




Fourier transform infrared spectroscopy-chemometric approach as a non-destructive olive cultivar tool for discriminating Portuguese monovarietal olive oils

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Abstract

Four Portuguese monovarietal olive oils (cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana) were evaluated, totalizing 24 independent samples. Olive cultivar significantly influenced (P values < 0.05 , one-way ANOVA) the oils' physicochemical and sensory attributes, fulfilling all oils the legal thresholds for extra-virgin classification. Oils from cv. Verdeal Transmontana had the highest oxidative stability (16.9 ± 1.0 h), whereas cvs. Cobrançosa and Santulhana oils have the highest total phenols content (204 ± 30 and 177 ± 33 mg GAE/kg, respectively). Concerning the sensory attributes, only cv. Santulhana oils fitted fruity greenly oils type, presenting, in general, significantly higher olfactory and gustatory intensities (P values < 0.05 , one-way ANOVA), with a higher number of perceived sensory attributes (e.g., herbaceous sensations), compared to the other monovarietal oils. Free acidity, peroxide value, extinction coefficients, total phenols and oxidative stability allowed the unsupervised differentiation (principal component analysis, PCA) of the four monovarietal oils, and in a less extent, the sensory data. Fourier transform infrared (FTIR) spectroscopy-chemometric procedure was developed based on raw or transformed forms of the recorded spectra. Raw, 1st and 2nd derivative spectra allowed the unsupervised (PCA) and supervised (linear discriminant analysis, LDA) identification of the olive cultivar of the studied oils. In fact, FTIR-LDA models, based on the raw transmittance or on their 1st or 2nd derivatives, recorded at 5, 4 or 3 selected wavenumbers, respectively, allowed to correctly classify all oils (sensitivities greater than $99 \pm 7\%$ for the repeated K-fold-CV internal validation). Therefore, FTIR spectroscopy could be used as a fast, reliable, environmentally friendly and non-destructive olive cultivar-classification tool for monovarietal oils.

Keywords Olive oil · Fourier transform infrared spectroscopy · Data processing · Portuguese cultivar discrimination · Authenticity

Introduction

Olive oil is widely used in the Mediterranean diet, being its consumption associated with several health benefits [1]. This vegetable oil is one of the most valued in the world, being commercialized as extra virgin or virgin olive oil (EVOO or VOO, respectively), which include both blend and monovarietal olive oils. The latter ones are usually appreciated by consumers and commercialized at higher prices, and thus,

more prone to deliberated adulterations. Several analytical techniques, including physicochemical, sensory, electrochemical, metabolic, and genomic based-strategies in combination with chemometrics, have been proposed, aiming to ensure the authenticity of monovarietal EVOO, guaranteeing the labeling correctness regarding the olive cultivar [2–6]. Although these analytical strategies can be used to accurately assess the cultivar/botanical origin of monovarietal olive oils, most of them rely on a destructive analysis, being needed to pretreat the oils' samples, requiring in some cases the combination of several analytical techniques, turning out into a time-consuming and expensive process [7].

Spectroscopy-based tools can be a feasible alternative, allowing a fast and non-destructive/non-invasive analysis of olive oils [7–9]. Among these, Fourier transform infrared (FTIR) spectroscopy has emerged as a key tool for the

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traceability and authenticity of foods [10]. In fact, FTIR spectroscopy has been widely applied for olive oils analysis since edible vegetable oils' spectrum (4000–600 cm^{-1} zone) contains well-resolved intense bands that correspond to the fundamental vibrations of specific functional groups of the main oil constituents [11]. To extract the valuable information gathered with the infrared absorption spectra, qualitative (e.g., principal component analysis, PCA; cluster analysis, CA; linear discriminant analysis, LDA; and soft independent modeling of class analogy, SIMCA) and quantitative (e.g., principal component regression, PCR; partial least square regression, PLSR; multiple linear regression models, MLRM; and artificial neural networks, ANN) chemometric tools coupled with different variable selection algorithms (e.g., successive projections algorithm, SPA; uninformative variable elimination, UVE; simulated annealing, SA; and genetic algorithms, GAs) are commonly employed [11].

FTIR-chemometric tools allowed the quantification of several characteristics of olive oils (e.g., fatty acids, wax, diacylglycerol, squalene, iodine values, free acidity, peroxide value, color pigment contents, sensory intensities and rancid markers, phenol content and antioxidant activity, among others) [12–19], the identification of olive oil adulteration/mixing with different vegetable oils as well as to discriminate olive oils from other vegetable oils [14, 20–26], to discriminate the geographical origin of olive oils [12, 27, 28], to evaluate the thermo/auto oxidation of olive oils [29, 30], and to differentiate/classify olive oils according to their commercial grade [31]. Besides these applications, olive oils were also successfully classified according to the olive cultivar/botanical variety, which constitute a major commercial interest due to the high value of monovarietal oils. In this sense, Turkish olive oils were correctly classified according to the cultivar (cvs. Ayvalik, Erkence, Gemlik, Memecik and/or Nizip) based on FTIR-PCA approaches [27, 32, 33]. Similarly, monovarietal Spanish olive oils were also correctly classified (sensitivity of 88%) according to the olive cultivar (cvs. Arbequina, Borriolenca, Canetera, Farga, Hojiblanca, Picual, or Serrana) using a FTIR-LDA approach [34]. Gouvinhas et al. [35] showed that FTIR spectra coupled with PCA or LDA allowed classifying, simultaneously, the maturation stage and the olive cultivar (two Portuguese and one Spanish) of three monovarietal oils (cvs. Cobrançosa, Galega and Picual), providing correct classifications in the range 74–88%, depending on the ripening stage. More recently, the same research group showed that this latter methodology could even allow the clonal assessment (i.e., phenotypes' selection) of cv. Cobrançosa oils, with a successful classification rate of 94% [36]. Cultivar discrimination (100% of correct classifications) of Tunisian extra-virgin olive oils (cvs. Chemchali, Chemlali, Dhokar, Fouji, Jemri, Zalmati and Zarrazi) based on FTIR-LDA was

also reported by Abdallah et al. [37]. Lastly, Revelou et al. [38] verified that FTIR spectroscopy combined with linear or quadratic discriminant analysis enables the correct discrimination (96%) of the botanical origin of three commercial Greek monovarietal olive oils (cvs. Koroneiki, Megaritiki and Amfissis).

In this context, four monovarietal Portuguese olive oils were studied. The olive cultivars were selected due to the high economic importance in terms of production and/or valorization of the extracted olive oils. Cobrançosa is a very spread olive cultivar; Madural, Santulhana and Verdeal Transmontana were included due to the highly appreciated sensory characteristics of the respective olives and monovarietal olive oils [39]. All olive oils were evaluated taking into account the physicochemical quality parameters (free acidity, FA; peroxide value, PV; and extinction coefficients, K_{232} and K_{268}), total phenols content (TPC), oxidative stability (OS) and sensory profile (olfactory and gustatory attributes) aiming to classify them according to the correct olive cultivar (cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana) based on an unsupervised multivariate approach (i.e., PCA). FTIR spectroscopy combined with PCA was also implemented to evaluate if this non-destructive analytical tool could be used as an alternative tool for cultivar discrimination. Finally, a FTIR-LDA-SA methodology was further used to identify the most discriminant infrared absorption wavenumber region(s).

Materials and methods

Samples

Four monovarietal Portuguese olive oils, from cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana, were obtained from different olive oil producers located in the region of Trás-os-Montes, northeast of Portugal. Olives were grown under the same agro-climatic conditions and collected at similar maturation index (MI greater than 3.5, which corresponds to a fruit with black epidermis and with a white to reddish pulp), aiming to restrict the known influences of the olive ripeness level and geographical origin on the oils composition and thus, to highlight the olive cultivar effect. Olives were harvested during the last week of November and the first week of December 2020. The olive oils were industrially extracted under similar conditions (time and temperature of malaxation) using two-phase olive mills and following the good practices of olive oils extraction. The correctness of the olive cultivar used to produce the monovarietal olive oils was guaranteed by the technicians of the Integrated Production Growers Association (APPITAD, Portugal). For each cultivar, six independent glass amber bottles were collected. Each bottle, of 500 mL of oil, was

filled during the extraction of olives from independent olive batches. The samples were coded and stored at room temperature and protected from light exposure until analysis, which was performed after 2 months of the oils' extraction.

Olive oil physicochemical analysis

Free acidity (FA, in % oleic acid), peroxide value (PV, in mEq O₂/kg) and specific coefficients of extinction at 232 nm and 268 nm (K_{232} , and K_{268}) were evaluated according to the European Union Regulation [40]. Oxidative stability (OS, in h) was determined under accelerated oxidation conditions (120 °C) using the Rancimat method (Rancimat 743, Metrohm CH, Switzerland), as previously described [41]. Total phenolic contents (TPC) were assessed following the methodology proposed by Capannesi et al. [42] with some modifications, as previously described [43]. Quantification (in mg of Gallic acid equivalents (GAE) per kg of oil) was carried out using external standard calibration in methanolic solution 80% ($R^2 \geq 0.9999$).

Olive oil sensory analysis

The olfactory and gustatory sensations of the olive oils were assessed by a trained sensory panel following the methodologies described by the European Union standard methods [40]. The analysis was performed by 8 trained panelists of the olive oil sensory panel of the School of Agriculture of the Polytechnic Institute of Bragança (Portugal) [39]. The intensity of the perceived sensations was scored according to an unstructured continuous intensity scale ranging from 0 (no sensory sensation perceived) to 10 (maximum intensity of the sensory sensation perceived). The descriptive profile was assessed using a test sheet, with some modifications, as recommended by the International Olive Council [44] and as previously reported by Rodrigues et al. [39].

ATR-FTIR spectroscopy: apparatus and olive oil analysis

FTIR analysis was carried out using a 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. For each monovarietal olive oil, the spectra were acquired in duplicate for two sub-samples collected from each of the six oil independent samples (bottles). Finally, for statistical analysis, raw and transformed forms (1st and 2nd

derivatives) of the FTIR spectra of the monovarietal olive oils were used [19].

Statistical analysis

The four monovarietal olive oils' composition was analyzed using the one-way ANOVA followed by Tukey's post hoc multi-comparison test. Prior to the multivariate analysis, the weight of each variable was normalized by applying variable scaling and centering procedures. Principal component analysis (PCA) was applied as an unsupervised multivariate classification technique for evaluating the possibility of identifying the olive cultivar using the physicochemical data or the sensory profiles. In addition, PCA was performed on the raw FTIR spectra and their transformed forms (i.e., 1st and 2nd derivatives) to infer which spectrum pretreatment(s) would allow a better differentiation of the studied monovarietal oils according to the olive cultivar. For PCA, mean values of the replicates made for each analysis were used. Thus, for each evaluated parameter, the dataset totaled 24 values, corresponding to 4 monovarietal oils × 6 independent bottles. The results are discussed based on the 2D or 3D plots of the first two or three principal components (PC) functions, respectively. Linear discriminant analysis (LDA) was applied to assess the studied oils' correct discrimination based on the FTIR data. The best subsets of wavenumbers (and related band intensities) were identified using the meta-heuristic simulated annealing (SA) variable selection algorithm. The SA is a probabilistic technique that aims a global optimization, being based on the physical annealing process of solids [45, 46]. In this sense, and oppositely to deterministic optimization techniques, the SA algorithm deals with non-optimal solutions during the optimization, is capable to cross along local optimums and to identify the global optimal solution [46, 47]. The SA algorithm is a robust variable selection algorithm that has been previously applied within the spectroscopy field allowing enhancing the robustness of the developed models in which concerns model transfer and predictive capability [46, 48, 49]. For the selected LDA models, the variables normality distribution was checked using the Kolmogorov–Smirnov normality test with the Lilliefors significance correction. The homogeneity/equality of the variance–covariance matrices was assessed using the Box's *M* test, and the multicollinearity was evaluated based on the correlation matrix. However, it should be noticed that LDA is fairly robust to a moderate violation of these assumptions [50, 51]. In which concerns the internal validation, both the leave-one-out cross-validation (LOO-CV) and the repeated K-fold-CV variants were considered, being the latter implemented with 4 folds ($K=4$) and 10 repeats (number of internal validations: 4 folds' 10 repeats = 40 validation runs, allowing that at each time, 25% of the dataset was randomly selected for validation purposes, being the remaining

75% of the data used for training purposes). To avoid the risk that replicates from the same independent olive oil sample (bottle) of each studied monovarietal oil could be simultaneously included in the training and internal validation sets, mean values were also used. The predictive performance was assessed for both variants based on the established classification model's sensitivity and specificity. In addition, 2D plots of the two main discriminant functions (DF) were used, being the class membership ellipses established using the posterior probabilities, computed using the Bayes' theorem [52]. All data processing and statistical analyses (5% significance level) were performed on the open-source statistical program R (version 3.6.2) and related software packages.

Results and discussion

Physicochemical, TPC, OS and sensory profile of cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana monovarietal olive oils

The physicochemical quality data (FA, PV, K_{232} and K_{268}), the OS and TPC of the four monovarietal Portuguese olive oils (cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana) are shown in Table 1. The sensory profiles and the intensities of the perceived olfactory and gustatory attributes are listed in Table 2. It can be inferred that all studied monovarietal oils fulfilled the legal thresholds for being classified as EVOO (FA ≤ 0.8 g oleic acid/100 g, PV ≤ 20 mEq O₂/kg, $K_{232} \leq 2.50$ and $K_{268} \leq 0.22$; fruity intensity greater than 0 and no sensory defect perceived) [53].

Regarding the quality and other chemical parameters evaluated (Table 1), the results showed that the olive cultivar had a significant influence on the mean values of all

studied parameters (P value < 0.0001 , one-way ANOVA). In general, cv. Santulhana olive oils showed the highest primary and secondary oxidation indices, and cv. Verdeal Transmontana olive oils had the lowest ones, thus having a slight superior quality level. Table 1 also shows that the oils' OS greatly depended on the cultivar (P value < 0.0001 , for one-way ANOVA), decreasing the order cv's values as: cv. Verdeal Transmontana $>$ cv. Cobrançosa = cv. Santulhana $>$ cv. Madural. This fact can be tentatively attributed to the higher oxidation degradation of monovarietal oils from the latter cultivar, which could be partially related to the lower TPC determined for cv. Madural. However, it should be noticed that oils' oxidation status do not only depend on the TPC level but also with the monounsaturated fatty acids content, mainly oleic acid. However, for the studied monovarietal oils, no direct correlation could be found between OS and TPC.

Concerning the sensory profile, the panelists identified 8 olfactory sensations and 11 gustatory sensations (Table 2), being also assessed the olfactory or gustatory harmony and overall complexity and persistence. The panelists perceived no sensory defect. The olive cultivar had a significant effect (P value < 0.0001 , one-way ANOVA) on the intensities of the majority of the olfactory and gustatory sensations, including fruitiness (green or ripe), basic tastes (sweet, bitter and pungent) and both fruity (apple, banana, dry fruits and tomato) and herbaceous (cabbage, grass and tomato leaves) attributes. Table 2 shows that cv. Santulhana oils were the only fruity greenly oil type among those evaluated, possessing a significantly different sensory profile, being the cultivar for which a significant number of different positive attributes could be perceived. For example, herbaceous sensations (e.g., cabbage, grass and tomato leaves) were only or primarily detected in cv. Santulhana oils, at considerable intensities (with mean intensities ranging from 4.6

Table 1 Mean values (\pm standard deviation) of free acidity, peroxide value, extinction coefficients, oxidative stability and total phenols contents of monovarietal olive oils from four Portuguese cultivars (cvs. Cobrançosa, Madural, Santulhana and Verdeal Transmontana) (for each oil, $n = 6$ bottles $\times 2$ analysis)

Monovarietal olive oils					
Parameters ¹	cv. Cobrançosa	cv. Madural	cv. Santulhana	cv. Verdeal transmontana	P values ²
Quality parameters					
FA (g oleic acid/100 g)	0.24 \pm 0.02 ^b	0.23 \pm 0.03 ^b	0.20 \pm 0.03 ^b	0.35 \pm 0.05 ^a	< 0.0001
PV (mEq O ₂ /kg oil)	3.88 \pm 0.41 ^c	4.98 \pm 0.70 ^b	7.07 \pm 0.83 ^a	3.32 \pm 0.01 ^c	< 0.0001
K_{232}	1.90 \pm 0.17 ^b	2.01 \pm 0.14 ^b	2.20 \pm 0.04 ^a	1.58 \pm 0.10 ^c	< 0.0001
K_{268}	0.15 \pm 0.01 ^b	0.17 \pm 0.02 ^a	0.18 \pm 0.03 ^a	0.13 \pm 0.02 ^b	< 0.0001
Other parameters					
OS (h)	9.2 \pm 0.6 ^b	4.8 \pm 0.3 ^c	9.2 \pm 1.0 ^b	16.9 \pm 1.0 ^a	< 0.0001
TPC (mg GAE/kg oil)	204 \pm 30 ^a	106 \pm 12 ^b	177 \pm 33 ^a	123 \pm 21 ^b	< 0.0001

¹FA free acidity, PV peroxide value, K_{232} and K_{268} UV-Vis extinction coefficients at 232 and 268 nm, respectively; OS oxidative stability, TPC total phenols content

² P values for the one-way ANOVA. Different letters in the same row show statistically differences from the given mean ($p < 0.05$)

Table 2 Intensities of olfactory, gustatory and global sensations (mean \pm standard deviation, for each cultivar, $n=6$ olive oil bottles \times 2 samples \times 8 panelists) perceived by the trained sensory panelists

Perceived sensory attributes		Monovarietal olive oils				<i>P</i> values ¹
		cv. Cobrançosa	cv. Madural	cv. Santulhana	cv. Verdeal transmontana	
Olfactory sensations						
Fruity	Greenly	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	5.9 \pm 0.6 ^a	0.0 \pm 0.0 ^b	< 0.0001
	Ripely	6.2 \pm 0.5 ^a	5.1 \pm 0.8 ^b	0.0 \pm 0.0 ^c	5.2 \pm 0.9 ^b	< 0.0001
Fruit sensations	Apple	5.8 \pm 0.7 ^a	4.1 \pm 1.0 ^c	5.3 \pm 0.5 ^{a,b}	4.5 \pm 1.1 ^{b,c}	< 0.0001
	Banana	0.0 \pm 0.0 ^b	3.0 \pm 1.5 ^a	0.0 \pm 0.0 ^b	2.6 \pm 2.0 ^a	< 0.0001
	Dry fruits	2.5 \pm 0.4 ^c	3.2 \pm 0.6 ^{bc}	3.7 \pm 0.6 ^{ab}	4.4 \pm 1.2 ^a	< 0.0001
	Tomato	3.4 \pm 1.8 ^b	4.2 \pm 1.1 ^{ab}	5.5 \pm 0.9 ^a	3.6 \pm 1.8 ^b	0.0052
Herbaceous sensations	Cabbage	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	5.6 \pm 1.2 ^a	0.0 \pm 0.0 ^b	< 0.0001
	Grass	0.0 \pm 0.0 ^c	0.8 \pm 0.8 ^b	4.6 \pm 1.0 ^a	0.0 \pm 0.0 ^c	< 0.0001
	Tomato leaves	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	5.7 \pm 0.8 ^a	0.0 \pm 0.0 ^b	< 0.0001
Harmony		7.2 \pm 0.2 ^a	7.7 \pm 0.6 ^a	7.6 \pm 0.3 ^a	7.4 \pm 0.5 ^a	0.1160
Gustatory sensations						
Fruity	Greenly	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	6.2 \pm 0.5 ^a	0.0 \pm 0.0 ^b	< 0.0001
	Ripely	6.2 \pm 0.5 ^a	3.5 \pm 1.7 ^a	0.0 \pm 0.0 ^c	6.2 \pm 0.6 ^a	< 0.0001
Basic tastes	Bitter	2.4 \pm 0.5 ^{bc}	3.1 \pm 0.8 ^b	4.6 \pm 0.6 ^a	2.2 \pm 0.6 ^c	< 0.0001
	Pungent	3.6 \pm 0.7 ^b	3.7 \pm 0.7 ^b	5.7 \pm 0.8 ^a	3.1 \pm 0.6 ^b	< 0.0001
	Sweet	7.2 \pm 0.6 ^a	6.4 \pm 0.4 ^b	2.2 \pm 0.6 ^c	6.9 \pm 0.3 ^a	< 0.0001
Fruit sensations	Apple	4.1 \pm 0.5 ^b	4.2 \pm 1.4 ^{ab}	5.0 \pm 0.9 ^{ab}	5.2 \pm 0.7 ^a	0.0099
	Banana	0.0 \pm 0.0 ^c	2.8 \pm 2.3 ^b	0.0 \pm 0.0 ^c	4.7 \pm 0.6 ^a	< 0.0001
	Dry fruits	3.8 \pm 0.7 ^a	3.0 \pm 0.8 ^b	3.9 \pm 0.6 ^a	3.8 \pm 0.6 ^a	0.0076
	Tomato	1.2 \pm 1.8 ^c	3.9 \pm 0.9 ^b	5.6 \pm 0.7 ^a	2.2 \pm 1.6 ^c	< 0.0001
Herbaceous sensations	Cabbage	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	5.4 \pm 1.6 ^a	0.0 \pm 0.0 ^b	< 0.0001
	Grass	0.0 \pm 0.0 ^c	1.4 \pm 1.1 ^b	5.2 \pm 0.9 ^a	0.0 \pm 0.0 ^c	< 0.0001
	Tomato leaves	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	5.2 \pm 0.9 ^a	0.0 \pm 0.0 ^b	< 0.0001
Harmony		6.6 \pm 0.5 ^b	7.6 \pm 0.6 ^a	7.4 \pm 0.2 ^a	7.1 \pm 0.6 ^{ab}	< 0.0001
Global sensations						
Complexity		6.0 \pm 0.3 ^{ab}	6.6 \pm 0.7 ^a	6.4 \pm 0.5 ^{ab}	5.8 \pm 0.7 ^b	0.0092
Persistence		7.0 \pm 0.3 ^a	7.1 \pm 0.4 ^a	7.4 \pm 0.4 ^a	7.2 \pm 0.4 ^a	0.0642

¹*P* values for the one-way ANOVA. Different letters in the same row show statistically differences from the given mean ($p < 0.05$)

to 5.7). Besides, in general, cv. Santulhana oils showed the highest intensities for most of the perceived positive olfactory and gustatory attributes (except for banana and sweet sensations). Consequently, they had the highest complexity and persistence values. The other three monovarietal oils were classified as fruity ripely oil type, being the cultivar effect highly dependent on the sensory attribute. Lastly, it should be noticed that several of the perceived attributes of the four studied monovarietal olive oils are in line with those previously reported for Portuguese monovarietal oils (e.g., apple, banana, cabbage, dry fruits, grass, tomato and/or tomato leaves sensations), namely of cvs. Cobrançosa, Madural and Verdeal Transmontana [39, 43, 54].

The observed cultivar significant effect on the studied physicochemical and sensory parameters was further evaluated based on an unsupervised classification approach. From

the PCA, it was found that the FA, PV, K_{232} , K_{268} , TPC and OS allowed the unsupervised differentiation of the studied monovarietal oils according to the olive cultivar recognition, explaining the first four of the six PCs more than 94% of the data variability (59.1, 16.9, 10.2 and 8.0%, respectively). This satisfactory differentiation can be visualized in Fig. 1A (2D-PCA plot). The unsupervised classification pointed out that TPC mainly contributed to the differentiation of cv. Cobrançosa oils; FA and OS to the differentiation of cv. Verdeal Transmontana oils and the primary and secondary oxidation-related parameters allowed the differentiation of cv. Santulhana oils. On the other hand, the sensory profiles allowed a less satisfactory unsupervised differentiation of the studied olive oils (Fig. 1B). In fact, the PCA results (the first four of the 22 PCs only explained 81% of the data variability, namely, 54.7, 13.3, 7.2 and 6.3%, respectively)

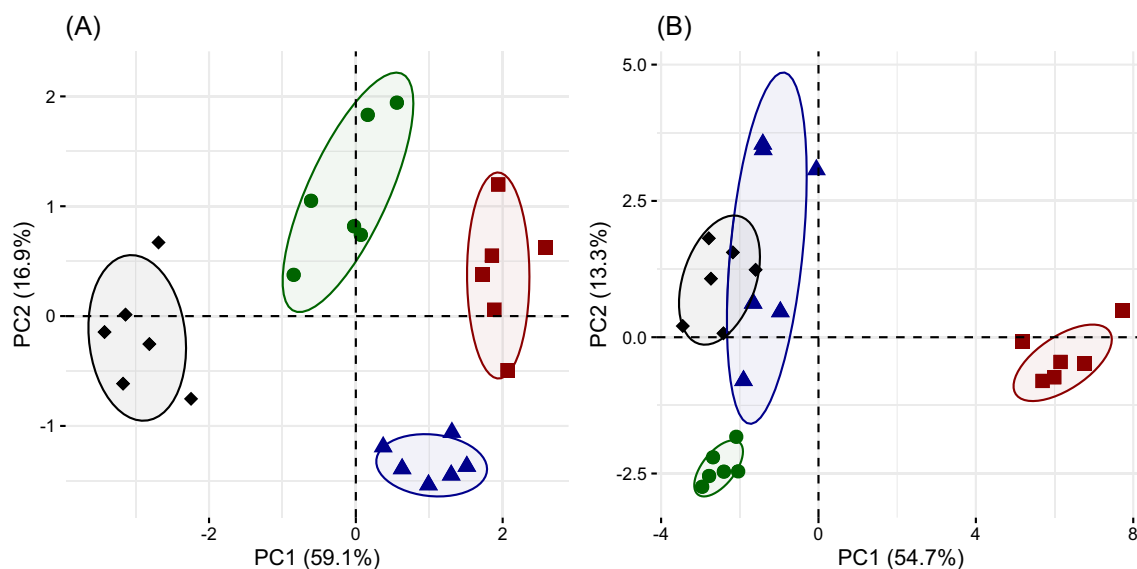


Fig. 1 Monovarietal olive oils unsupervised classification (2D-PCA plot) according to the four studied olive cultivars (● cv. Cobrançosa, ▲ cv. Madural, ■ cv. Santulhana, and ◆ cv. Verdeal Transmontana) based on: **A** six physicochemical parameters (*FA* free acidity, *PV* peroxide values, K_{232} and K_{268} extinction coefficient values at 232 and 268 nm, respectively; *OS* oxidative stability, and *TPC* total phenols

content); and **B** intensities of 8 olfactory and 11 gustatory sensations (fruity green or ripe, apple, banana, dry fruits, tomato, cabbage, grass, tomato leaves, sweet, bitter and pungent) perceived by the sensory panel as well as harmony, complexity and persistence attributes (color figure online)

evidenced that cv. Santulhana oils were the most easily differentiated, mainly due to attributes like fruity greenly, bitter, pungent, tomato, tomato leaves, grass and cabbage, followed by cv. Cobrançosa oils, being a partial overlap observed for cvs. Madural and Verdeal Transmontana oils. Although physicochemical or sensory data could be used as cultivar-differentiation biomarkers, their implementation would require several analytical techniques (e.g., titration, spectrophotometry and Rancimat) or trained sensory panels, turning out into time-consuming, destructive and/or rather expensive procedures. Therefore, alternative, fast, cost-effective and non-destructive monovarietal cultivar identification tools are needed.

ATR-FTIR spectroscopy

FTIR analysis was performed using the ATR technique, a straightforward technique where the olive oil sample is directly placed onto the ATR crystal without any previous pretreatment. The vibration bands of the FTIR spectra (Fig. 2) recorded for the cvs. Cobrançosa, Madural, Santulhana, and Verdeal Transmontana monovarietal EVOO were in line with those reported in the literature for olive oils from different cultivars, geographical origins, quality grades, analyzed in fresh or after storage [27, 28, 37, 38], in particular with those described by Machado et al. [36] for cv. Cobrançosa oils produced in the South region of Portugal (Alentejo region). Typical olive oil's absorption

bands were assigned at different wavenumber regions and correlated with vibration modes of the molecular bonds, in agreement with the literature data, widely summarized in recent years [19, 25, 27, 34, 37]. In general, for all studied oils, the raw FTIR spectra showed bands at 721 cm^{-1} , associated with the bending rocking vibration of aliphatic -CH_2 groups; 914 cm^{-1} , related to the bending out of plane vibration of *cis* -HC=CH- group of disubstituted olefins; region between 1051 and 1161 cm^{-1} , which could be related to the stretching vibrations of saturated C–O aliphatic ester groups; 1236 cm^{-1} related with the stretching vibration of C–O acetate groups or of bending vibration of -CH_2 ; 1377 cm^{-1} linked to the bending vibration of =CH ; 1431 cm^{-1} due to the asymmetrical bending vibration of -CH_3 ; 1464 cm^{-1} associated with the scissoring bending vibration of aliphatic -CH_2 groups; 1653 cm^{-1} associated to the symmetric stretching vibration of -CH_2 ; 1744 cm^{-1} due to the stretching vibration of the ester carbonyl groups (-C=O) of triglycerides; 2853 cm^{-1} linked to the symmetrical stretching vibration of aliphatic -CH_2 and -CH_3 groups; 2922 cm^{-1} associated with the asymmetrical stretching vibration of aliphatic -CH_2 groups; 2951 cm^{-1} related with the asymmetrical stretching vibrations of -CH_3 groups; and finally 3007 cm^{-1} regarding the CH stretching vibration of *cis* double bonds (=C-H).

The visual examination of the ATR-FTIR spectra as well as of the respective 1st and 2nd derivatives (Fig. 2) revealed slight differences between the spectral features

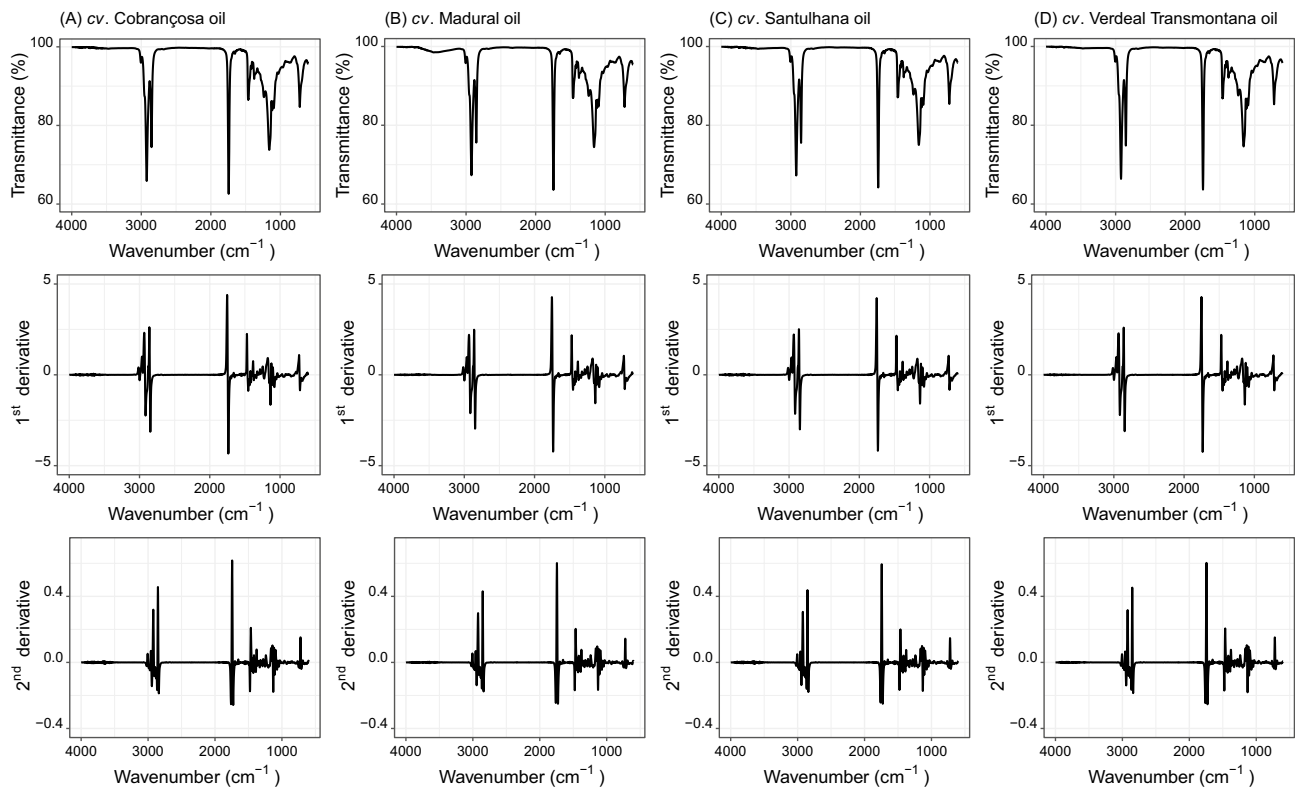


Fig. 2 FTIR spectra (wavenumbers ranging from 4000 to 500 cm^{-1}) and respective 1st and 2nd derivatives for monovarietal olive oils of: **A** cv. Cobrançosa, **B** cv. Madural, **C** cv. Santulhana, and **D** cv. Verdeal Transmontana (color figure online)

(4000–500 cm^{-1}), more evident for the 3700–3100 cm^{-1} region, in which, contrary to the other studied oils, the cv. Madural oils showed a large and slight intense band. This band could be associated with the hydroperoxide moieties, which exhibit characteristic absorption bands between 3600 and 3400 cm^{-1} due to the $-\text{O}-\text{O}-\text{H}$ stretching vibrations, depending on the polarity's peak intensity and the extent of hydrogen interactions [12]. This observed enhancement in the $-\text{OH}$ stretching region of the spectrum (in the 3200–3600 cm^{-1}) may be related to a possible higher oxidation level of cv. Madural oils, which is in line with the lower TPC and OS values, compared to the other studied monovarietal oils, as previously discussed for thermoxidized virgin olive oils [55]. The other minor differences observed among the FTIR spectra, according to the cultivar, may be tentatively attributed to the known differences in the chemical composition of the studied monovarietal olive oils, namely in which concerns their volatiles, fatty acids and phenolic profiles [56–59].

Finally, from Fig. 2, two noise regions were identified based on the recorded ATR-FTIR spectra, namely from 2800 to 2000 cm^{-1} and 4000–3700 cm^{-1} . Thus, to minimize the noise effect on the analysis, these two wavenumber regions were not considered for the multivariate statistical analysis. To evaluate the possibility of using the FTIR

spectra and the 1st and 2nd derivatives data to differentiate the monovarietal oils under analysis, according to the four olive cultivars, a PCA was carried out as a first approach using the data recorded between 2000 and 500 cm^{-1} and 3700–2800 cm^{-1} . Three unsupervised classification models were established based on the raw, 1st derivative and 2nd derivative data, comprising all of them 23 PCs, allowing the first three PCs to explain 95.1, 75.4, and 59.6% of the data variability. The 3D-PCA plots (Fig. 3) clearly showed that the 1st and 2nd derivatives of the recorded FTIR spectra (Figs. 3B, C) enabled a better unsupervised classification of the four monovarietal oils, compared with the raw spectra-based procedure (Figs. 3A). Moreover, the analysis based on the 2nd derivative data allowed a full differentiation of all monovarietal oils, while that based on the 1st derivative only provided a clear split of cvs. Madural and Santulhana oils. The easier differentiation of cv. Madural oils (achieved using both raw and derivative data) may be tentatively attributed, as previously discussed, to the oils' FTIR spectra main differences observed in the 3600–3100 cm^{-1} region. The satisfactory classification performance is in-line with the literature, which showed that FTIR-PCA approaches could be successfully used to recognize the cultivar of Turkish monovarietal olive oils [27, 32, 33] or Portuguese and Spanish monovarietal olive oils [35].

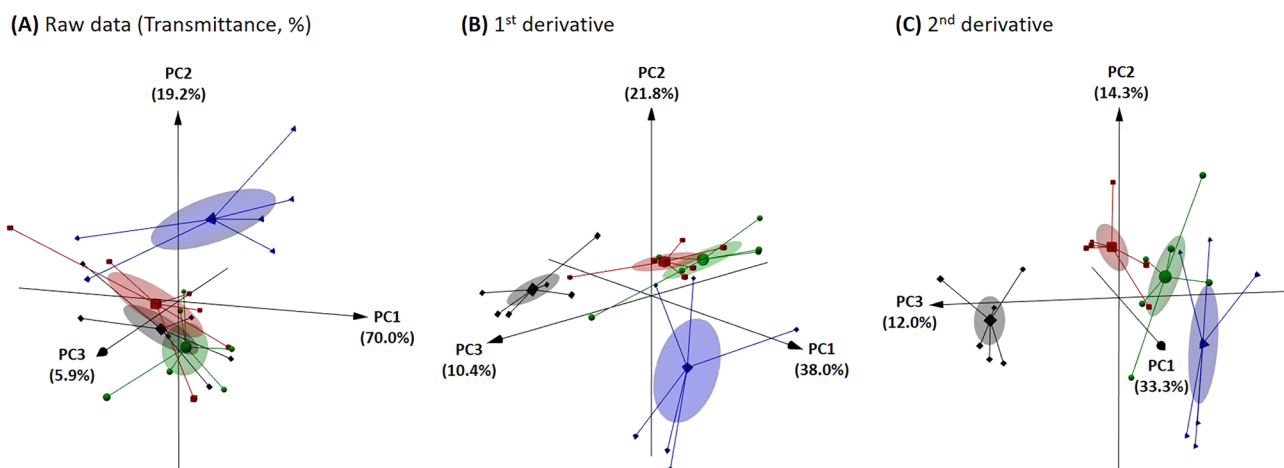


Fig. 3 Monovarietal olive oils unsupervised classification (3D-PCA plot) according to the four studied olive cultivars (● cv. Cobrançosa, ▲ cv. Madural, ■ cv. Santulhana, and ◆ cv. Verdeal Transmontana) based on the recorded: **A** FTIR spectrum raw data (transmittance, %; wavenumbers selected regions: 3700–2800 cm^{-1} and 2000–

500 cm^{-1}), **B** 1st derivative of the FTIR spectrum data (wavenumbers selected regions: 3700–2800 cm^{-1} and 2000–500 cm^{-1}), and **C** 2nd derivative of the FTIR spectrum data (wavenumbers selected regions: 3700–2800 cm^{-1} and 2000–500 cm^{-1}) (color figure online)

To further evaluate the potential use of FTIR spectroscopy as a fast and non-destructive olive oil cultivar-recognition tool, LDA-SA was implemented, using the FTIR raw experimental data and both 1st and 2nd derivative values. A LDA-SA-FTIR raw data model, for which the two first DFs explained 99.98% of the data variability, was established based on the transmittance values (%) from at five non-redundant wavenumbers (1055, 1092, 1117, 1292, and 1311 cm^{-1}), selected using the SA algorithm as those with the most discrimination power among those belonging to the non-noise regions of the spectra (2000–600 cm^{-1} and 3700–2800 cm^{-1} , based on Fig. 2). However, data normal distribution was only verified for two of the five selected wavenumbers, namely 1292 and 1311 cm^{-1} ($0.0692 \leq P$ values ≤ 0.1466 , for the Lilliefors–Kolmogorov–Smirnov normality test). On the other hand, for the selected variables and according to the Box’s M test, the homogeneity of variance–covariance matrices was verified (P value = 0.2091). Although the variables showed a high correlation (greater or equal to 0.97), the selected variables had a collinearity lower than the tolerance of the LDA. It should be highlighted that LDA is fairly robust to moderate violations of these assumptions [50, 51] and so, the established model can still be applied. The selected wavenumbers may be tentatively related with the bending or stretching vibrations of aliphatic saturated C–O aliphatic ester groups, C–O acetate groups, O–H groups, $-\text{CH}_2$ groups, or $=\text{CH}$ groups. The multivariate model allowed the correct classification of all samples for the original grouped data (training; Fig. 4A) as well as for LOO-CV, and of $99 \pm 7\%$ for the repeated K-fold-CV. For this latter CV variant, the misclassifications were observed between cvs. Cobrançosa and Madural oils, in two of the

40 random repeated resampling. If the 1st derivative dataset was used, a LDA-SA-1st derivative model could be established, based on four selected wavenumbers (1105, 2810, 3061, and 3352 cm^{-1}), which two first DFs explained 99.99% of the data variability. For the first three selected wavenumbers, data followed a normal ($0.3365 \leq P$ values ≤ 0.6680 , for the Lilliefors–Kolmogorov–Smirnov normality test). According to the Box’s M test, the homogeneity of variance–covariance matrices was verified for the four wavenumbers (P value = 0.6026). Although the variables showed a high correlation (greater or equal to 0.97), the selected variables had a collinearity lower than the tolerance of the LDA. Moreover, the four selected wavenumbers showed low correlation values (ranging from 0.18 to 0.51 in absolute values), pointing out that the variables showed a low collinearity. Overall, the LDA-SA-1st derivative model better fulfilled the main assumptions of the LDA compared to the LDA-SA-FTIR raw data model. The selected wavenumbers could be tentatively associated with the stretching vibration of aliphatic C–O ester groups, the symmetrical stretching vibration of aliphatic $-\text{CH}_2$ and $-\text{CH}_3$ groups or to the $-\text{O}-\text{O}-\text{H}$ stretching vibrations. The model also allowed to correctly classify all monovarietal olive oils (sensitivity and specificity of 100%) according to the olive cultivar for the training (Fig. 4B) as well as for LOO-CV, and of $99 \pm 5\%$ for the repeated K-fold-CV. Similarly to the raw data-based model, misclassifications between cvs. Cobrançosa and Madural oils were detected, in 3 of the 40 random repeated resampling. Finally, a LDA-SA-2nd derivative model was also established based on the data derived from only three wavenumbers (1111, 1209 and 2829 cm^{-1}), explaining the two first DFs 99.98% of the data variability.

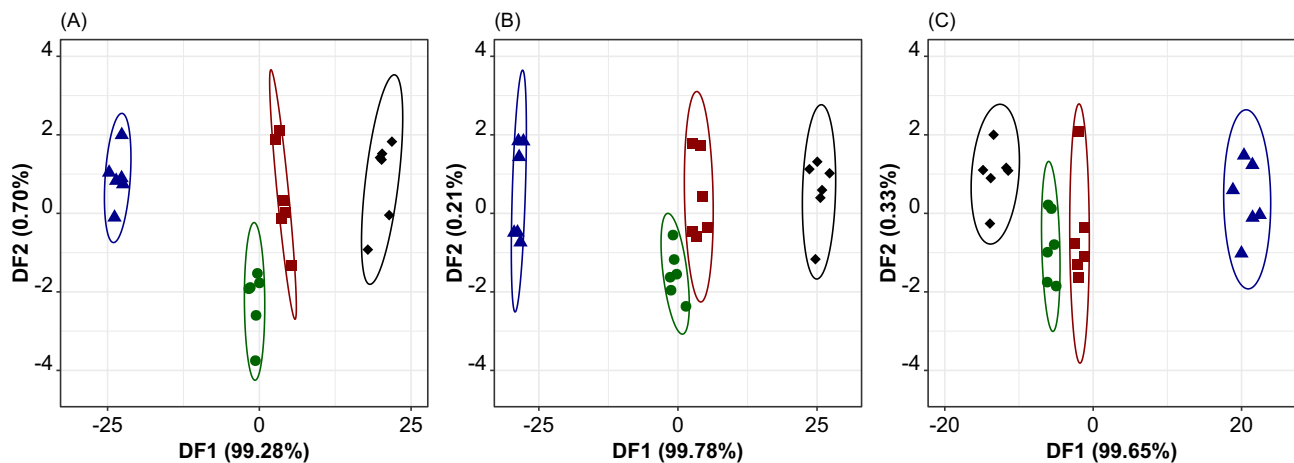


Fig. 4 Monovarietal olive oils supervised discrimination (2D-LDA-SA plot) according to the four studied olive cultivars (● cv. Cobrançosa, ▲ cv. Madural, ■ cv. Santulhana, and ◆ cv. Verdeal Transmontana) based on: **A** LDA-SA-FTIR raw data model based on the transmittance (%) values recorded at five wavenumbers (1055, 1092, 1117, 1292, and 1311 cm^{-1}) selected by the SA algorithm, **B** LDA-

SA-1st derivative model based on the 1st derivative of the transmittance (%) values recorded at four wavenumbers (1105, 2810, 3061, and 3352 cm^{-1}) selected by the SA algorithm, and **C** LDA-SA-2nd derivative model based on the 2nd derivative of the transmittance (%) values recorded at three wavenumbers (1111, 1209 and 2829 cm^{-1}) selected by the SA algorithm (color figure online)

The selected wavenumbers were tentatively related with the stretching vibrations of saturated C–O aliphatic ester groups, to the bending vibration of $-\text{CH}_2$ and to the symmetrical stretching vibration of aliphatic $-\text{CH}_2$ and $-\text{CH}_3$ groups. The model correctly classified the monovarietal olive oils (sensitivity and specificity of 100%) according to the olive cultivar for the original grouped data (Fig. 4C) as well as for LOO-CV, and showed an average sensitivity of $99 \pm 2\%$ for the repeated K-fold-CV. Similarly to the other models, misclassifications were observed between cvs. Cobrançosa and Madural oils, in only one of the 40 random resampling. However, it should be remarked that even if the homogeneity of variance–covariance requirement was fulfilled (P value = 0.2793, for the Box’s M test), only the derived data related with the wavenumber of 1111 cm^{-1} had a normal distribution. Moreover, two of the selected wavenumbers (1209 and 2829 cm^{-1}) showed a high correlation (equal to 0.97).

Although the three approaches were feasible and even considering that the LDA models are robust to moderate violations towards the variables’ normal distribution and non-colinearity, it can be inferred that the LDA model based on the 1st derivative data would be the most adequate to be used as a predictive tool for discriminating the four studied monovarietal oils according to the correct olive cultivar. Also, it should be referred that, in general, the predictive discrimination performances of the developed FTIR based models are similar or slightly better than the supervised classifications obtained with FTIR-LDA models previously developed by Concha-Herrera et al. [34] for discriminating seven Spanish monovarietal oils (sensitivities of 74–88%),

by Abdallah et al. [37] for classifying Tunisian monovarietal olive oils (sensitivity of 100%) or by Revelou et al. [38] to assess the botanical origin of Greek monovarietal olive oils (sensitivity of 96%).

Lastly, it could be stated that the PCA and LDA models developed in this study, using FTIR raw or transformed data, could be used as a practical and accurate tool to discriminate cvs. Cobrançosa, Madural, Santulhana, and Verdeal Transmontana oils allow verifying these Portuguese monovarietal oils’ authenticity, which is of major relevance for high demanding consumers of monovarietal olive oil producers.

Conclusions

The present study evaluated the effect of the olive cultivar on the physicochemical and sensory characteristics of monovarietal olive oils from four different Portuguese cultivars, industrially obtained in similar extractions conditions. The findings confirmed that free acidity, peroxide value, extinction coefficients, total phenols content and oxidative stability could be used as biomarkers to successfully differentiate the four extra-virgin monovarietal olive oils, and to a less extent, the sensory profiles (positive olfactory and gustatory sensations). However, both strategies are time-consuming and invasive approaches. In contrast, Fourier Transform Infrared spectroscopy, coupled with unsupervised and supervised chemometric techniques, and based on raw transmittance spectra or their 1st or 2nd derivative, allowed good discrimination of the monovarietal olive oils, enabling to correctly predict the olive cultivar, among the four considered olive

cultivars (cvs. Cobrançosa, Madural, Santulhana or Verdeal Transmontana). In conclusion, the spectroscopy approach showed to be a promising, reliable, fast and non-destructive olive cultivar-traceability tool, being foreseen its possible practical application, in the near future, ensuring the monovarietal labeling correctness and thus, enhancing the confidence of the demanding olive oil consumers. Nevertheless, to validate and strengthen the predictive performance of the proposed spectroscopy-chemometric procedure, it would be essential to expand the study to include other potential interesting monovarietal olive oils produced in different geographical regions.

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Authors contribution Conceptualization: MFB, JAP, and AMP. Methodology: NR, MFB, JAP, and AMP. Data analysis and interpretation: MFB, JAP, and AMP. Investigation: SL, IPF, and NR. Data Curation: SL and NR. Writing and editing: MFB, JAP, and AMP. Revision and final approval: all the authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human participants or animals, as ruled by the Directive 2010/63/EU, performed by any of the authors.

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