



10 ^{AL} 13 ^{TO} MAYO
MAY / 2023

21ª
FERIA INTERNACIONAL
DEL ACEITE DE OLIVA
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FTIR coupled with chemometrics as a non-invasive tool for PDO olive oils' discrimination

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ABSTRACT

Quality schemes protect the diversity of traditional European foods, such as the Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI). Only the olive oils from extra virgin and virgin commercial categories can be labelled with a PDO or PGI. In Portugal, currently, there are six PDOs. Olive oils labelled as PDO present a superior chemical-sensory quality. Nevertheless, from a commercial point of view it is of paramount importance to be able to identify them according to the correct label, avoiding fraud and ensuring the consumer regarding the exact origin of the purchased oil. Non-invasive and fast techniques, like Fourier transform infrared spectroscopy (FTIR), have been applied to assess olive oil origin and to detect fraud and adulterations. Thus, this work aimed to use FTIR spectra coupled with linear discriminant analysis-simulated annealing algorithm (LDA-SA) to classify commercial olive oils belonging to three Portuguese PDOs, namely, 'Alentejo Interior', 'Beira Interior', and 'Trás-os-Montes'. The results showed that a FTIR-LDA-SA model could classify 30 independent oils according to the correct PDO with a sensitivity and specificity of 100% (training, leave-one-out cross-validation) and a sensitivity of 97.5% for the repeated K-fold cross-validation, based on the transmittance values recorded at six selected wavenumbers.

Keywords: *olive oil, quality schemes, FTIR, authenticity.*

INTRODUCTION

There is an increasing demand from consumers for products with beneficial, nutritional and sensory differentiated characteristics, which in some cases are related to their geographical origin (Hajdukiewicz, 2014).

The identification and protection of the designations of agricultural products and foods, with specific quality schemes were launched by the European Union as part of its complex agricultural quality policy, implementing the Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Speciality Guaranteed (TSG). These schemes were created to protect the identity and the differentiating quality that are strongly related to the product's geographical origin and its natural and human characteristics (Castelló, 2020).

Virgin olive oil is the oil extracted from freshly harvested healthy fruits of the olive tree (*Olea europaea* L.), through different mechanical processes, preserving their physicochemical and sensorial characteristics. The benefits that come from its consumption have been gaining interest on the part of the consumer due to its antioxidant properties, high content of monounsaturated fatty acids, and differentiated sensory characteristics (Lolis et al., 2020).

Only olive oils belonging to the extra virgin and virgin commercial categories (EVOO and VOO, respectively) can be certified as PDO or PGI, provided they comply with Commission Delegated Regulation (EU) 2022/2104.

In Portugal, from the 377 234 hectares occupied by olive groves, 98.9% produce olives from oil extraction, being Alentejo as the main producing region, followed by Trás-os-Montes and Beiras regions (INE, 2022). In this country, six PDOs distributed throughout the national territory, were

created for olive oil, namely the PDOs of 'Trás-os-Montes', 'Beira Interior', 'Ribatejo', 'Norte Alentejano', 'Moura', and 'Alentejo Interior'.

Olive oil is a highly valued food product, which price has been increasing due to the high worldwide demand, making it susceptible to frauds and adulterations. A common practice is mixing high-quality olive oils with other vegetable oils or lower-quality oils to obtain higher profit (Uncu et al., 2019).

Several studies describe techniques and methods to identify adulterations in oils through sensory analysis, chemical analysis, chromatography (fatty acids, tocopherol), spectroscopy, electronic nose, electronic tongue and DNA-based methods (Meng et al., 2023).

Spectroscopy-based methodologies, like FTIR, can be an asset, being a key non-invasive/non-destructive tool for the traceability and authenticity of foods, showing high precision and sensitivity, low cost, fast analysis, not requiring any sample pre-treatment (Hou et al., 2021; Lamas et al., 2021; Vieira et al., 2021).

In this context, olive oils from three Portuguese PDOs ('Trás-os-Montes', 'Beira Interior', and 'Alentejo Interior'), were studied. The oils' spectra (i.e., transmittance values) were acquired by FTIR spectroscopy, in the wavenumber region of 4000 and 500 cm^{-1} . The transmittance data were further used with LDA-SA to develop a multivariate classification model based on the spectral information gathered by the minimum number of wavenumbers, in order to obtain the most robust and non-redundant predictive model.

MATERIAL AND METHODS

PDO commercial olive oils from 'Trás-os-Montes', 'Beira Interior', and 'Alentejo Interior' were gathered from certified olive oil producers within each of the referred geographical regions. From each region, 10 independent oils were obtained.

FTIR analysis was carried out using an MB300 FTIR from ABB (Zurich, Switzerland) operating in attenuated total reflectance (ATR) mode using a horizontal cell equipped with a diamond crystal. The spectra acquisition was done using 32 scans/min at a resolution of 4 cm^{-1} , being the reading range between 4000 and 500 cm^{-1} . Spectra were acquired and treated using the software Horizon MB version 3.4. The background was acquired every two consecutive assays. The spectra were acquired in duplicate for each olive oil (30 in total) for two sub-samples collected from each of the ten oil independent samples (bottles).

Finally, raw transmittance values of the FTIR spectra, recorded during the olive oils spectroscopic analysis, were used for statistical analysis. Linear discriminant analysis (LDA) was applied to establish multivariate classification models based on the spectral information related to subsets of wavenumbers selected using the simulated annealing (SA) variable selection algorithm. The LDA classification was discussed based on the leave-one-out cross-validation (LOO-CV) and the repeated K-fold-CV (4 folds \times 10 repeats), allowing this latter variant to keep 25% of the dataset for validation, being used, at each repeat, the remaining 75% of the data for training purposes. The predictive capability was checked by calculating the sensitivity, the specificity and, visually, by plotting the class membership ellipses, computed by the Bayes' theorem (Bishop, 2006), for the first two main discriminant functions (DFs). The open-source statistical program R (version 3.6.2) was used.

RESULTS AND DISCUSSION

As can be inferred from **Figure 1**, the vibration bands of the FTIR spectra recorded for the studied Portuguese olive oils of the three PDOs were in line with those reported in the literature for other Portuguese olive oils from different cultivars and geographical origins, namely from the geographical regions studied in the present work (Machado et al., 2017; Lamas et al., 2021; Rodrigues et al., 2023). Two main absorption regions could be identified in all olive oils, namely between 3100 and 2750 cm^{-1} and 1850–600 cm^{-1} , showing typical bands that could be related to the known vibration modes of the molecular bonds (Üçüncüoğlu & Küçük, 2019; Uncu et al., 2019; Socaciu et al., 2020).

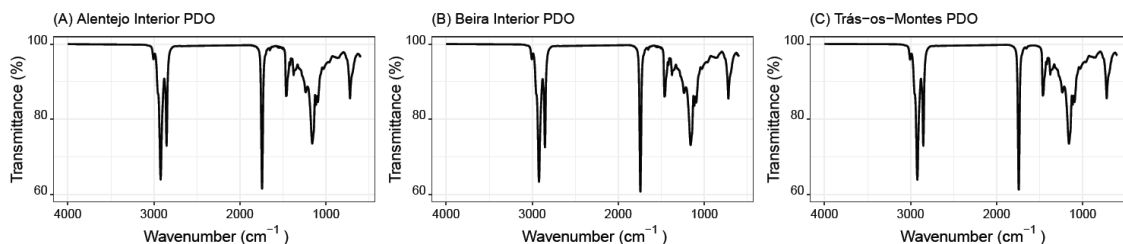


Figure 1. FTIR spectra: transmittance (%) values recorded for olive oils from three Portuguese PDOs olive oils in the wavenumber region of 4000 to 500 cm^{-1} . (A) 'Alentejo Interior', (B) 'Beira Interior', and (C) 'Trás-os-Montes'.

The transmittance raw data (**Figure 1**) were further treated by applying LDA-SA allowing to establish a classification model based on the spectral information related to 6 selected wavenumbers (868, 872, 1126, 1134, 1724, and 2881 cm^{-1}). The model allowed to correct classify all the olive oils according to the certified PDO (100% of sensitivity and specificity), for training (**Figure 2**) and LOO-CV internal validation. The predictive classification performance was further checked with the repeated K-fold CV variant. A correct classification average rate of 97.5% was obtained for the 40 random subsets of data used (4 folds \times 10 repeats, in which one, 25% of the data, i.e., 2-3 olive oils per PDO region, were kept for assessing the predictive performance of the model established using the remaining 75% of the data), confirming the potential use of the proposed FTIR-chemometric approach. It should be highlighted that the few misclassifications observed were related to olive oils from 'Beira Interior' and 'Trás-os-Montes', being all oils from 'Alentejo Interior' successfully classified. However, it is expected that the correct classification rate could be even enhanced either by including the transmittance information of some extra wavenumbers or by developing multivariate classification models based on data related to the 1st or 2nd derivatives of the raw spectral data.

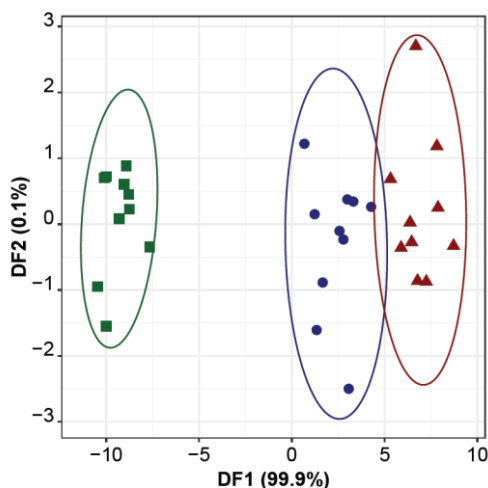


Figure 2. PDO olive oils supervised discrimination (2D LDA-SA plots) achieved with the FTIR-LDA-SA model based on the transmittance (%) data recorded at six wavenumbers selected by the SA algorithm; ■ 'Alentejo Interior' PDO's olive oils; ● 'Beira Interior' PDO's olive oils; and, ▲ 'Trás-os-Montes' PDO's olive oils.

The overall results clearly pointed out the feasibility of applying the developed FTIR- LDA-SA model as a predictive tool to identify the correct PDO of the studied Portuguese olive oils, confirming that FTIR is a powerful non-invasive tool for olive oil authentication in terms of geographical origin.

ACKNOWLEDGMENTS

The authors thank the Foundation for Science and Technology (FCT, Portugal) for the financial support of the national funds FCT/MCTES to CIMO (UIDB/00690/2020 and UIDP/00690/2020) unit and to the Associate Laboratory SusTEC (LA/P/0007/2020). Nuno Rodrigues thanks the FCT-Foundation for Science and Technology, P.I., for the National funding through the institutional program contract for scientific employment. Sandra Lamas also acknowledges the Ph.D. research grant (2022.10070.BD) provided by FCT.

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