Tracing the Geographical Origin of Moroccan Saffron by Mid-Infrared Spectroscopy and Multivariate Analysis

Omar Elhamdaoui*1, Aimen El Orche2, Amine Cheikh3, Khalid Laarej4, Khalid Karrouchi1, Miloud El Karbane1, Mustapha Bouatia1

1Laboratory of Analytical Chemistry & Bromatology, Team of Formulation and Quality Control of Health Products, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco
2Laboratory of Chemical Processes and Applied Materials, Faculty of Science and Technology, Sultan Moulay Slimane University, Beni-Mellal, Morocco
3Faculty of Pharmacy, Abulcasis University-Rabat, Morocco
4Department of Toxicology-Pharmacology, Laboratory of Research and Medical Analyzes (LRAM), the Fraternal of the Royal Gendarmerie, 10100 Rabat, Morocco

This work aims to investigate the potential of mid-infrared spectroscopy (MIR) and chemometrics algorithms for the determination of geographical origin and detection of adulteration of Moroccan saffron samples. First, the determination of the geographical origin of five saffron varieties was analyzed by linear discriminant analysis (PCA-LDA) and partial least squares discriminant analysis (PLS-DA). As a result, the developed models correctly classified saffron samples in a subset of external validation with 100% predictive ability. Next, partial least squares regression (PLS-R) was conducted to estimate the amount of adulterants (safflower) in the saffron samples. A good performance was found with Coefficient of Determination (R²) between 0.97 and 0.99. Compared to other techniques, the main advantage of the proposed methods are non-destructive, fast and sensitive which allows to achieve very precise and accurate results.

Keywords: Saffron, Geographical origin, Adulteration, FTIR spectroscopy


Submitted 12 April 2022, Resubmitted 03 June 2022, Accepted 07 June 2022, Available online June 2022.
INTRODUCTION

Saffron is the red dried stigma with attached yellowish style of *Crocus sativus* L., family Iridaceae (ISO 3632-1. 2011), and is regarded as one of the most extensively used and expensive spices, as 1 kg of saffron is obtained from about 150 000 flowers, which requires a lot of time and laborious handling and processing. The quality of the spice depends mainly on many factors such as geographical origin and varietal, which also has an important influence on its price.

Saffron is only available in a minority of countries and in limited quantities due to the particular growing conditions of saffron (climate, latitude and longitude), hence its name of red gold. Saffron has been cultivated in Morocco for centuries in the Taliouine area (Taroudant province), and more recently in the Tazenakht area (Ouarzazate province) and in other areas in Azilal. But the saffron of Taliouine is highly reputed at national and international level. It constitutes one of the main supports of the economy of the region.

In response to growing food safety concerns, food authenticity and traceability are becoming a vital issue and a major challenge for consumers and regulatory authorities. Foods with high nutritional and economic value are particularly important for food authenticity. The search for a fast and reliable method of food authentication has therefore become an important need of the food industry. For this reason, food scientists assist this type of research by developing analytical techniques to improve the ability to identify the geographical origin of foods and to detect their adulteration.

Producers and consumers are nowadays increasingly interested in high-quality food products with a clear geographical origin. Therefore, several methods are employed to assess the authenticity and geographical origin of saffron, such as spectroscopic techniques including ultraviolet-visible, mid infrared (MIR), nuclear magnetic resonance, and chromatographic techniques such as high and ultra-high-performance liquid chromatography, head-space-gas-chromatography, gas chromatography-mass spectrometry and high-performance thin-layer chromatography have been applied to ensure the quality control of saffron according to its chemical main components.

Each of the above analytical tools poses its own problems, such as they are costly, usually destructive, demand a high level of skill in technical knowledge of data interpretation, use of solvents and therefore require considerable time. Alternatively, FTIR spectroscopy is a non-destructive, rapid and sensitive tool involving minimal sample preparation. When coupled with multivariate calibrations, FTIR spectroscopy can be utilized as a strong tool for geographic origin determination and adulterant detection in saffron. Currently, coupling FTIR spectroscopy with the user-friendly sampling of attenuated total reflectance (ATR) is a novel technology for saffron analysis due to its potential as a “fingerprinting technique” for quantitative or qualitative analysis. Food analysis using FTIR spectroscopy also can be regarded as “green analytical chemistry” since this technique minimizes the use of chemical reagents that are harmful to human health and the environment.

The aim of the present work is to develop a rapid analytical approach based on the combination of MIR spectroscopy with multivariate analysis methods enabling to classify and predict Moroccan saffron according to their geographical origin and also, to detect and determine quickly saffron adulteration at levels of practical interest. The construction of a database by this technique constitutes a real novelty presenting considerable advantages in terms of rapidity and simplicity for the monitoring of the quality of Moroccan saffron.

MATERIALS AND METHODS

Sample preparation

A total of 230 saffron samples from five different regions of Morocco (Table I) were collected directly from farmers, guaranteeing their origin and the absence of fraud. All samples were purchased in the 2021 harvest season, packaged in boxes directly after harvest, and stored at room temperature. The samples were then transferred to the laboratory, where the analysis was performed at room temperature (25 °C, 60% relative humidity).
Table I. Geographic origin and number of Moroccan saffron samples employed in this work

<table>
<thead>
<tr>
<th>Code</th>
<th>Number of samples</th>
<th>Origin</th>
<th>Altitude (m)</th>
<th>Price (€/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>AIT-MAZIGH (Azilal province)</td>
<td>1100 - 1300</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>AIT-OUMDIS (Azilal province)</td>
<td>1100 - 1300</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>ATRGI (Azilal province)</td>
<td>1100 - 1300</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>TALIOUINE (Taroudant province)</td>
<td>1900 - 2200</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>TAZENAKHT (Ouarzazate province)</td>
<td>1450 - 1650</td>
<td>4</td>
</tr>
</tbody>
</table>

Safflower (*Carthamus tinctorius* L) was used as an adulterant of saffron powder due to its similar physical properties, low price, and lack of significant effects on human health.

The totality of samples (saffron and adulterants) was ground into a powder with an agate mortar, then stored in a dark room equipped with a dehumidifier to prevent humidity and ambient light.

To establish the geographical origin of five varieties of saffron, the calibration model was built with a set of 180 samples, and then validated by external validation with new 10 samples of each variety (new set of tests not used in the calibration of the models).

For the assessment of adulteration, a series of 43 samples was prepared by mixing authentic saffron (Taliouine origin) with adulterating safflower at different levels w/w (2.5% to 30%).

\[
\% \text{ Adulteration} = \frac{\text{mass of adulterant in sample}}{\text{total mass of sample}} \times 100
\]

**Spectral Acquisition**

For each MIR analysis, spectra were registered on a single reflection diamond ATR (JASCO FTIR 460 PLUS (Pike Technologies, Madison, USA)), by placing the saffron powder sample directly on the ATR cell (diamond crystal). A quantity of powder equivalent to one milligram provides good spectra, recorded from 4000 to 600 cm\(^{-1}\), by taking a total of 60 scans with a spectral resolution of 4 cm\(^{-1}\). After each measurement, the crystalline surface was washed with isopropanol solution and dried with a soft paper. The treatment of the obtained spectra was carried out with the software (Spectra manager) in order to eliminate the effect of moisture (at 3756 cm\(^{-1}\), 3652 cm\(^{-1}\), and 1595 cm\(^{-1}\)) and to eliminate the effect of carbon dioxide in three bands (at 2349 cm\(^{-1}\), 1388 cm\(^{-1}\), and 667 cm\(^{-1}\)).

**Data Analysis**

Multivariate statistics of the MIR spectra were performed using Unscrambler X, v10.4 (CAMO Software AS, Oslo, Norway, 2016). An exploratory analysis of the data is usually performed as the first step. For this purpose, PCA is generally used as a common unsupervised recognition method, to visualize the data to search for inconsistencies and aberrant values before performing regression techniques.\(^{19}\)

The geographical origin of saffron was traced in this study by using two algorithms, linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA).

LDA is a technique for supervised classification that builds on linear discriminant functions, maximizing the inter-class variance ratio and minimizing the intra-class variance ratio. It analyzes the different variables associated with a particular object and affects the object to a class or group according to the differences and similarities between the variables.\(^{20}\)

PLS-DA is a linear classification technique that integrates the properties of PLS regression and the discrimination capability of a classification approach. PLS-DA is based on the PLS regression algorithm (PLS1 when dealing with a single dependent Y variable and PLS2 when dealing with several dependent
The main advantage of PLS-DA is that the pertinent sources of data variability are modeled by the latent variables (LVs), representing the linear combinations of the original variables, and as a result it allows visualization and understanding of the different data patterns and linkages via the LV scores and loadings. The optimal number of LVs is commonly identified by using a cross-validation method to minimize the classification error.

The predictive quality of the LDA and PLS-DA model was evaluated by determining the performance indicators, including the sensitivity (number of real positive cases successfully identified), specificity (number of real negative cases successfully identified), the accuracy (proportion of correctly identified cases as true positives (TP) and true negatives (TN)), the precision (number of true positives among all individuals that were predicted to be positive) and the correct classification rate (CCR) which is considered as the overall accuracy.

\[
\text{Sensitivity}(\%) = \frac{TP}{TP + FN} \times 100 \\
\text{Specificity}(\%) = \frac{TN}{TN + FP} \times 100 \\
\text{Accuracy}(\%) = 1 - \frac{FP + FN}{TP + TN + FP + FN} \times 100 \\
\text{Precision}(\%) = \frac{TP}{TP + FP} \times 100 \\
\text{CCR}(\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100
\]

For quantitative analysis partial least squares regression (PLS-R) was applied to build a calibration model able to predict saffron adulteration, it is recognized as a standard technique for calibration and prediction using the relationship between X and Y matrices. It is widely employed in fluorescence, Raman and infrared spectroscopy for the prediction and quantification of some chemical parameters in agri-food.

To examine the predictive ability of these models, we considered the Coefficient of Determination ($R^2$), root mean square error of calibration (RMSEC) and root mean square error of cross-validation (RMSECV). The RMSEC, RMSECV values of a strong model must be low and the $R^2$ should be high.

\[
\text{RMSEC} = \sqrt{\frac{\sum (Y_i - \bar{Y}_i)^2}{A - 1}} \\
\text{RMSECV} = \sqrt{\frac{\sum (Y_i - \bar{Y}_i)^2}{B - 1}}
\]

\[
R^2 = 1 - \frac{\text{RSS}}{\text{TSS}}
\]

Where:
- $Y_i$ and $\bar{Y}_i$ indicate the actual and predicted values, while A and B indicate the number of samples used in the calibration and cross-validation datasets.
- RSS represents the residual sum of squares and TSS represents the total sum of squares.

RESULTS AND DISCUSSION

Spectra Analysis

The MIR spectra of all samples were similar by visual inspection and presented the same profile over the full wavelength range (Figure 1), indicating that there are no significant qualitative differences between the saffron samples. All spectra show common absorption bands as illustrated in Table II. In the spectra, however, small differences are observed between 1800 and 700 cm$^{-1}$ in the relative absorbance intensities of the different cultivars. These minor differences in relative intensity values were exploited in the classification for the determination of the geographical origin of saffron, as discussed below.
The multivariate analysis method is nowadays an essential chemometric tool to analyze data coming from many observations performed on numerous variables. The main objective of classification is to find a mathematical relationship between a set of descriptive variables (such as physico-chemical characteristics, spectral measurements, etc.) and a qualitative variable (sample categories). In this study, two classification approaches such as LDA and PLS-DA were employed to construct classification models of saffron origin.

<table>
<thead>
<tr>
<th>Location of wave numbers (cm⁻¹)</th>
<th>Vibrations</th>
<th>Band assignment of functional group/component</th>
</tr>
</thead>
<tbody>
<tr>
<td>3400 (Broad)</td>
<td>O-H stretch</td>
<td>Hydroxy group</td>
</tr>
<tr>
<td>2900 and 3000</td>
<td>C-H asymmetric and symmetric stretching</td>
<td>Aliphatic methylene group</td>
</tr>
<tr>
<td>1800 -1500</td>
<td>C=O stretch</td>
<td>crocetin / aminoacids</td>
</tr>
<tr>
<td>1645</td>
<td>C=C and C=O stretch</td>
<td>Picrocrocin / amide I</td>
</tr>
<tr>
<td>600 - 1500 (fingerprint region)</td>
<td>–CH2–, CH3–, –OH, C–C, C–O, C–O–C groups</td>
<td>glycosidic linkages of oligo and polysaccharides or in triacylglycerols</td>
</tr>
</tbody>
</table>
**Geographical origin discrimination**

**Principal component analysis**

Principal component analysis (PCA) was applied to the MIR spectral results to investigate the structure of the saffron data and also to study the similarity between individuals. The PCA shows that the first three principal components represent 93% of the total variability of the data, the observation of the score plot Figure 2 shows the presence of clusters of saffron samples according to their geographical origin, we can distinguish the presence of five groups indicating that the samples belonging to each of the five groups had similar FT-MIR properties.

![Figure 2. Score plot of the PCA model with three principal components (PC1-PC2-PC3) for the saffron MIR data. The first three PCs can explain more than 90% of the variation.](image)

**Linear discriminant analysis (LDA)**

The linear discriminant analysis was applied to the MIR spectra of saffron samples, previously processed by principal component analysis (PCA). LDA was applied on the first 10 principal components obtained by PCA. The application of this method shows good discrimination between the 5 groups of saffron coming from different regions of Morocco (Figure 3). This plot shows that groups 1, 2, and 3 are close to each other while groups 4 and 5 are far away, this remark is explained by the distance that separates these regions, the more the distance is great more inter-group variability is high the more the groups are well separated in the discrimination plot obtained by LDA and vice versa.
Figure 3. LDA scores plot in the analysis of the FTIR spectra of saffron samples. A strong discrimination of five varieties is displayed by an accuracy of 100%.

The use of the LDA method demonstrates successful results for the classification of different saffron samples according to their geographical origin using MIR spectral data, this discrimination capacity is summarized in Table III in which it can be observed that this model is able to classify the five saffron groups with specificity, sensitivity, and CCR of 100%.

Table III. LDA confusion matrix on the test saffron data set, including the calculation of the calibration performance (sensitivity, precision, specificity, accuracy and CCR)

<table>
<thead>
<tr>
<th>Confusion matrix</th>
<th>Actual</th>
<th>Sensitivity (%)</th>
<th>Precision (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>CCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Predicted</td>
<td>1 39 0 0 0 0 100 100 100 100 100</td>
<td>2 0 33 0 0 0 100 100 100 100 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 0 0 39 0 0 100 100 100 100 100</td>
<td>4 0 0 0 37 0 100 100 100 100 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 0 0 0 0 33 100 100 100 100 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These results reveal that the developed model successfully classify the five groups of saffron according to their geographical origin with an accuracy of 100%. Authentications were based on various discriminant variables including crocins, picrocrocin, triacylglycerols, oligo, and polysaccharides.
Partial Least Square-Discriminate Analysis (PLS-DA)

The PLS-DA model was built considering the FTIR spectra as X variables, while the Y variables were associated with the five different saffron classes (one different Y variable for each group's class, with 1 or 0 depending on whether it belongs or not to the considered data group).

The use of the PLS-DA method (on the first 10 principal components) shows a high capacity for the classification of the saffron groups according to their geographical origin as shown in Figure 4 in which we observe clear and perfect discrimination between the saffron groups following the PC2-PC5 score plot.

![Scores](image)

**Figure 4.** 2D scores plot of PLS-DA in the analysis of the FTIR spectra of saffron samples. Group 1, 4 and 5 are well separated while group 2 and 3 are not far apart on the figure.

This discrimination ability is represented by the high value of the sensitivity, specificity, and accuracy which reach 100%, indicating that all samples are correctly assigned to their classe (Table IV).

**Table IV.** Confusion matrix for the classification five types of saffron samples using the PLS-DA method

<table>
<thead>
<tr>
<th>Confusion matrix</th>
<th>Actual</th>
<th>Sensitivity (%)</th>
<th>Precision (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>CCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>39 0 0 0 0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0 33 0 0 0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0 0 39 0 0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0 0 0 37 0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0 0 0 0 33</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

As shown in the loading plot (Figure 5), the most important absorbances are respectively situated between 900 and 1700 cm\(^{-1}\) corresponding to the -CH\(_2\)-, CH\(_3\)-, -OH, C-C, C–O and C-O-C groups.
Validation of discriminant Models

The classification and predictive ability of the constructed models was verified by external validation of the models with samples other than those used for models construction (10 samples for each group).

According to the PLS-DA rules when the predicted value of Y is between 0.5 and 1.5 for a class, the sample is regarded as belonging to this class.\cite{33}

The confusion matrix (Table V) achieved by external validation shows that for the validation samples, a 100% correct classification was achieved and all the saffron spectra were correctly matched to the five corresponding classes. These results confirm that the predictive ability of PLS-DA and LDA models was satisfactory good. These results demonstrated the ability of the proposed technique to discriminate between the five different cultivars of saffron samples used in this study.
Table V. Results of the external validation of the PLS-DA and LDA model of the saffron samples

<table>
<thead>
<tr>
<th>Confusion matrix</th>
<th>Actual set</th>
<th>Sensitivity (%)</th>
<th>Precision (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>CCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted set</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PCA-LDA)</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Predicted set</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PLS-DA)</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

According to the obtained results, the geographical origin determination of the five groups of saffron has been well done based on the MIR spectral data, since the application of the PLS-DA and LDA approaches shows a great discrimination performance, which revealed no misclassification in all cases.

Detection of adulteration by Partial Least Square regression (PLS-R)

The development of a quantification model based on PLS regression can therefore extend the potential of this work for the control of saffron falsification using MIR spectroscopy.

The prediction of the added adulterant (safflower) in saffron powder was performed using a PLS-R model. 43 adulterated saffron samples were used for the creation of the calibration model, and 10 new adulterated samples were used for the validation of this model. The linearity of the method was evaluated in the PLS-R calibration model to demonstrate that the predictor variables (band intensity) were proportionally related to the percentage of adulterants.

According to the results obtained by the PLS regression we observed that there is a good fit between the reference values and the prediction values (Figure 6). This ability is displayed by the low value of the root mean square error of calibration (RMSEC=1.59), cross-validation (RMSECV=2.45) and the high value of R-square for the calibration (R²cal=0.97) and cross-validation (R²cv=0.92). Based on the results obtained by the cross validation 7 latent variables were selected to build the quantification model.
In order to evaluate the capacity of the model for accurate prediction of the adulteration rate an external validation is performed. The validation results (Table VI) show a strong capacity for the prediction of the adulteration rate. This capacity is represented by the R-square of prediction which reaches 0.95 and the low value of RMSEP=1.75.

**Table VI.** Prediction of adulterant content (safflower) by external validation of PLS-R model

<table>
<thead>
<tr>
<th>Reference (w/w)</th>
<th>Predicted (w/w)</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.20</td>
<td>1.75</td>
<td>1.94</td>
</tr>
<tr>
<td>4.1</td>
<td>3.11</td>
<td>1.72</td>
</tr>
<tr>
<td>5.5</td>
<td>2.8</td>
<td>2.53</td>
</tr>
<tr>
<td>7.2</td>
<td>6.68</td>
<td>1.75</td>
</tr>
<tr>
<td>11.5</td>
<td>13.39</td>
<td>1.95</td>
</tr>
<tr>
<td>14</td>
<td>15.85</td>
<td>2.95</td>
</tr>
<tr>
<td>16.31</td>
<td>15.97</td>
<td>2.31</td>
</tr>
<tr>
<td>20.4</td>
<td>18.27</td>
<td>1.71</td>
</tr>
<tr>
<td>21.2</td>
<td>24.20</td>
<td>1.98</td>
</tr>
<tr>
<td>31.2</td>
<td>32.32</td>
<td>3.16</td>
</tr>
</tbody>
</table>

In the light of the results obtained, the strategy proposed in this study is not only applicable to determine whether a saffron sample is adulterated or not, but it can also be used to assess the level of adulteration of samples.
In comparing the results of this study with previous research, it is important to underline that the performance criteria (accuracy, specificity and sensitivity) in this study were the highest compared to the other studies.\textsuperscript{34,35,18} However, compared to previous work, a higher number of saffron samples in this work were utilized (n=230). Furthermore, it should be noted that it is for the first time that the authentication of the varieties of Moroccan saffron was carried out by this technique, thus this work will make it possible to build a database for the control of the quality of Moroccan saffron.

**CONCLUSION**

This project proves that the use of FTIR spectroscopy, combined with PLS-DA and LDA algorithms, is able to classify saffron according to its geographical origin. Sample analysis was carried out directly on the saffron powder to provide a fingerprint of the saffron without the preparation of the sample. This approach represents a practical and easy tool to verify the origin of saffron, offering advantages such as speed and ease of use, and liable to represent a good alternative to the method of DNA markers.

The combination of FTIR spectroscopy and PLS-R algorithm reveals to be effective to determine and identify adulteration in saffron, since it is difficult to detect saffron fraud according to ISO 3632 methods, in particular when using identical-looking plants for adulteration and when the spice is marketed as a powder.

As a result, the combined use of MIR spectroscopy and multivariate data analysis was confirmed as a powerful and valid tool to evaluate the authenticity and quality control of saffron by offering unique fingerprint spectra. However, it is necessary to dispose of more robust origin models, able to better detect regional varieties. Specifically, a large dataset representing high variability should be analyzed to create a comprehensive database of Moroccan saffron varieties (geographic origin, harvest year).

**Conflicts of interest**

The authors have declared no conflicts of interest for this article.

**Acknowledgements**

The authors are grateful to the laboratory of analytical chemistry, for providing the equipment for the studies.

**Funding**

No funds, grants, or other support was received.

**REFERENCES**


