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Development of fast analytical method for the detection and quantification of Moroccan picholine extra virgin olive oil adulteration using MIR spectroscopy and chemometrics tools

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A B S T R A C T

In this study, the adulteration of Moroccan Picholine extra virgin olive oil with Arbequina virgin olive oil was monitored using the Fourier transform mid-infrared (FT-MIR) spectroscopy technique and chemometrics methodologies. To discriminate between olive oil that has been adulterated and unadulterated, principal component analysis (PCA) was utilized for qualitative analysis. We created the best calibration models for quantitative analysis using principal component regression (PCR) and partial least-squares regression (PLS). The first three principal components account for 95% of the overall variability, according to PCA analysis. PCA allows for the classification of the dataset into two groups: adulterated and unadulterated Moroccan Picholine olive oil. The application of the PLS and PCR calibration models for the quantification of adulteration demonstrates high-performance capabilities, as indicated by high values of correlation coefficients R^2 greater than 0.999 and 0.995 and lower values of root mean square error (RMSE) less than 0.767 and 2.16 using PLS and PCR, respectively. According to our results, FT-MIR spectroscopy combined with chemometrics approaches can be used successfully as a simple, quick, and non-destructive method for the quantification and discrimination of adulterated olive oil.

1. Introduction

Olive oil (OO) is a liquid vegetable fat derived from the olive fruits of the olive tree (*Olea europaea* L. which has been cultivated since biblical times. The olive tree supported the Pax Romana during the Roman Empire by providing the conquered countries with a good product for their commerce, economy, and health. Indeed, for two thousand years, the olive tree was a symbol of the Mediterranean region, from Greece to Spain via Egypt, Italy, Tunisia, France, Spain, and Morocco.[1] Virgin olive oil (VOO) is mechanically extracted straight from olive fruits. The process includes washing and crushing olives, combining dough, separating phases by pressing and settling or centrifugation, and filtering to obtain pure VOO.[2] Due to its great nutritional value and status as a key lipid source in the Mediterranean diet, olive oil is also significant economically. Because of its high content of unsaturated fatty acids like oleic and linoleic acids, it does really offer needed fatty acids and has positive effects on health. Also known to contain anti-inflammatory, anti-atherogenic, antibacterial, antiviral, and anti-aging properties, OO, and neuroprotective effects. [3-7] Fatty acids make up 99% of the chemical components of OO., with the remaining 1% consisting of minor substances including squalene, triterpenic alcohols, sterols, phenolic compounds, chlorophylls, b-carotenes, and tocopherols.[2] Phenolic and tocopherol molecules are significant minor components of OO, which are among the primary chemicals providing OO its high nutritional, pharmacological, and therapeutic properties. Extra-virgin olive oil is a premium vegetable oil that has been produced for centuries in the countries of the Mediterranean basin. It does not require purifying before consumption. Extra-virgin olive oil is well recognized to

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have a chemical makeup that is more variable than other vegetable oils since it is greatly influenced by agronomic methods, geographic origins, harvesting times, and processing processes.[8,9] Additionally, several extra-virgin olive oil varieties are marketed based on the type of olive fruit used. There are over 20 different botanical kinds of olive trees in Morocco, but only Picholine and Arbequina play a significant role in the marketing of olive oil. Their olives differ in terms of size, oil content, flavor, chemical properties, ripening period, and numerous other aspects. They could differ in terms of appearance as well as developing traits and interests. Although historically each variety was specific to a particular geographical region, today different varieties are grown in the same area, making it impossible to draw a direct correlation between a variety and geographical origin.

Food adulteration for economic purposes is becoming a serious issue in many countries as a result of the growth of international trade. For the purpose of evaluating the quality and authenticity of OO, numerous analytical methods have been developed. These methods include chemical, sensory, chromatographic, and others.[10,11] However, because they are time-consuming and destructive, most chemical and chromatographic methods have limitations. Therefore, it is crucial to develop quick, non-contact, non-destructive, and real-time measurements. Food safety control puts a greater emphasis on spectroscopic techniques like visible spectroscopy (Vis), near-infrared spectroscopy (NIRS), mid-infrared spectroscopy (MIR), and fluorescence in combination with suitable chemometrics multivariate methodologies.[12-17] Since it gives valuable information on the presence of specific functional groups, these methods have been regarded as a quick and non-destructive alternative to detect and quantify the presence of adulterants. It is also known as a "fingerprinting technique," meaning that no two varieties of olive oil have exactly the same FTIR spectra in terms of peak counts or peak intensities.

FTIR is a great instrument for quantitative analysis because the intensities of the spectral bands are related to concentration. For this reason, FTIR has been utilized in non-supervised classification approaches to differentiate oils from various botanical origins.[18,19] Additionally, FTIR has been utilized to differentiate EVOOs from various geographic sources,[20-22] and different genetic varieties.[23] FTIR applications designed to identify adulteration of olive oil with low-cost edible oils,[24,25] to evaluate olive oil freshness and to assess oil oxidation have been also described.[26,27]

However, there is no information on the use of FTIR spectroscopy in combination with chemometrics for analysis of adulterated Picholine extra virgin olive oil (EVOO) with Arbequina olive oil. The objective of this study is to provide a quick method for detecting and quantifying Picholine olive oil adulteration using FT-MIR spectroscopy with supervised and unsupervised chemometrics tools and to compare the predictive capability of the two regression methods PLS and PCR. This method offers a simple and practical way to keep track of the quality of olive oil, with the benefits of simplicity of use, quick sample turnover, and no sample pretreatment.

2. Results and Discussion

2.1. Physicochemical analysis

In **Table 1** the physicochemical parameters of the two samples of virgin olive oil are given.

Table 1. physicochemical analysis

Sample	Acidity (%)	Peroxyde value	K232	K270	ΔK	Polyphenols
Picholine EVOO	0.57	4.5604	0.5135	0.134	0.0024	8.76
Arbequina VOO	1.02	11.413	0.821	0.162	0.0053	6.12

Based on these results, and the quality criteria of The International Olive Council COI/T.15/NC No 3/Rev. 7, we can conclude that the sample of Picholine olive oil was extra vierge, and the sample of Arbequina olive oil was vierge.

2.2. Spectra analysis

Chemically speaking, fatty acids and glycerol are esterified to form fats and oils. Physically detecting fats and oils that have been adulterated is frequently challenging because some of the fats and oils may have relatively comparable compositions.[3] However, because of its ability as a fingerprint technique, MIR spectroscopy may discriminate between authentic oils and those adulterated with others by analyzing spectral changes caused by the adulteration.[26]

For OO samples, Fourier transform infrared (FT-IR) spectra were acquired and first visually inspected. The entire spectral range 4000-600 cm^{-1} served as the investigation's starting point. According to the literature,[27] the region between 2400 and 2300 cm^{-1} was excluded prior to the development of the chemometrics tools because its signal-to-noise ratio was very poor and the signal variation was found to be independent of the sample composition. The region between 4000 and 3100cm^{-1} is also removed due to the water subtraction band which contains a lot of instrumental noise and brings useless information

Fig. 1 exhibited the FTIR spectra of Picholine extra virgin olive oil (EVOO) , Arbequina virgin olive oil (VOO) and adulterated one. The assignment of functional groups responsible for IR absorption is as follows: 3006 cm^{-1} (trans =C–H

stretch), 2920 and 2852 (symmetrical and asymmetrical stretching of –CH_2), 1743 (–C=O stretch), 1463 (–CH_2 bending), 1377 (–CH_3 bending), 1237 (–C–O stretch), 1161 (–C–O stretch; –CH_2 bending), 1118 (–C–O stretch), 1096 (–C–O stretch), and 722 cm-1 (cis-CH=CH– bending out of plane).[28]

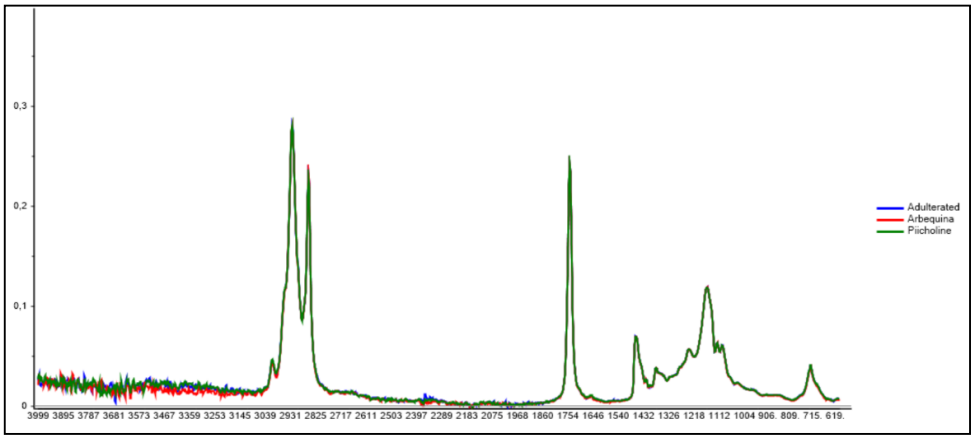

Fig. 1. Mid-infrared spectrum of Picholine extra virgin olive oil (EVOO) , Arbequina virgin olive oil (VOO) and adulterated one

2.3. Classification

We utilized certain unsupervised chemometrics techniques, such as principal component analysis, to effectively analyze the spectrum dataset (PCA).

PCA was first used using spectral data that had been normalized and treated using the Savitzky-Golay algorithm to lessen unwanted spectral effects. The spectra were split into two groups: pure Picholine olive oil and olive oil that had been tampered with Arbequina.

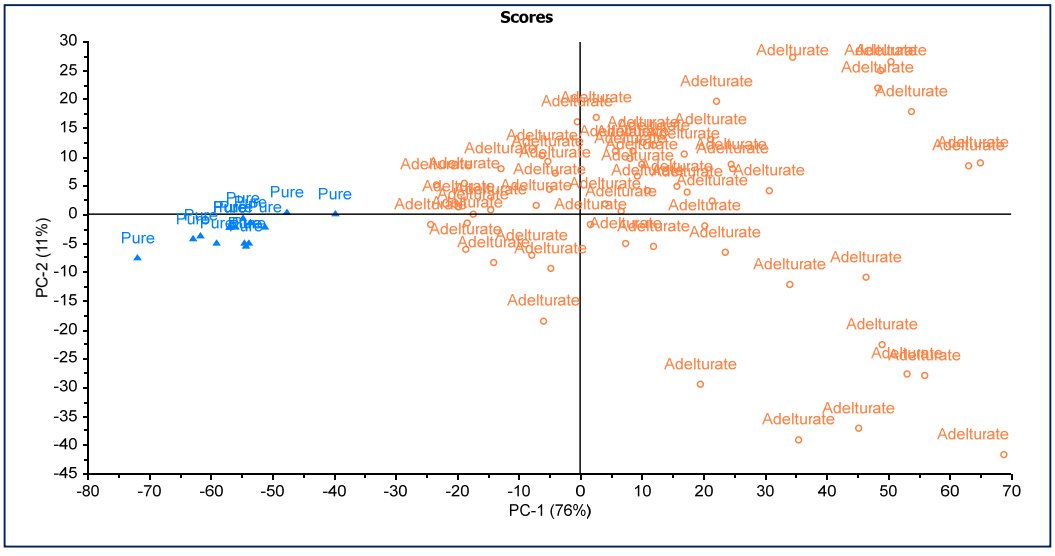

Fig. 2. PCA scores plot of samples of pure and adulterated Picholine olive oil.

First, two categories pure EVOO and adulterated oils were established for pure Picholine EVOO and that in combination with the adulterant of Arbequina VOO. PCA was used to classify both groups. The score plot in Fig. 2 clearly differentiates between the EVOO group and the Arbequina-adulterated group. In this investigation, 100% of the samples were correctly identified by the PCA model according to their group, indicating that no samples were incorrectly placed in the wrong group, which could occasionally happen due to the strong similarities in chemical composition between groups.

2.4. Quantification:

Partial least square (PLS) and principal component regression (PCR) methods were used to quantify the amounts of Arbequina VOO present in the adulterated Picholine EVOO samples. The samples of EVOO adulterated with Arbequina VOO were divided into the calibration and validation sets for PLS and PCR methods. The calibration set contained 45 samples, while the validation set contained 15 samples.

PLS has the capacity to take data from sample spectra at a variety of spectrum frequencies, and then link spectral absorption change with analyte concentration while simultaneously computing additional spectra that can interfere with analyte spectra.[29] Meanwhile, PCR is a type of factor analysis where the spectral and concentration data are incorporated into the model in one step.[30]

The PLS and PCR regression models were applied to a medium infrared spectral area ranging from 3100 cm^{-1} to 600 cm^{-1} without and with spectral preprocessing.

2.4.1. Calibration model

Table 2 contains a list of the PLS and PCR regression's parameters, both with and without spectral correction. The multiple determination coefficient (R^2), the root means square error of calibration (RMSEC), and the root mean square error of cross-validation (RMSECV) are also summarized in this table. The good performance and accuracy of the PLS and PCR generated models are indicated by the high value of R^2 and the low values of RMSEC and RMSECV.

Table 2. Performance parameters of PLS and PCR.

Regression	Preprocessing	Calibration		Cross-validation	
		R^2	RMSEC	R^2	RMSECV
PLS	Without preprocessing	0.999	0.057	0.989	3.232
	Centred and normed	0.999	0.267	0.947	4.282
	Detrend polynomial degree 1	0.999	0.034	0.991	2.990
	Detrend polynomial degree 2	0.999	0.026	0.990	3.1
	1st derivative	0.999	0.767	0.994	2.523
	2nd derivative	0.999	0.641	0.990	3.185
PCR	Without preprocessing	0.985	4,546	0.910	6,053
	Centred and normed	0.972	3.091	0.929	5.019
	Detrend polynomial degree 1	0.995	2.203	0.991	2.248
	Detrend polynomial degree 2	0.995	2.016	0.993	2.647
	1st derivative	0.995	2.161	0.991	2.963
	2nd derivative	0.991	3.005	0.983	4.236

As shown in **Fig. 3**, the PLS and PCR regression models demonstrate a strong correlation between the MIR-TF spectra of Picholine EVOO and their rate of adulteration by Arbequina VOO. Typically, a number of mathematical models have been built using various spectral preprocessing techniques to create high-performance models with good quality prediction of the adulteration rate in Picholine EVOO. Figure 3 shows the very excellent performance of these constructed PLS and PCR regression models, which have correlation coefficients between 0.972 and 0.999 and calibration result errors between 0.026 and 4.546. Additionally, the correlation coefficient and error ranges for the cross-validation results are 0.910 to 0.994 and 2.248 to 6.053, respectively.

From these results, we also note that the calibration models developed by the PLS regression have better regression parameters than those developed by the PCR regression. **Fig. 3** shows that for the calibration findings, the PLS regression has a correlation coefficient better than 0.999 and an error of less than 0.026, and for the cross validation results, it has a correlation coefficient greater than 0.994 and an error of less than 2.248. Compared to the PCR regression results, these results are thought to be more reliable.

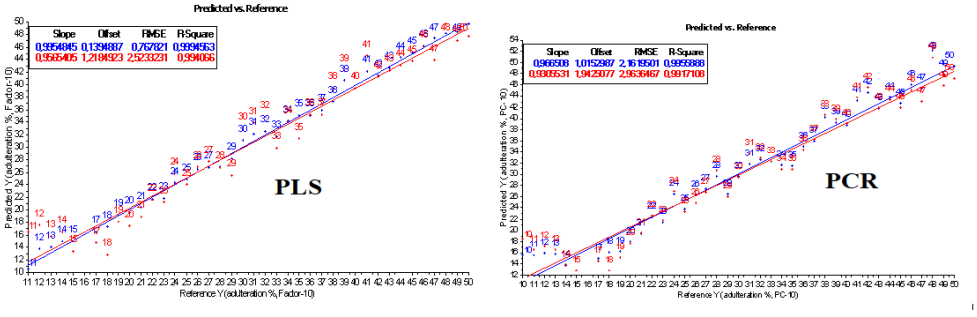

Fig. 3. The calibration models of PLS and PCR for the relationship between actual and FTIR predicted values of Picholine EVOO adulterated with Arbequina VOO using 1st derivative spectra.

2.4.2. Validation model

15 samples were utilized for external validation of these models, the test sample was examined, and the outcomes of the test group data were predicted using the PLS and PCR models. This was done to confirm the models' ability to quantify EVOO adulteration. The concordance between actual and anticipated levels of adulteration is clearly displayed in **Fig. 4.** With a mean square error of less than 0.74 for PLS and 1.69 for PCR regression, the prediction quality R^2 values are more than 99% for PLS regression and 98% for PCR regression. The projected values and actual values agree very well.

The results show that the approach suggested in this study can be used to detect and quantify the adulteration of Picholine EVOO with Arbequina VOO. As was already mentioned in the calibration and cross-validation findings, the PLS-R regression outperforms the PCR regression in terms of performance.

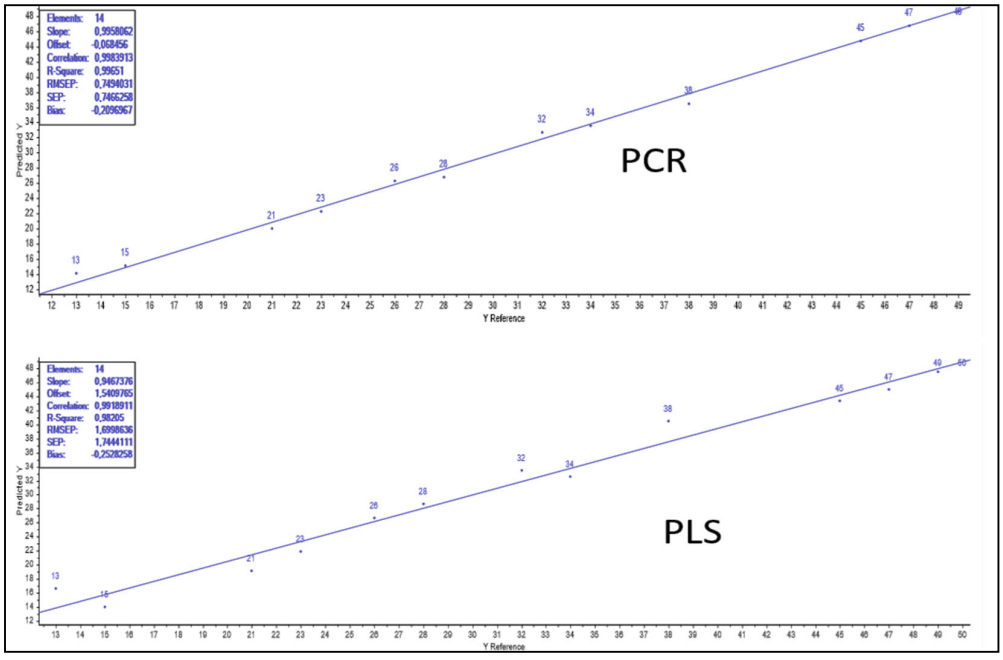

Fig. 4. The validation models of PLS and PCR for the relationship between actual and FTIR predicted values of Picholine EVOO adulterated with Arbequina VOO using 1st derivative spectra.

The results demonstrated the viability of the method described in this study for identifying and measuring adulteration of Picholine EVOO with Arbequina VOO. As was already mentioned in the calibration and cross-validation findings, the PLS-R regression outperforms the PCR regression in terms of performance.

3. Conclusion

To identify and quantify the adulteration of Moroccan Picholine extra virgin olive oil with Arbequina virgin olive oil, we used in this study a MIR spectroscopy in combination with chemometrics algorithms. The obtained data from multivariate analysis and classification techniques like PCA have indicated that the percentage of discrimination accuracy can achieve 100%. Additionally, the use of PLS and PCR regression methods has demonstrated a very high capacity for quantifying the percentage of adulterated Moroccan Picholine extra virgin olive oil, as demonstrated by the high correlation coefficient and low prediction error values obtained through cross-validation and external validation. It has been effectively proven that the suggested procedure can quantify low adulteration. These findings demonstrate that the use of this technique, which is quick, nondestructive, and easy to use, can be a promising tool to control the quality of Picholine extra virgin olive oil.

4. Experimental

4.1. Sample preparation

Extra virgin olive oil (EVOO) of Picholine and VOO of *Arbequina* collected in olive crop 2021-2022 from Beni Mellal area (center of Morocco) were used for quantitative analysis using PLS and PCR calibrations, a set of 60 samples containing Picholine EVOO and *Arbequina* VOO was mixed together in accurately weighted proportions of 1–70% wt/wt and shaken vigorously to ensure the total homogenization. For validation, 15 independent samples were built. Picholine EVOO and *Arbequina* VOO were mixed to obtain a series of standard or trained sets of 15 pure and 60 adulterated samples containing 1–70% of *Arbequina* VOO. The samples containing *Arbequina* VOO were assigned as adulterated, while a series of pure Picholine EVOO was marked with EVOO and classified using FTIR spectra.

4.2. Physicochemical parameters

In this study, five physicochemical analyses of Extra virgin olive oil (EVOO) of *Picholine* and Arbequina samples were carried out following the analytical methods described by Regulation COI/T.15/NC No 3/Rev. 7 of the International Olive Council.[2] Free acidity was given as percentage of oleic acid and determined by titration with 0.1N KOH of an oil solution in a previously neutralized solvent (ethanol: ethyl ether, 1:1) and using phenolphthalein as indicator. Peroxyde value was expressed as milliequivalents of active oxygen per kg of oil (meq O^2/kg) and determined by a mixture of oil, chloroform and acetic acid left to react with potassium iodide in darkness. Free iodine was then titrated with a 0.01 N sodium thiosulfate solution. The specific extinction coefficients, K270 and K232, were measured from absorption in cyclohexane solution at 232 and 270 nm, respectively, with 1 cm path length in an UV/VIS spectrum 2100 series spectrophotometer (J.P SELECTA s. a).The Folin–Ciocalteu method was used to determine total phenolic content.[31]

4.3. Acquisition of Mid infrared (MIR) spectra

Spectra were recorded on a single reflection diamond ATR (JASCO FTIR 460 PLUS (PIKE Technologies, Madison, USA)). Analyses were carried out at room temperature. After each measurement, the crystalline surface was washed with ethanol solution and dried with a soft paper. Without any prior preparation, the olive oil sample was applied directly to the ATR cell. Using the best signal to noise ratios, a drop of 20 µL is adequate to obtain excellent spectra. With a spectral resolution of 4 cm^{-1}, the spectra were captured from 4000 to 600 cm^{-1}.

4.4. Data Analysis

In this study, spectral data produced by ATR FT-MIR spectroscopy have been processed and evaluated using a variety of statistical approaches. We started the findings PCA analysis to make sure the dataset was fully represented and explored.

In order to identify groups in the obtained data, principal component analysis (PCA), an unsupervised model recognition technique, is typically used as the initial step in exploratory data analysis. When there is a lot of quantitative data that needs to be processed and understood, PCA is highly helpful. Its goal is to identify the most significant data from the data table and project it onto a collection of new orthogonal variables known as principal components (PCs).

The PCs describe, in descending order, the largest variations between characteristics, and because they are calculated to be orthogonal to each other, each PC can be interpreted independently. This gives an overview of the data structure by revealing the relationships between objects and the detection of deviant features.[32]

Regression algorithms like PLS and PCR are primarily utilized for multi-variate data, including spectral data. They are frequently used to predict and quantify specific chemical parameters in agri-food or pharmaceutical products.[33] Calibration and validation were the two phases in the calibration approach used to quantify the adulteration.[25] The root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and regression coefficient (R^2) are used to evaluate the model's performance. The concentration of samples is then calculated using a separate (or external) set of predictions using the chosen model. The root mean square of prediction (RMSEP) is used to assess the model's predictive power. A good quality of the prediction is indicated by the lower RMSE and higher R^2.[34]

4.5. Chemometrics Software

Unscrambler software, Version 10.1, was utilized to do the quantitative analysis utilizing the partial least-squares (PLS) and principal component regression (PCR) procedures. To identify adulterants and discriminate between authentic and adulterated Picholine EVOO, discriminant analysis (PCA) was used.

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