics like fatty acid and triacylglycerol compositions. In 2011, using to the hierarchical ascendant clustering analysis (HAC), derivative FTIR spectroscopy have allowed De Luca M. and al. [18] the classification of Moroccan VOOs. A recent work of El bir M. and al. has used a coupling between gas chromatography and chemometric methods (PCA, K-Means and HAC) for automatic classification of Moroccan virgin olive based on its fatty acids and sterols contents [19]. In Morocco, there is a need of non expensive and fast tool for the quality control and the detection and the quantification of the adulteration of the virgin olive oil by refined oil. That is why the present work has aimed to contribute to the control of the quality of the VOO adulterated by refined oil (ROO) in some informal markets.

# 2 MATERIALS AND METHODS

Olives from the Moroccan Picholine cultivar were collected in the "Tadla-Azilal" Moroccan area in December 2012. The olives have been submitted to mechanical press in the laboratory to assure the extraction of virgin olive oil samples. The virgin olive oil extracted has been stored at 10°C. Fourier Transform Middle Infrared (FT-MIR) spectroscopic parameters of 17 prepared "VOO+ROO" mixtures (table 1) were explored to predict adulteration. These 17 prepared adulterated samples were analyzed by using a Brücker Vector 22 ATR Diamond FT-MIR spectrometer. However, the FT-MIR spectroscopy could not allow the discrimination between the 17 adulterated samples.

So, we have used the Euclidean distance method to remedy this problem. The square of Euclidian distance "d" between a spectrum of unknown sample and a "VOO+ROO" mixture spectrum is given by the following equation (1):

$$d^{2} = \sum \left( Ab_{i(unknownsam ple)} - Ab_{i(knownsampl e)} \right)^{2}$$
(1)

Where  $Ab_{i(unknownsample)}$  and  $Ab_{i(knownsample)}$  are the absorbency corresponding to the wave number  $1/\lambda_i$  for the unknown sample and for the known one, respectively. (i) is raising from 1 to 1764. Table 2 illustrates a type of a table for the FT-MIR data base of the 17 samples.

Mixture number	1	2	3	4	5	6	7	8
VOO (g)	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004
"VOO + ROO" (g)	4.9004	4.9238	4.9492	4.9734	5.0042	5.0310	5.0534	5.0760
Adulteration (% w/w)	0.00	0.48	0.99	1.47	2.07	2.60	3.03	3.46

 Table 1. Weight and percentage of adulteration for the 17 "VOO+ROO" mixtures

Mixture number	9	10	11	12	13	14	15	16	17
VOO (g)	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004	4.9004
"VOO + ROO" (g)	5.1074	5.1334	5.1604	5.1860	5.2098	5.2414	5.2662	5.3000	5.3302
Adulteration (% w/w)	4.05	4.54	5.04	5.51	5.94	6.51	6.95	7.54	8.06

Column 1	Column 2	Column 3			Column 17	Column 18	Column 19 (unknown sample)
•	В	С			Q	R	S
A	(0% w/w)	(0.48% w/w)	•	•	(7.54% w/w)	(8.06% w/w)	(% to determine)
1/λ (cm <sup>-1</sup> )			•	•			
1/λ =3999		•	•		•	•	
•	•	•	•		•	•	
•	•	•	•		•	•	
			•			•	
			•	•			
			•	•			
		•	•	•	•	•	

#### Table 2. Type of a table for the FT-MIR spectroscopic data base of 17 "VOO+ROO" mixtures

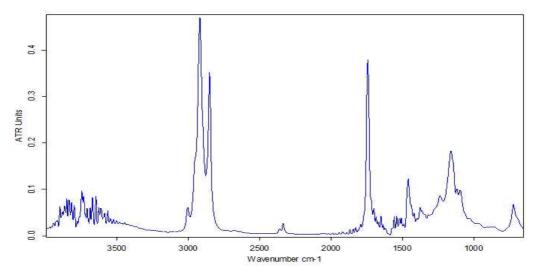
Finally, we applied the Euclidean distance method to predict 10 samples of VOO, eventually adulterated by ROO, collected in the informal markets in the Moroccan Tadla Azilal area.

In order to enrich the results of the Euclidean Distance method we have applied the HAC method on the Fourier Transform Middle Infrared spectroscopic data extracting from the 17 "VOO+ROO" mixture spectra of the virgin olive oil adulterated by the refined olive oil.

# 3 RESULTS

# **3.1** EUCLIDEAN DISTANCE

The FT-MIR spectra of the "VOO+ROO" mixtures present the same bands that one can detect by MIR spectroscopy. The type of the spectrum of these 17 spectra is given in the figure 1. The adulteration of VOO by a low quantity of ROO is not easy to be detected. In fact, the figure 2 shows that it is difficult to distinguish between the 17 "VOO+ROO" mixtures. So, it was necessary to couple the FT-MIR spectroscopy with the Euclidean distance method. We checked the equation (1) of Euclidean distance method by testing it using the sample which corresponds to the column number 6. As it is observed in column 6 (G) in table 3, Euclidean distance is equal to zero, so it means that the adulteration percentage of this testing sample would be 2.6%. The result of the predicted Euclidean Distance concerning the 10 adulterated VOO by ROO samples collected in the informal markets is reported in the table 4.



#### Fig.1. FT-MIR spectrum of virgin olive oil adulterated by refined oil

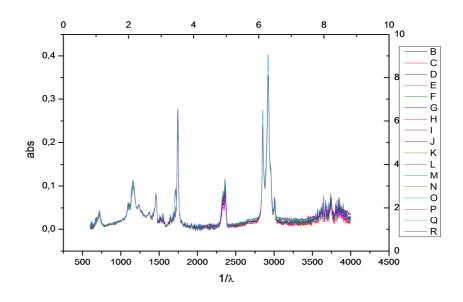


Fig. 2. FT-MIR spectra of the 17 prepared "VOO+ROO" mixtures

 Table 3. Euclidean Distance (ED) between testing sample spectrum and each one of 17 FT-MIR mixtures spectra

	1	2	3	4	5	6	7	8	9	10
Α	В	С	D	E	F	G	Н	I	J	К
d	0.2002	0.2634	0.1927	0.2111	0.2009	0	0.1949	0.3094	0.1874	0.2143
11		12	13	14		15		16	17	1
L		Μ	Ν	0	0 P Q		R			
0.352	6 0.	2456	0.2374	0.223	1 (	0.3238		.3246	0.14	43

 Table 4. Predicted Euclidean distance between each one of the 10 collected adulterated VOO sample spectrum and each one of 17 FT-MIR mixtures spectra.

Real adulteration percentage (% w/w)	HT1	HT2	HT3	HT4	HT5	HT6	HT7	HT8	HT9	HT10
H1 (0.00)	0.1433	0.1261	0.1312	0.2209	0.0967	0.0944	0.2303	0.1417	0.2809	0.1312
H2 (0.48)	0.0159	0.2333	0.1604	0.2923	0.2131	0.0897	0.3471	0.1760	0.3504	0.2383
НЗ (0.99)	0.1660	0.0839	0.1150	0.1954	0.0864	0.0897	0.1980	0.1500	0.2504	0.1073
H4 (1.47)	0.1241	0.2292	0.1777	0.2707	0.1883	0.1252	0.3159	0.1011	0.3362	0.2175
H5 (2.07)	0.1518	0.1263	0.1283	0.2127	0.0978	0.1010	0.2184	0.1390	0.2734	0.1327
H6 (2.60)	0.2527	0.2216	0.2323	0.2688	0.2007	0.2122	0.2524	0.1095	0.3214	0.1595
H7 (3.03)	0.1822	0.1521	0.1295	0.1706	0.1094	0.1368	0.2163	0.1351	0.2410	0.1546
H8 (3.46)	0.3683	0.1877	0.2586	0.2126	0.2006	0.3046	0.0665	0.3142	0.2266	0.2106
H9 (4.05)	0.1877	0.1144	0.1295	0.1809	0.0519	0.1301	0.1890	0.1395	0.2418	0.1177
H10 (4.54)	0.2077	0.0900	0.1502	0.2028	0.0757	0.1475	0.1738	0.1820	0.2549	0.0810
H11 (5.04)	0.3672	0.2468	0.2253	0.1281	0.2651	0.3174	0.2449	0.3461	0.0851	0.2829
H12 (5.51)	0.2523	0.0413	0.1618	0.1889	0.1071	0.1872	0.1413	0.2313	0.2245	0.1167
H13 (5.94)	0.2329	0.0816	0.1638	0.2052	0.0716	0.1710	0.1600	0.2134	0.2504	0.1127
H14 (6.51)	0.2353	0.0785	0.1408	0.1596	0.0873	0.1709	0.1447	0.2004	0.2065	0.1127
H15 (6.95)	0.3422	0.2348	0.2018	0.0731	0.2445	0.2943	0.2324	0.3133	0.0221	0.2656
H16 (7.54)	0.3461	0.2106	0.1960	0.1107	0.2298	0.2923	0.1990	0.3185	0.0741	0.2478
H17 (8.06)	0.3056	0.2049	0.2568	0.2816	0.2158	0.2524	0.2107	0.2082	0.3164	0.1380
Predicted adulteration percentage	0.48	5.51	0.99	6.95	4.05	0.48	3.46	1.47	6.95	4.54

# 3.2 HIERARCHICAL ASCENDING CLUSTER (HAC)

We have, also, preceded to the hierarchical ascending cluster (HAC) method and the result is given by the dendrogram in the following figure 3.

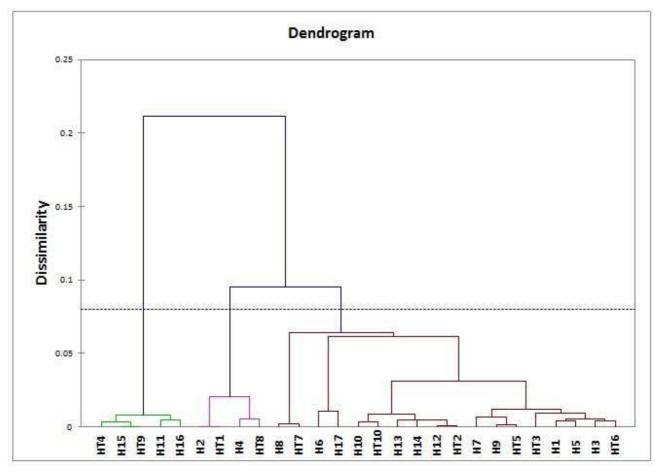


Fig.3. Classification dendrogram by the HAC method

# 4 DISCUSSION

It was important to try to determine adulteration degree by a rapid and simple tool that is coupling between FT-MIR spectroscopy and chemometrics. As it is shown in figure 2, it has been actually difficult to discriminate between the 17 FT-MIR spectra of the prepared adulterated virgin olive oil ("VOO+ROO"). In fact, these 17 spectra present the same olive oil characteristic bands. The two intense bands at 2924 and 2853 cm<sup>-1</sup> are attributed respectively to the asymmetric and symmetric vibrations of the pulled  $CH_2$  group. Another band having medium intensity at 2953 cm<sup>-1</sup> is due to the asymmetric stretching vibration of the  $CH_3$  group. At the low frequency region, only the bending vibration of  $CH_2$  is observable. The bands at 1458 and 1232 cm<sup>-1</sup> are due to vibrations of shear deformation out of the plane. The band which appears at 724 cm<sup>-1</sup> is representative of the deformation vibrations of swinging and corresponds to the fact that the latter is the most apparent in the unsaturated fatty acids.

The =CH mode vibration corresponds to a band of low intensity is observed around 3008 cm<sup>-1</sup>. In contrast, one can observe an intense band at about 970 cm<sup>-1</sup>. It is due to the bending vibration of the =C-H group. The Mode C=O vibration appears around 1750 cm<sup>-1</sup>. The C-O mode vibration which appears at 1150 cm<sup>-1</sup> is due to the stretching vibration of C-O group of esters. The band line to 2350 cm<sup>-1</sup> is related to the conditions of the spectra registration.

FT-MIR spectroscopy coupled to Euclidian distance method has finally allowed recognizing that an testing sample which was adulterated VOO by ROO, arbitrarily chosen as the one corresponding to the column 7 (2.6%).

The Euclidean distance (ED) is a statistical method used for the classification in the field of information processing (image processing, data mining and chemometrics), More studies have been done using this Euclidian distance method as discriminative parameter for classification of sud-Paris waters. The same approach and others have been to represent data objects by their similarity or dissimilarity with respect to other objects, The Euclidean distance and incremental linkage methods used by A.G. Cruz et al. [20] in the pre-processing to elaborate the Hierarchical Cluster Analysis in chemometric techniques to discriminate low-fat and full-fat yogurts.

Elbir and al. have recently applied algorithms (PCA, K-Means an HAC) for discrimination between virgin olive oils on the basis of their contents in terms of fatty acids and sterols [19]. Also, classification algorithms like LDA (linear discriminant analysis), CART, and Treenet and SVM (support vector machines) have been carried out by A.S. Melissa [21] so that he PCCM method performed well for different tests over large sets of classification experiments. These last authors have established discriminating PCCM classifier that provides a quick visualization tool to diagnose complex classification problems. Many techniques used in soil Vis–NIR sensing are based on the measurement of the similarity or distance between samples [22]. The authors evaluated the following distance metrics: Euclidean (ED), Mahalanobis (MD), spectral angle mapper (SAM), surface difference spectrum (SDS), spectral information divergence (SID), principal component distance (PC-M), optimized PC distance (oPC-M), locally linear embedding distance (LLE-M) and  $\sigma$ -locally linear embedding ( $\sigma$ LLE-M) [22]. The experimental results of these last authors showed that the Vis–NIR similarity measures reflecting better the soil compositional similarity are those corresponding to the oPC-M, LLE-M and  $\sigma$ LLE-M methods. The United States and Canada currently use exposure-based metrics to protect vegetation from O<sub>3</sub> [23]. Using 5 years (1999 to 2003) of commeasured O<sub>3</sub>, meteorology and growth response, the two countries have developed exposure-based regression models that predict Populus tremuloides growth change within the context of North American ambient air quality. These models comprised growing season fourth-highest daily maximum 8-h average O<sub>3</sub> concentration, growing degree days, and wind speed.

So, in the present work, to discriminate between the 17 FTIR spectra of the adulterated virgin olive oil, a coupling between the MIR spectroscopy and the method of Euclidian distance was an efficient, rapid, less expensive and determining for routine analyses, particularly for the olive oil quality control.

Finally, such results of the present work are very important as a first level in the control of the quality of the olive oil, thanks to its optimizing in terms of time, exactitude and price.

The results of the HAC were found corresponding to those of the Euclidean distance method. Overall, the Euclidean distance between the FT-MIR spectrum of each of the 10 samples (HT1 to HT10) coming from the informal market and that of a spectrum FT-MIR coming from a 'VOO + ROO' mixture, is minimal (hatched values in Table 4) when the two spectra are those of samples that are close neighbors on the HAC dendrogram (Figure 3). In fact, only the HT6 sample coming from the informal market is the exception of this observation since it is not a neighbor of the H2 mixture sample on the HAC dendrogram.

# 5 CONCLUSION

Adulteration of virgin olive oil by adding a very small quantity of refined olive oil is difficult to be detected. Coupling between Middle infrared spectroscopy and Euclidian distance method contributes to the detection and the prediction of this adulteration. Thanks to its rapidity and relatively low cost, coupling between FT-MIRS and Euclidean distance and Hierarchical Ascending Cluster methods would be an efficient chemometric tool to ensure authentication and traceability of virgin olive oil, foods, medicines or other products.

In order to control the quality of the olive oil at the industrial laboratory and the research one, such a coupling may be a reference chose of rapid and less expensive tool.

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