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Application of the FTIR technique as a non-invasive tool to discriminate Portuguese olive oils with Protected Designation of Origin

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Three Portuguese olive oils with PDO ('Azeite do Alentejo Interior', 'Azeites da Beira Interior' and 'Azeite de Trás-os-Montes') were studied considering their physicochemical quality, antioxidant capacity, oxidative stability, total phenols content, gustatory sensory sensations and Fourier transform infrared (FTIR) spectra. All oils fulfilled the legal thresholds of EVOOs and the PDO's specifications. Olive oils from 'Azeite da Beira Interior' and 'Azeite de Trás-os-Montes' showed greater total phenols contents and antioxidant capacities, while 'Azeites da Beira Interior' presented higher oxidative stabilities. Linear discriminant models were developed using FTIR spectra (transmittance and the 1st and 2nd derivatives), allowing the correct identification of the oils' PDO (100% sensitivity and specificity, repeated K-fold-CV). This study also revealed that multiple linear regression models, based on FTIR transmittance data, could predict the sweet, bitter, and pungent intensities of the PDO oils ($R^2 \geq 0.979 \pm 0.016$; RMSE $\leq 0.26 \pm 0.05$, repeated K-fold-CV). This demonstrates the potential of using FTIR as a non-destructive technique for authenticating oils with PDO.

Introduction

The increasing concern with food safety, health, and environmental issues has increased the demand for products with beneficial and differentiated nutritional and sensory properties, often related to their geographical origin. To recognise and valorise the products' origin, European Union implemented labels like the Protected Designation of Origin (PDO), the Protected Geographical Indication (PGI) and the Traditional Specialty Guaranteed (TSG).[1] These labels guarantee that consumers purchase food with a differentiating quality strongly related to the specific natural and human factors of the region where it is produced. Specifically, PDO label is used for products whose differentiated quality characteristics depend exclusively on the region where they are produced, such as cheeses, wines, olive oils and other gastronomic specialities.[1] This certification is a guarantee of the product's quality and genuineness, as well as of its geographical origin. At the same time, PDOs help preserve the tradition and authenticity of ancient production techniques in specific regions, promoting these regions' economy, development and sustainability. These standards impose strict rules for production, processing, and labelling, which help to guarantee the authenticity and quality of products for consumers,[1] contributing to combat fraud.

In Europe, there are a total of 113 PDOs and 22 PGI registered for olive oils distributed by different countries and regions,[2] certifying olive oils belonging to the extra virgin or virgin commercial categories (EVOO and VOO, respectively) as PDO or PGI.[3]

In Portugal, olive growing is a relevant economic activity. In total, 377,234 hectares are occupied by olive groves, with 98.9% of the production destined for oil extraction. 'Alentejo' is the main producing region (82.1%), followed by 'Trás-os-Montes' (9.0%) and 'Beira Interior' (8.4%).[4] Six PDOs are registered for Portuguese olive oils, distributed throughout the national territory, namely the PDOs of 'Azeite de Trás-os-Montes', 'Azeites da Beira Interior', 'Azeite do Ribatejo', 'Azeite do Norte Alentejano', 'Azeite de Moura' and 'Azeite do Alentejo Interior'. Therefore, at both a national and international level, it is of utmost relevance to be able to guarantee olive oils geographical origin and quality, to ensure the consumers' confidence when purchasing this product, as well as for market transparency. Indeed, olive oil is one of the most adulterated products in the world.[5] Thus, several studies describe the development of methodologies to identify adulterations of olive oils, as well as their geographical and/or botanical origin, based on sensory analysis, chemical analysis, spectroscopy, electrochemistry, and nuclear magnetic resonance.[6–8] For example, recently, a lab-made electronic nose with metal oxide semiconductor sensors allowed discriminating Portuguese olive oils according to the respective production region.[9] Other studies described the use of olive oils' volatile profiles, established by solid-phase micro-extraction (SPME) coupled

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with gas chromatography–mass spectrometry (GC–MS), for assessing their geographical origin.[10–12] However, most of these tools are invasive/destructive techniques, expensive, time-consuming, and require skilled technicians.

Currently, spectroscopy-based techniques have proven to be a key tool for food traceability and authenticity. These techniques have high precision and sensitivity, are low cost and allow a fast analysis, possess non-destructive characteristics, and do not require complex sample preparation procedures, providing spectral data that can be related to the olive oils' chemical composition.[13–16] Spectroscopy-based techniques, like Fourier Transform Infrared Spectroscopy (FTIR), have been widely used in the analysis of olive oils, efficiently assessing their authenticity and quality. FTIR permits the identification of different chemical components present in olive oil, such as fatty acids, sterols, phenols, and other important compounds, for its characterization.[17] Thus, the analysis of olive oils using the FTIR technique can be a valuable tool for identifying frauds and adulterations, namely to evaluate the authenticity and quality of the oils.[18–19] In this context, olive oils from three Portuguese PDOs ('Azeite do Alentejo Interior', 'Azeites da Beira Interior' and 'Azeite de Trás-os-Montes') were studied with respect to their quality physicochemical parameters, antioxidant capacity, oxidative stability, and total phenol content. Additionally, FTIR analysis was performed to evaluate its potential as a tool to assess the authenticity of the olive oils' labels regarding their geographical origin. The oils' FTIR spectra (i.e., transmittance values) were acquired in the wavenumber region from 4000 to 500 cm^{-1}. The transmittance data were further treated by linear discriminant analysis (LDA) coupled with the simulated annealing (SA) algorithm to develop multivariate classification models using spectral data, or their derivatives, based on a minimum number of selected wavenumbers, to obtain the most robust and non-redundant predictive models.

Results and Discussion

Physicochemical quality of PDO olive oils

The minimum-maximum values of the physicochemical quality data (FA, PV, K_{232} and K_{268}) for the studied olive oils (10 independent oils per region) are shown in Table 1. The maximum limit values are also presented according to the specifications of each PDO declaration and according to the EU requirements for EVOO classification. It can be verified that all oils fulfilled the legal specifications of each PDO's declaration as well as the legal thresholds for being classified as EVOO.[3] A large amplitude was observed for each studied parameter and PDO oil (Table 1), even if all of them could be classified as EVOOs. This highlights the intrinsic variability of the olive oils' composition and quality for each geographical origin, tentatively attributed to the fact that, according to the legal specifications, it is allowed to obtain a PDO oil with olives from different cultivars and at different contents. Moreover, these amplitudes could also arise since the studied PDO olive oils were collected from different producers of each demarcated geographical region, which could be extracted from fruits at different stages of maturation, grown in different olive groves and also with different quality degrees, for example, levels of pests and diseases attacks. In fact, the oils' quality parameters are known to be greatly related to the quality of the olive fruits, harvesting practices, transport and storage conditions before extraction, and the oil extraction process (e.g., malaxation time and temperature).

Antioxidant capacity, oxidative stability, and total phenols content

Figure 1 shows the antioxidant capacity, OS, and TPC of the studied olive oils. As can be inferred, the geographical origin significantly influenced those parameters. 'Azeites da Beira Interior' and 'Azeite de Trás-os-Montes' olive oils showed similar average DPPH activities (33 ± 13 and 34 ± 8 %, respectively) and TPC (360 ± 138 and 412 ± 84 mg GAE/kg, respectively), which were significantly greater than those determined for 'Azeite do Alentejo Interior' olive oils (15 ± 2 % and 137 ± 9 mg GAE/kg). Furthermore, 'Azeites da Beira Interior' olive oils showed a higher stability, with average OS values (15 ± 4 h) significantly greater the other two PDO olive oils ('Azeite de Trás-os-Montes': 11 ± 1 h; 'Azeite do Alentejo Interior': 10 ± 1 h).

The characteristics of the studied PDOs olive oils (i.e., 'Azeite do Alentejo Interior', 'Azeites da Beira Interior', and 'Azeite de Trás-os-Montes' oils) are, in general, in-line with those reported in the literature for other olive oils produced in the same

Table 1. Minimum and maximum values of free acidity, peroxide value, and extinction coefficients of olive oils from PDOs 'Alentejo Interior', 'Beira Interior', and 'Trás-os-Montes'.

Quality parameters	PDOs' olive oils									
	'Azeite do Alentejo Interior'			'Azeites da Beira Interior'			'Azeite de Trás-os-Montes'			Reg. (EU) 2022/2104[d]
	Min	Max	Spec.[a]	Min	Max	Spec.[b]	Min	Max	Spec.[c]	
FA (g oleic acid/100 g)	0.34	0.45	≤0.80	0.17	0.45	n.a.	0.17	0.23	≤1	≤0.80
PV (mEq O_2/kg oil)	4.14	7.50	≤15.0	3.32	6.64	≤19.0	2.49	5.83	≤15	≤20.0
K_{232}	1.70	2.14	≤2.40	1.59	2.09	n.a.	1.61	2.03	≤2.00	≤2.50
K_{268}	0.15	0.18	≤0.20	0.11	0.16	n.a.	0.12	0.14	≤0.20	≤0.22

Min: minimum value; Max: maximum value; Spec.: limit values referring to the specifications; Spec.:[a][20] Spec.:[b][21] Spec :[c][22] Reg. (EU) 2022/2104:[d] n.a.: information not available (i.e., not established from the PDO's specifications).

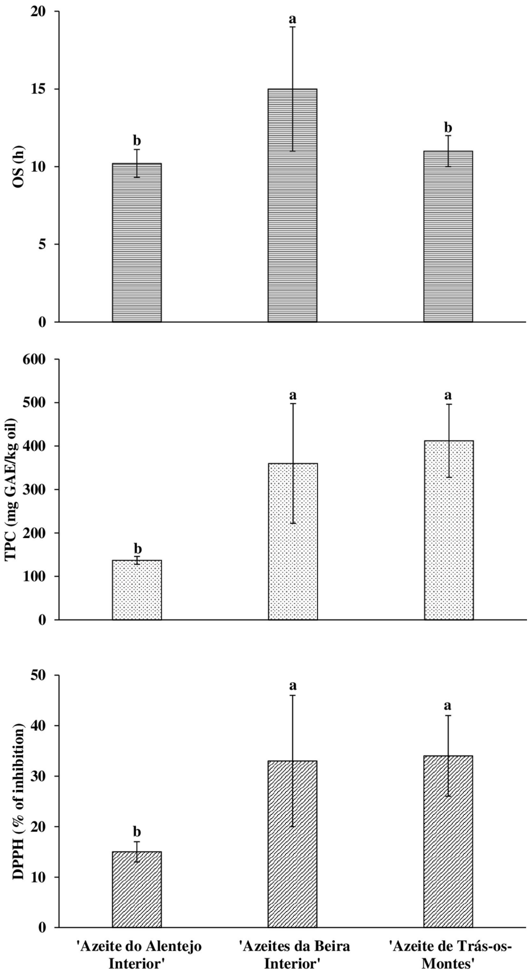

Figure 1. Oxidative stability (OS), total phenols contents (TPC) and DPPH (%) of three Portuguese PDO's olive oils: 'Azeite do Alentejo Interior', 'Azeites da Beira Interior', and 'Azeite de Trás-os-Montes'.

geographical regions and from olives of the predominant cultivar of each PDO (cv. 'Galega vulgar' predominates in the PDOs of 'Azeite do Alentejo Interior', and 'Azeites da Beira Interior'; while cv. 'Verdeal Transmontana' is predominant in the PDO of 'Azeite de Trás-os-Montes'). For example, TPC values of 118.0 ± 3.9, 657.2, and 505 ± 188 mg GAE/kg were previously reported for oils produced in the region of Alentejo, with PDO olive oil where the cv. Galega Vulgar predominates,[29] 'Azeites da Beira Interior', where cv. Galega Vulgar is also used,[18] and 'Azeite de Trás-os-Montes', with a different range of cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana) without the inclusion of cv. Galega Vulgar,[9] respectively. It should be highlighted that, only for the region of Beira Interior, the literature values were higher than those found in the present study.

In which concerns the antioxidant capacity assessed by the DPPH method, expressed in % of inhibition, the values reported in Figure 1 (ranging from 15 ± 2% to 34 ± 8%), are low compared with those reported for PDO olive oils from Trás-os-Montes, produced from olives from cv. Verdeal Transmontana (44.9 ± 1.3 %),[30] or olive oils extracted from olives of cv. Santulhana (55 ± 7%).[31]

Regarding the OS, the average values found for the three PDO's oils (varying from 11 ± 1 to 15 ± 4 h) are similar or slightly greater than those previously reported for oils produced in Trás-os-Montes region (~12 h) when PDO olive oils from 'Azeite de Trás-os-Montes' were studied (extracted mainly from olives of cvs. Cobrançosa and Verdeal Transmontana, with a 10% percentage of olives of cv. Madural),[25] in Beira region (~17 ± 7 h),[18] or in Alentejo region (~6 h) (extracted from olives of cv. Galega Vulgar).[5]

However, as said before, it should be emphasised that TPC, DPPH and OS values greatly depend on several factors, these factors greatly influence the behaviour and shelf life of the olive oils.

ATR-FTIR spectroscopy analysis and relationship with gustatory sensations

The main vibration bands of the FTIR spectra (Figure 2, corresponding to the mean spectra values for the 10 independent samples of each PDO) recorded for the PDOs' olive oils from 'Azeite do Alentejo Interior', 'Azeites da Beira Interior', and 'Azeite de Trás-os-Montes', were the same previously described for other olive oils.[14,32,33] In fact, the transmittance bands were observed within the same wavenumber regions, i.e., from 3100–2750 cm^{-1} and 1850–600 cm^{-1}.[33–35]

In more detail, olive oil raw FTIR spectra (transmittance data) showed characteristic bands at different wavenumbers, namely the band at 656 cm^{-1} related to the bending out of plane vibration of cis $-HC{=}CH-$ group of disubstituted olefins. Other typical bands were identified, namely in the regions: 1136–1047 cm^{-1}, which can be associated to the stretching vibrations of saturated $-C-O-$ aliphatic ester groups; 1200–1146 cm^{-1} due to the stretching vibration of $-C-O-$ acetate groups or of the bending vibration of $-CH_2-$; 1298–1259 cm^{-1} attributed to the bending vibration of cis $=C-H$; 1761–1720 cm^{-1} related to the stretching of the $-C{=}O$ functional group of ester compounds or to the stretching of acid $-C{=}O$; and 2841–2770 cm^{-1} that can be explained by the stretching of trans $=C-H$ group or by the stretching of cis $=C-H$ group.[14,32,34]

Figure 2 shows that, for the studied oils, the ATR-FTIR spectra and the respective 1st and 2nd derivatives have similar spectral profiles in the region of 4000 to 500 cm^{-1}. Nevertheless, looking at the spectral fingerprint region intervals (3100–2750 cm^{-1} and 1850–600 cm^{-1}), the transmittance and the respective transformed derivative values at some specific wavenumbers have slightly different intensities, probably related to differences in the chemical composition of the oils (e.g., phenolic compounds, tocopherols, carotenoids, and sterols), which could be used for olive oils' discrimination. Indeed, peaks in the regions of 3400–3200 cm^{-1} are typical of polyphenolic compounds and acids;[36] whereas peaks in the regions of 2850–2750 cm^{-1}, 1820–1660 cm^{-1}, and 1300–1000 cm^{-1} can be related to aldehydes, carbonyls, and esters functional groups, respectively.[37–38]

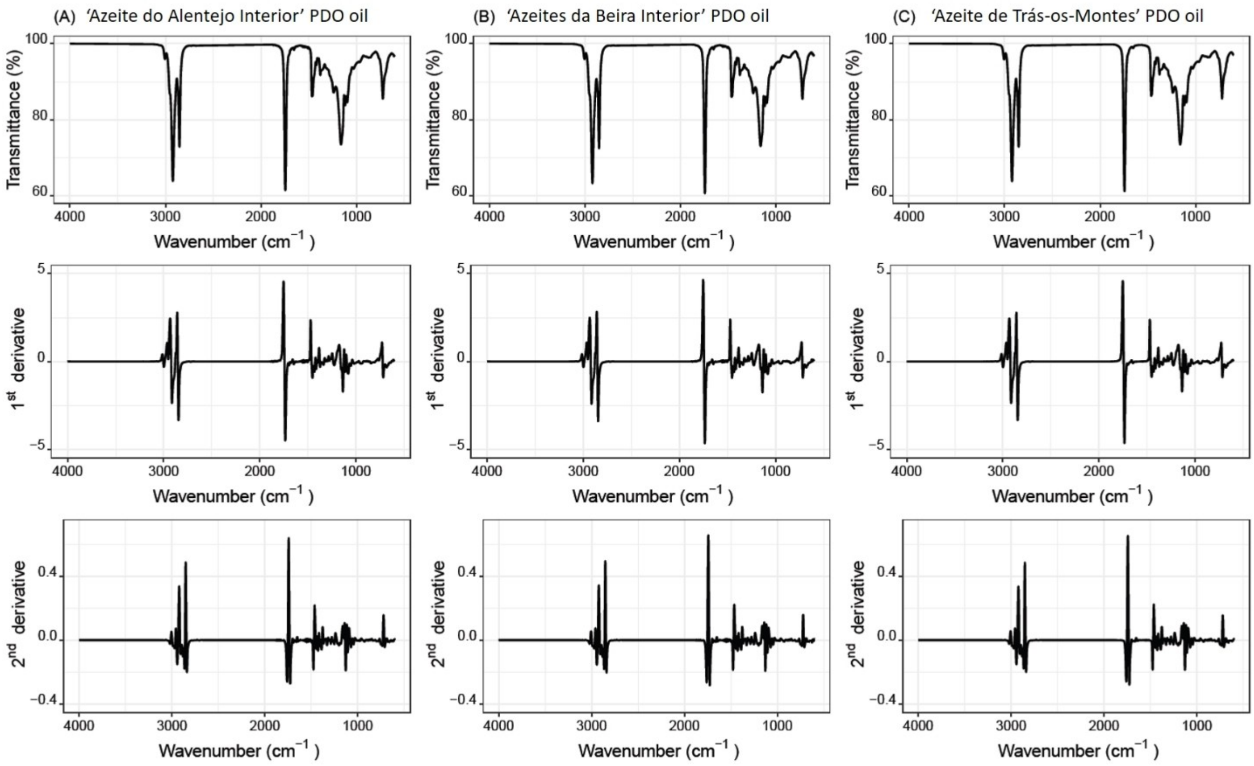

Figure 2. FTIR mean spectra obtained by averaging those recorded for the 10 independent oils per PDO (wavenumbers ranging from 4000 to 500 cm^{-1}) – transmittance (%) and respective 1st and 2nd derivatives for olive oils from three Portuguese PDOs: (A) 'Azeite do Alentejo Interior', (B) 'Azeites da Beira Interior', and (C) 'Azeite de Trás-os-Montes'.

Thus, the FTIR spectra, i.e. transmittance, 1st and 2nd derivatives data (Figure 2) were further treated by applying LDA-SA, allowing to establish classification models based on the spectral information related to 10, 8 and 6 selected wavenumbers, respectively (transmittance: 656, 1200, 1331, 1533, 1587, 1720, 2770, 2781, 2789, and 2820 cm^{-1}; 1st derivative: 1146, 1151, 1259, 1265, 1761, 2826, 3022, and 3084 cm^{-1}; and, 2nd derivative: 1020, 1047, 1136, 1298, 2841, and 2878 cm^{-1}). All models allowed to correctly classify all the olive oils according to the certified PDO (100% of sensitivity and specificity), for training (Figure 3), leave-one-out cross-validation and repeated K-fold cross-validation procedures. The proposed models present an overall sensitivity and specificity similar to or slightly better than those reported in the literature, also developed using FTIR spectra. For example, Revelou et al.[39] studied the discrimination of the botanical origin of Greek olive

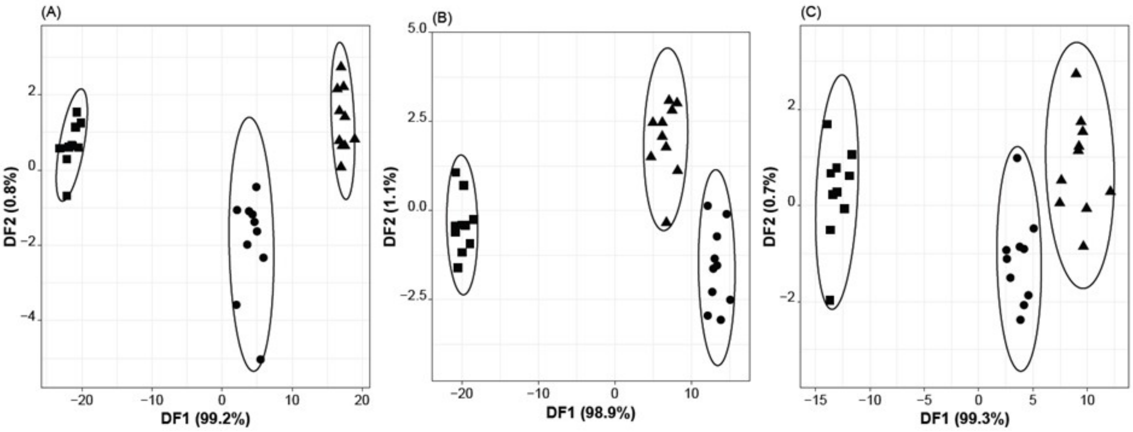

Figure 3. PDO olive oils supervised discrimination (2D LDA-SA plots) achieved with the FTIR-LDA-SA models, according to the three certified geographical origins studied (■ 'Azeite do Alentejo Interior' PDO olive oils; ● 'Azeites da Beira Interior' PDO olive oils; and, ▲ 'Azeite de Trás-os-Montes' PDO olive oils) based on: (A) Transmittance raw data (%) of 10 selected wavenumbers; (B) 1st derivative data of 8 selected wavenumbers; and, (C) 2nd derivative data of 6 selected wavenumbers.

oils, reporting a sensitivity and specificity of 97.4%. Lamas et al.[14] successfully discriminated Portuguese monovarietal olive oils according to the olive cultivar, with 100% of sensitivity and specificity. Also, Rodrigues et al.[18] developed multivariate discrimination models that allowed the correct classification of olive oils from cv. 'Galega Vulgar' according to the geographical origin, with a mean sensitivity of 99 ± 3%.

Concerning the present study, the model based on raw transmittance data does not require spectral data pre-treatment but needs a higher number of wavenumbers, which decreases from 10 to 8 and 6 for raw, 1st and 2nd derivative-based models, respectively. Models based on transformed forms of the recorded transmittance data are simpler, pointing out that, for the studied PDO olive oils, 1st and mainly 2nd derivatives may contribute to reveal/extract spectral information with greater discrimination potential compared to that comprised within the raw spectra. On the other hand, although all three developed models showed similar predictive classification performances, Figure 3 shows that the use of the model based on the raw data provides a clearer differentiation of the 3 PDO's olive oils. Thus, overall, it is clearly pointed out the feasibility of applying the developed FTIR- LDA-SA models as predictive tools 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.

The satisfactory performance in categorizing PDO of the olive oils can tentatively be ascribed to the correlation between certain FTIR bands and the chemical composition of the oils, encompassing volatile and phenolic compounds. These chemical constituents, in turn, are responsible for several gustatory sensations (e.g., sweet, bitter, and pungent) commonly experienced when tasting olive oils.[40–42] To validate this assumption, PDO oils were evaluated by a sensory panel, which established their gustatory profiles. The aim was to determine the feasibility of developing MLRMs to quantify the intensity of basic tastes such as sweetness and bitterness, along with pungency (a trigeminal sensation), utilizing FTIR transmittance data at selected wavenumbers. The conducted sensory analysis did not detect any negative gustatory sensations. Instead, 13 positive gustatory sensations were identified, including fruity-ripe, fruity-green, sweet, bitter, pungent, apple, banana, tomato, dry fruits, cabbage, fresh herbs, dry herbs, and tomato branches. Table 2 presents the mean intensities (± standard deviation) for each of these gustatory sensations detected by the trained panelists in the analyzed olive oils, assessed using an unstructured continuous scale ranging from 0 (undetected) to 10 (maximum detected intensity).

The gustatory sensory analysis, as presented in Table 2, showed distinct taste profiles for olive oils based on their PDO classification. Olive oils under the 'Azeite do Alentejo Interior' PDO were characterized as fruity-ripe, exhibiting significantly sweeter flavors and lower levels of bitterness and pungency compared to oils from the other two PDO (*P*-value < 0.05). On the other hand, the 'Azeites da Beira Interior' PDO and 'Azeite de Trás-os-Montes' PDO oils had a fruity-green profile with pronounced bitterness and pungency, accompanied by higher notes of banana, cabbage, fresh herbs, and tomato. This overview is further substantiated by the 2D PCA biplot depicted in Figure 4, illustrating the distinct taste profile of 'Azeite do Alentejo Interior' PDO oils in comparison to the other PDO oils under study.

This study also aimed, for the first time to the authors' best knowledge, to test the hypothesis that the sweetness, bitterness and pungency intensities, perceived by the sensory panel,

Table 2. Intensities of the gustatory sensations (mean ± standard deviation, using an unstructured continuous scale from 0 to 10) perceived by trained panelists.

Gustatory-retronasal sensations	PDO olive oils			*P*-value#
	'Azeite do Alentejo Interior'	'Azeites da Beira Interior'	'Azeite de Trás-os-Montes'	
Fruity ripe	7.52 ± 0.38[a]	1.96 ± 2.66[b]	n.d.	< 0.0001
Fruity green	n.d.	1.90 ± 1.69[b]	4.48 ± 0.91[a]	0.0002
Sweet	5.49 ± 0.95[a]	2.36 ± 1.04[b]	2.29 ± 0.62[b]	< 0.0001
Bitter	1.53 ± 0.43[b]	3.77 ± 0.59[a]	3.70 ± 0.97[a]	< 0.0001
Pungent	1.46 ± 0.26[b]	4.29 ± 0.53[a]	4.14 ± 1.04[a]	< 0.0001
Apple	4.94 ± 0.40[a]	3.93 ± 0.54[b]	4.13 ± 0.65[b]	0.0005
Banana	0.45 ± 1.41[b]	2.47 ± 2.00[a]	1.06 ± 1.71[a,b]	0.0407
Cabbage	n.d.	0.77 ± 1.32[b]	4.36 ± 1.13[a]	< 0.0001
Dry fruits	3.69 ± 0.87[a]	3.02 ± 0.70[a]	3.78 ± 0.57[a]	0.0508
Dry herbs	3.85 ± 0.85[a]	1.25 ± 1.67[b]	n.d.	0.0002
Fresh herbs	n.d.	1.58 ± 1.40[b]	3.09 ± 1.39[a]	0.0131
Tomato	1.32 ± 2.24[b]	3.12 ± 1.37[a]	3.69 ± 1.04[a]	0.0084
Tomato branches	n.d.	1.74 ± 1.87[a]	2.04 ± 1.48[a]	0.6967

n.d.: not detected/perceived by the sensory panel; # Different lowercase letters in the same row mean a statistical significant effect (*P*-value < 0.05) of the PDO on the olive oil content of a specific volatile class or on the intensity of the olfactory sensation perceived by the sensory panel (when olive oils from two PDO are compared the *t*-Student test is applied; when olive oils from three PDO are compared the one-way ANOVA followed by the Tukey's test is performed).

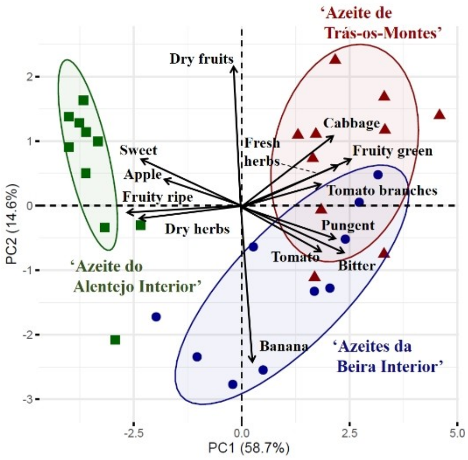

Figure 4. 2D PCA biplot (PC1 versus PC2) showing the unsupervised differentiation of the studied PDO olive oils according to the demarcated geographical origin, based on the intensities of 13 gustatory-retronasal positive sensations (fruity-ripe, fruity-green, sweet, bitter, pungent, apple, banana, tomato, dry fruits, cabbage, fresh herbs, dry herbs and tomato branches) perceived by the sensory panel: 'Azeite do Alentejo Interior' (●); 'Azeites da Beira Interior' (▲); or, 'Azeite de Trás-os-Montes' (■).

Table 3. Quantification of the sweet, bitter and pungent intensities perceived in the 30 PDO olive oils evaluated by the sensory panel, using FTIR-MLRMs developed based on the transmittance data from selected sub-sets (SA algorithm) of the wavenumbers.

Gustatory-retronasal sensation	Intensity range[a]	N° of feature variables[b]	FTIR-MLRM (repeated K-fold-CV)[c]	
			Determination coefficient (R^2)	Root mean square errors (RMSE)
Sweet	[1.45, 6.75]	12[d]	0.985 ± 0.009	0.26 ± 0.06
Bitter	[1.01, 5.64]	11[e]	0.979 ± 0.016	0.20 ± 0.05
Pungent	[1.18, 6.09]	12[f]	0.989 ± 0.006	0.19 ± 0.05

[a] Intensities were assessed by trained panelists using an unstructured continuous scale ranging from 0 (not perceived) to 10 (maximum intensity level); [b] Number of wavenumbers included in the MLRMs; [c] Repeated K-fold-CV: cross-validation variant used for validation of the established MLRMs (4 folds×10 repeats), which uses 25% of the dataset for internal validation; [d] Wavenumbers selected: 611.4, 709.8, 744.5, 790.8, 808.1, 823.5, 1047.3, ad 1288.4 cm^{-1}; [e] Wavenumbers selected: 702.0, 804.3, 821.6, 956.6, 966.3, 1074.3, 1153.3, 1307.6, 2758.0, 2773.4, and 3037.7 cm^{-1}; [f] Wavenumbers selected: 599.8, 609.5, 702.0, 705.9, 918.0, 1060.8, 1093.6, 1147.6, 1199.6, 1487.0, 1510.2, and 1828.4 cm^{-1}.

during the evaluation of the studied PDO oils, could be quantified using the FTIR raw transmittance data recorded at selected/specific wavenumbers. Recently, Lobo-Prieto et al.[43] showed that the increase of the concentration of olive oils rancid markers was in agreement with the increase of the intensity of the aldehyde band recorded using a mesh cell-FTIR, having been some aldehydes associated with the referred negative sensation. In the present study, MLRM were developed based on sub-sets FTIR raw transmittance data, measured at wavenumbers selected using the SA algorithm. For each one of the three gustatory sensations under analysis, a FTIR-MLRM was developed. Table 3 gives the details regarding the number of wavenumbers included in each model as well as the R^2 and RMSE for the repeated K-fold-CV internal validation variant.

The FTIR-MLRMs developed from the transmittances recorded at 11–12 wavenumbers can accurately estimate the sweetness, bitterness and pungency intensities, perceived by the trained panellists on the the studied PDO olive oils ($0.979 \pm 0.016 \leq R^2 \leq 0.989 \pm 0.006$ and $0.19 \pm 0.05 \leq \mathrm{RMSE} \leq 0.26 \pm 0.06$, for the repeated K-fold-CV). It should be noticed that the wavenumbers selected by the SA algorithm fall in several specific wavenumber ranges related to acids, aldehydes, esters, hydrocarbons, phenolics and unsaturated or saturated fatty acids (e.g., 3400–3200; 2850–2750, 1820–1660, and 1400–1000 cm^{-1}).[36–38] These compounds, individually or combined, are in turn responsible for basic tastes like sweet and bitter, as well as by the trigeminal sensation of pungency, which may explain the successful performance of the FTIR-MLRMs. The accuracy in quantifying the sweet, bitter and pungent intensities was further confirmed by the regression lines (Figure 5) that can be satisfactorily established between predicted (FTIR, repeated K-fold-CV) and experimental (trained panellists) sensory intensities. The fitted single linear regression lines had R^2 values ranging from 0.973 to 0.982. The slope values, were

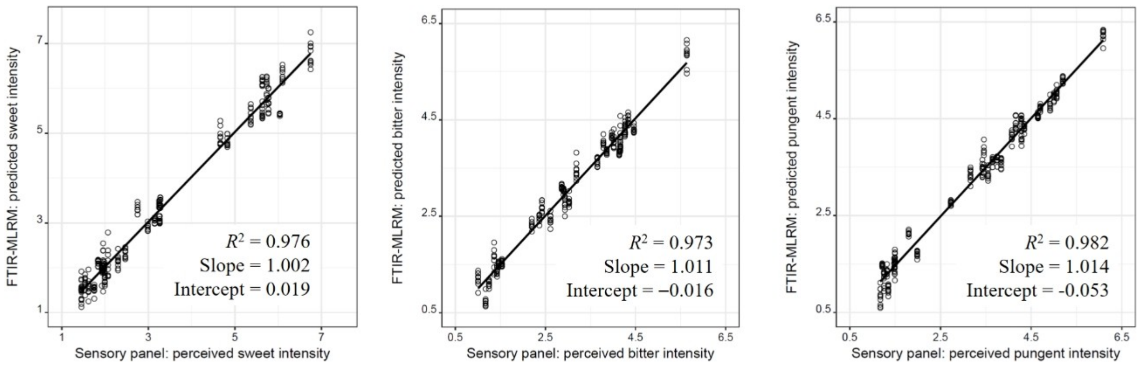

Figure 5. Intensities of sweet, bitter and pungent sensations of the three Portuguese PDO olive oils: predicted values (repeated K-fold-CV variant) using the developed FTIR-MLRMs based on the transmittance data measured at 12, 11 and 12 selected (SA algorithm) wavenumbers versus the experimental data according assessment performed by the sensory panel.

statistically equal to one (ranging from 1.002 to 1.014), indicating a perfect fitting. Similarly, the intercept values were statistically equal to zero (ranging from −0.053 to 0.019), in agreement with the theoretical intercept value for a perfect fit.

Conclusions

This work allowed a comparative study of olive oils from three Portuguese PDOs from 'Azeite do Alentejo Interior', 'Azeites da Beira Interior' and 'Azeite de Trás-os- Montes'. The results showed that the quality physicochemical parameters (free acidity, peroxide value, and extinction coefficients) were within the established limits of the current regulation regarding olive oil marketing standards and the specifications for each PDO. Furthermore, the values obtained for the oxidative stability and the total phenol content were similar to those found in the literature. At the same time, studies on antioxidant capacity had higher values than those obtained in this study. On the other hand, FTIR coupled with unsupervised and supervised chemometric techniques and based on raw transmittance spectra or the 1st or 2nd derivative, allowed a satisfactory discrimination of oils according to the correct PDO. Moreover, for the first time, it was demonstrated that the FTIR raw transmittance data could be used to establish multivariate linear regression models to quantitatively predict the sweetness, bitterness and pungency intensities of the studied PDO oils. In conclusion, FTIR spectroscopy proved to be a promising, fast, and non-destructive tool, providing to the consumer with a guarantee of authenticity for PDO certificated olive oils allowing a straightforward assessment of basic tastes and trigeminal sensation. However, to verify the robustness of the proposed methodology the study must be extended in order to include Portuguese PDO oils as well as non-PDO oils.

Experimental Section

Samples

PDO commercial olive oils from 'Azeite do Alentejo Interior', 'Azeites da Beira Interior', and 'Azeite de Trás-os-Montes' were gathered from certified olive oil producers within each of the referred geographical regions (Figure 6).

According to the producers, all selected samples were extracted in the 2021 campaign of production. After acquisition, the samples were coded, stored at room temperature, and protected from light exposure until analysis. From each region, 10 independent oils were obtained from 10 producers, being from each one purchased three bottles (500 mL), which were considered as non-independent replicates of the same oil. To be classified as PDO, each olive oil needs to accomplish the approved regulations with respect to olive groves located in a delimited geographical area within each region, permitted olive cultivars, and in some cases, specific production roles. The ' Azeite do Alentejo Interior' PDO olive oil must be extracted from olives of cvs. 'Galega Vulgar' (minimum 60%), 'Cordovil de Serpa' and/or 'Cobrançosa' (maximum 40%), although olives from other cultivars may be included (maximum 5%), foreign cultivars, such as 'Picual' and 'Maçanilha' are prohibited.[20] The ' Azeites da Beira Interior' PDO olive oil must be obtained from cvs. 'Galega', 'Verdeal Transmontana', 'Cobrançosa' and 'Cordovil'.[21] The ' Azeite de Trás-os-Montes' PDO olive oils can be extracted from olives of cvs. 'Verdeal Transmontana', 'Cobrançosa', 'Madural', 'Cordovil', 'Santulhana', 'Borreira', 'Redondil', 'Negrinha', 'Carrasquenha', 'Bical', 'Lentisca' and other varieties in smaller percentages.[22] All PDO oils were produced under a Mediterranean climate, with dry and hot summers, and rainy winters. Rain is mainly concentrated in the winter time-period. The oils under the 'Azeite de Trás-os-Montes' PDO are produced in a region marked by a brief hot and dry summer with predominantly clear skies. Winters in this area are cool, with precipitation and partially cloudy conditions. Temperature fluctuations throughout the year generally range from 4 °C to 32 °C, seldom dropping below −1 °C or exceeding 37 °C. In the case of 'Azeites da Beira Interior' PDO oils, the region is typified by a short, hot, and dry summer with mostly cloud-free skies. Winters are prolonged, cool, accompanied by precipitation and partly cloudy skies. The overall temperature range for the year typically spans from 2 °C to 32 °C, with rare instances of temperatures falling below −3 °C or surpassing 37 °C. Meanwhile, 'Azeite do Alentejo Interior' PDO oils originate from an area boasting a Mediterranean climate. The summer is characterized by a dry period lasting approximately 80 to 100 days, during which the average temperature fluctuates between 28 °C and 30 °C. Winter temperatures are comparatively lower in this region.

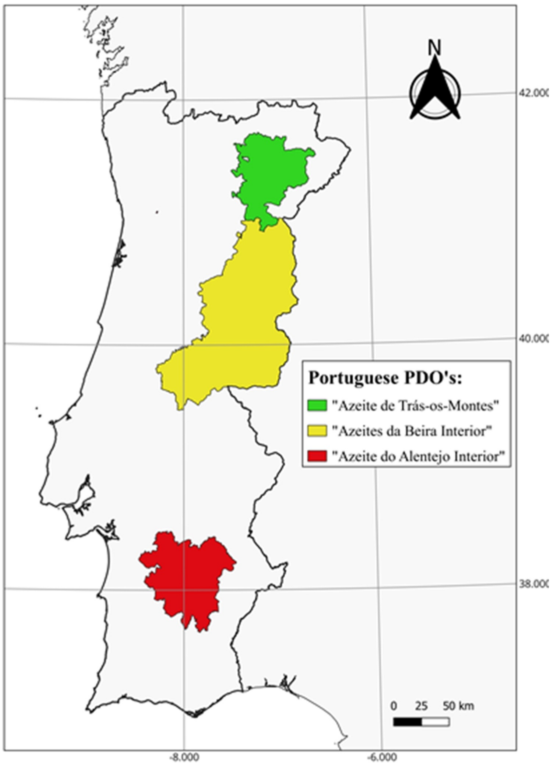

Figure 6. Sampling geographical areas associated with the PDO quality classification system for the chosen olive oil samples ('Azeite de Trás-os-Montes', 'Azeites da Beira Interior' and 'Azeite do Alentejo Interior').

Physicochemical quality parameters, antioxidant capacity, oxidative stability, total phenols content and gustatory-retronasal sensory analysis

All olive oil samples were analyzed following the guidelines of the European Commission methodologies,[23] for the physicochemical parameters, namely the free acidity (FA, expressed in g of oleic acid per 100 g), peroxide value (PV, in mEq O_2/kg oil), and the specific extinction coefficients at 232 nm and 268 nm (K_{232} and K_{268}). The antioxidant capacity of the olive oil samples was spectrophotometrically assessed regarding the radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) (DPPH). The samples were evaluated by UV-Vis spectrophotometry at 517 nm (UV-1280 Shimadzu spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany)), following the methodology described by Cherif et al.[24] with some modifications, namely 0.5 mL of the olive oil extract were mixed with 3.5 mL of DPPH solution (0.06 mM), vortexed, and the absorbance measured after 30 min in the dark. The DPPH radical scavenging was expressed as the reduction percentage of the DPPH activity. The oxidative stability (OS, in h) was determined under accelerated oxidation conditions ($120 \pm 1.6\,^{\circ}C$) using a Rancimat 743 apparatus (Metrohm CH, Switzerland).[25] Total phenolic contents (TPC) were assessed following the methodology by Pizarro et al.[26] with some modifications, specifically, a solution was prepared with 1.5 mL of water, 0.1 mL of olive oil extract, and 0.1 mL of reagent Folin-Ciocalteu, vortexed and allowed to react for 3 min. Subsequently, 0.3 mL of 20% sodium carbonate (w/v) was added, vortexed and allowed to react for 60 min in the dark. The results were expressed in mg of gallic acid equivalents (GAE) per kg of olive oil.

The gustatory-retronasal sensory analysis of the PDO's olive oils were assessed by trained sensory panel, in accordance with the guidelines set by the International Olive Council (IOC) with some adaptations.[44,45] They utilized a modified assessed using an unstructured continuous intensity scale, ranging from 0 (no perception) to 10 (maximum perception). The sensory panel assessed the following sensory aspects, fruitiness (ripe or green), fruit sensations, herbal sensations, along with sensations of sweetness, bitterness, and pungency sensations. Furthermore, they also assessed harmony, complexity, and persistence.

ATR-FTIR spectroscopy: apparatus and olive oil analysis

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 made 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 (ABB, Zurich, Switzerland). The background was acquired every two consecutive assays. The spectra were acquired in duplicate for each olive oil.

Statistical analysis

The effect of the type of PDO on the antioxidant capacity (DPPH), oxidative stability (OS), total phenols contents (TPC) and gustatory sensations was statistically inferred using a one-way ANOVA followed by the Tukey's post-hoc multi-comparison test. When an attribute was only identified in two of the three PDO oils, a t-Student test was performed instead. Principal component analysis (PCA) was also applied to evaluate the unsupervised differentiation of the oils by PDO, using the gustatory sensory profile.

The raw (transmittance values) and the transformed forms (1st and 2nd derivatives) of the FTIR spectra of the olive oils were used, as independent variables, to establish multivariate statistical models for classifying the olive oils according to the three PDOs studied. Linear discriminant analysis (LDA) was applied together with the meta-heuristic simulated annealing (SA) algorithm, which allows selecting the minimum number of non-redundant independent discriminant features (i.e., wavenumbers and related raw or transformed signals). The SA algorithm decreases the error value, allowing to increase the robustness of the developed models.[27] Briefly, SA is a local search optimization algorithm with a probabilistic and stochastic nature, functioning as a meta-heuristic approach. It is able to escape from local optima by incorporating the Markov chain, a random search engine. SA can be applied in both discrete and continuous optimization problems, whether unconstrained or bound-constrained. The algorithm identifies the best solution, or global minimum, within a predefined random search region. This is achieved by accepting suboptimal solutions and employing Monte Carlo simulations based on a predetermined probability schedule. The SA algorithm can overcome local optimum conditions through the acceptance of less-than-ideal solutions, determined by utilizing Boltzmann and Cauchy functions. The architecture of the algorithm provides control over the exploration of the search space while ensuring convergence to the global minimum.[46,47] In this study, the SA algorithm was initially employed in conjunction with LDA. This implementation facilitated the selection of the most informative and non-redundant wavenumbers, thereby enabling the development of multivariate classification models. These models utilized data corresponding to specific optimal subsets of wavenumbers (i.e., of independent variables), ranging from 2 to k independent variables. Here, k was limited to 25, a fixed value determined by considering the degrees of freedom, which took into account the total number of independent olive oils (30) and the number of groups (3). For each type of dataset (raw transmittance, 1st or 2nd order derivatives), a total of 24 classification models were established. These models were based on data from 2 to 25 wavenumbers. In each case, the model demonstrating the highest classification performance (with higher sensitivity) was selected. Finally, the LDA classification was discussed based on the leave-one-out cross-validation (LOO-CV) and the repeated K-fold-CV (4 folds ×10 repeats), allowing this latter variant to use 25% of the dataset for validation (i.e., 2–3 olive oils from each of the three regions considered), being used at each iteration the other 75% of the data for training purposes. The predictive capability was assessed based on the sensitivity, the specificity and, visually, by plotting the class membership ellipses, computed by the Bayes' theorem,[28] for the first two main discriminant functions (DFs). Lastly, multiple linear regression models (MLRM) were developed, based on selected subsets of wavenumbers from the FTIR raw transmittance spectra (SA algorithm), and used to predict (repeated K-fold-CV) the sweetness, bitterness, and pungency intensities of the PDO oils. The selection of the wavenumbers' subsets (varying from 2 to 25) was performed similarly to the process previously described for the LDA.

The predictive performance of the established models was assessed using the determination coefficients (R^2) and the root mean square errors (RMSE), being selected among the 24 models developed, the model based on the low-dimension subset and that returned the highest R^2 and the minimum RMSE. The open-source statistical program R (version 3.6.2) was used, at a 5% significance level.

Author Contributions

Sandra Lamas: Investigation, Data curation, Writing - Original Draft. Daniela Ruano: Investigation, Data curation, Writing - Review & Editing. Francisco Dias: Resources, Writing - Review & Editing. Filomena Barreiro: Resources, Writing - Review & Editing. José A. Pereira: Conceptualization, Funding Acquisition; Resources, Writing - Review & Editing. António M. Peres: Conceptualization, Funding Acquisition; Resources, Methodology, Formal Analysis, Software, Writing - Original Draft. Nuno Rodrigues: Conceptualization, Funding Acquisition; Resources, Validation, Writing - Review & Editing.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Olive oil · quality schemes · region of origin · authenticity FTIR

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