Food Chemistry 398 (2023) 133945

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Geographical discrimination of olive oils from Cv. 'Galega Vulgar'

Nuno Rodrigues [a,b], Fátima Peres [c,d], Susana Casal [e], Arantzazu Santamaria-Echart [a,b], Filomena Barreiro [a,b], António M. Peres [a,b], José Alberto Pereira [a,b,*]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal
[b] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal
[c] Instituto Politécnico de Castelo Branco, Escola Superior Agrária, 6001-909 Castelo Branco, Portugal
[d] LEAF—Linking Landscape, Environment, Agriculture and Food-Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[e] LAQV/REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, Porto 4050-313, Portugal

ARTICLE INFO

Keywords:
Fatty acids
Hydroxytyrosol
Tocopherols
Oxidative stability
FTIR
Linear Discriminant Analysis

ABSTRACT

Olive oils from seven Portuguese regions were selected to study the effect of the geographical origin on the oils' composition. Quality parameters, fatty acids, tocopherols, hydroxytyrosol and tyrosol derivatives, and oxidative stability were evaluated. All olive oils could be classified as extra virgin, and the geographical origin significantly affected the oils chemical composition. Principal component analysis further confirmed the significant impact of the geographical origin on the composition and, indirectly, on stability of the oils, showing that the evaluated parameters could be used as markers for geographical origin identification. Alternatively, Fourier transform infrared spectroscopy was applied, allowing to establish a linear discriminant model that correctly identified the geographical origin of the olive oils with a mean sensitivity of 99 ± 3 % (internal validation), confirming the impact of the oil origin on its characteristics. This finding allowed foreseeing the future application of the spectroscopy approach as a green, fast and non-invasive authentication tool.

1. Introduction

The olive tree (*Olea europaea* L.) is one of the oldest agricultural crops, and olive fruits are used to extract olive oil (OO) and for preparing table olives. This species is originated from the Mediterranean Basin, a region that represents almost 98 % of the world's olive trees (Rallo et al., 2018). Olive oil and table olives play an important role in the diet of the populations of the Mediterranean region, being considered key constituents of the Mediterranean diet, with a high economic, social, and cultural relevance (Ramos-Román et al., 2019). In addition, olive cultivation also has environmental and landscape importance contributing to the maintenance of populations in rural areas (Rodrigues et al., 2022). In Portugal, the olive tree is found throughout the national territory, with a dominance in Alentejo (south), the main producing region, followed by Trás-os-Montes region (north) and the Beira Interior and Ribatejo regions (centre) (INE, 2021). One of the strengths of the Portuguese olive sector lies in the diversity of autochthonous cultivars, with high diversity in the main producing regions, some of them uncharacterised. From that 'Galega' and 'Cobrançosa' are widely distributed along the national territory. Nevertheless, low differentiation occurs in the market, with oils usually commercialised as blends, and only a small amount is labelled as monovarietal olive oils. The cultivar 'Galega Vulgar' is a genuinely Portuguese olive tree, that integrates five of the six Protected Denominations of Origin (PDO) for olive oil, and one for table olives. This cultivar is extremely important within the Portuguese olive growing heritage. From an agronomic point of view, it is considered resistant to drought and sensitive to cold, salinity and limestone. It has a precocious entry into production, and its productivity is high but alternating (Cordeiro et al., 2008). In terms of fruit ripening, it is an early cultivar and is primarily intended for obtaining olive oil, being also highly appreciated as table olives (Peres et al., 2010). Olive oil composition depends not only on the cultivar, but also on different factors like geographic origin, climatic conditions, agronomic techniques and processing technologies (Cubero-Leon et al., 2014;

---

* Corresponding author at: Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal.
E-mail address: jpereira@ipb.pt (J. Alberto Pereira).

https://doi.org/10.1016/j.foodchem.2022.133945
Received 16 June 2022; Received in revised form 8 August 2022; Accepted 10 August 2022
Available online 13 August 2022

Kalogeropoulos & Tsimidou, 2014). According to the literature, geographical origin has a marked influence on tree behaviour. One of the effects of the geographical region is in the fruit ripening process. Also, olive trees from the same cultivar, grown in different regions, will give rise to olives with different chemical compositions (Borges et al., 2017; Serrano et al., 2021). This influence is observed at phenolic composition (Ghorbal et al., 2018), volatile composition (Üçüncüoğlu & Sivri-Özay, 2020), as OO of the same cultivar produced in different regions will possess different chemical profiles (Youssef et al., 2011). Consequently, the geographical origin indirectly influences the oxidative stability (OS), which is usually greater for oils extracted from olives grown in colder regions (Mansour et al., 2015). Also, it has been reported that oils extracted from olive fruits grown in colder regions are richer in monounsaturated fatty acids (Borges et al., 2017). Altitude is another factor that also influences the composition of olive oils. Therefore, the physicochemical composition of oils can be used as a possible geographical biomarker, allowing the authentication of the olive oils. However, the analytical techniques used to determine the referred parameters are time-consuming, expensive, non-green and invasive procedures.

Fourier transform infrared (FTIR) spectroscopy, coupled with chemometric tools, has been used as a fast and non-destructive/non-invasive technique for olive oils analysis. FTIR has been used either as a qualitative or a quantitative approach. Indeed, this technique was successfully applied to classify olive oils according to their botanical or geographical origin (Bendini et al., 2007; Abdallah et al., 2016; Üçüncüoğlu & Küçük, 2019; Uncu et al., 2020; Lamas et al., 2021; Revelou et al., 2021), or to quantify the oils physicochemical composition, namely the free acidity and peroxide values (Bendini et al., 2007; Maggio et al., 2009; Tarhan et al., 2017), colour pigment contents (Uncu et al., 2019), sensory intensities and rancidity markers (Bendini et al., 2007; Maggio et al., 2009), fatty acids abundance (Maggio et al., 2009; Tarhan et al., 2017), waxes contents (Uncu et al., 2019) and phenolic contents (Cerretani et al., 2010). Thus, the present work aims to study the effect of the geographical origin on the physicochemical composition of olive oils from cv. 'Galega Vulgar', grown in seven locations spread across Portugal (north, centre and south regions). For this purpose, quality parameters (free acidity, peroxide value, specific extinction coefficients), fatty acid profile, tocopherols composition, the total content of hydroxytyrosol and tyrosol derivatives and oxidative stability were evaluated. The possible use of this physicochemical information as putative geographical origin fingerprints was evaluated. Alternatively, FTIR spectroscopy was also used to assess the possibility of developing a non-invasive/non-destructive tool based on a chemometric approach to identify the geographical origin of the Portuguese 'Galega Vulgar' monovarietal olive oils.

2. Material and methods

2.1. Sampling

2.1.1. Tree selection and harvest

Seven olive groves of cv. 'Galega Vulgar', located in different geographical regions of Portugal were selected for this study. The olive groves were distributed in the north (2 locations), centre (4 locations) and south (1 location) of Portugal, as follows: Mirandela (41°26′21.25″N 7°16′34.428″W; altitude: 415 m), Alijó (41°12′8.928″N 7°29′42.504″W; altitude: 170 m), Covilhã (40°14′39.156″N 7°29′17.484″W; altitude: 500 m), Penamacor (40°9′19.008″N 7°10′10.812″W; altitude: 450 m), Castelo Branco (39°49′22.368″N 7°27′32.868″W; altitude: 384 m), Vila Velha de Rodão (39°38′59.316″N 7°39′44.1″W; altitude: 91 m) and Elvas (38°53 ′11.22″N 7°8′35.736″W; altitude: 244 m). During the harvest season of 2019, in each olive grove, five trees of cv. 'Galega Vulgar' were selected, and from each tree, 4 kg of fruits were manually picked. Independent of the geographical origin, all olives were harvested with a ripening index (RI) between 3.5 and 4.5, determined according to the International Olive Council guidelines (IOC, 2011). After being picked, olives were immediately transported to the laboratory of the Escola Superior Agrária of the Polytechnic Institute of Castelo Branco (IPCB), Castelo Branco (Portugal) for oil extraction.

2.1.2. Oil extraction

Olive oils were extracted using an Abencor extraction line (Comercial Abengoa SA, Seville, Spain) comprising a mill, a malaxation unit and a centrifuge as detailed by Marx et al. (2022). For each sample (i.e., olive tree) at least four batches were prepared, and after extraction, the olive oils obtained from the same tree were mixed in the same bottle. The oils were filtered in the presence anhydrous sodium sulphate to remove any solid particles and the residual water. The oils were stored in amber glass bottles (125 mL), protected from light and at 20–25 °C.

2.2. Physicochemical quality parameters

All olive oil samples were analysed according to the methodologies described by the European Community Regulation EEC/2568/91 of 11 July and subsequent amendments. Thus, the following physicochemical parameters were evaluated: free acidity (FA, in % of oleic acid), peroxide value (PV, in mEq O_2 kg^{-1}), as well as the specific extinction coefficients at 232 nm and 270 nm (K_{232} and K_{270}, respectively). Additionally, all oils were sensory assessed for classifying them according to the quality grade category.

2.3. Tocopherols composition

Tocopherols were evaluated following the international standard ISO 9936 (2006). The contents of α-, β-, γ-, and δ-tocopherol, were quantified by the internal standard method, using high-performance liquid chromatography (HPLC) coupled to a fluorescence detector and a Luna Silica column (3 μm, 100 × 3.0 mm from Phenomenex, USA), as described by Rodrigues et al. (2021).

2.4. Olive oils total content of hydroxytyrosol and tyrosol derivatives after acid hydrolysis of secoiridoids

The contents of the phenolic compounds were determined after acid hydrolysis with hydrochloric acid, according to the method proposed by Romero & Brenes, (2012), with the modifications previously described by Marx et al. (2021). Briefly, the tyrosol and hydroxytyrosol contents after hydrolysis were quantified by HPLC with a diode-array detector (DAD), using a C18 reversed-phase column. Since only the tyrosol and hydroxytyrosol moieties were detected after the acid hydrolysis, their contents were recalculated by applying the correction factors proposed by Tsimidou et al. (2019) (hydroxytyrosol: 2.2; and, tyrosol: 2.5).

2.5. Fatty acid composition

Fatty acids were evaluated by gas chromatography according to the Commission Regulation (EEC 2568/91 of 11th July), and the profile as previously described by Rodrigues et al. (2021). The composition was expressed as the relative abundance in percentage.

2.6. Oxidative stability (Rancimat)

Oxidative stability (OS) was determined following the method described by Rodrigues et al. (2021). A Rancimat 743 apparatus (Metrohm CH, Switzerland) was used to evaluate the oxidation induction time of 3.00 g of olive oil, which were heated to 120.0 ± 1.6 °C, under an airflow rate of 20 L/h (filtered, clean and dry). The resulting volatile compounds were collected in water and the increase in water conductivity (mS/cm) was measured continuously. The time (in hours) required to reach the inflection point of the conductivity curve was recorded, which corresponded to the OS value.

2.7. ATR-FTIR spectroscopy of olive oil

FTIR analysis was performed in attenuated total reflectance (ATR) mode as previously described by Lamas et al. (2021), being the transmittance values (in %) recorded from 4000 to 500 cm^{-1}. Raw, 1st and 2nd derivatives of the FTIR spectra of the ‘Galega Vulgar’ olive oils were used (Uncu et al., 2019).

2.8. Statistical analysis

One-way ANOVA was implemented to evaluate the possible statistical significance of the geographical origin on quality parameters and chemical composition (fatty acids, tocopherols and phenolic compounds) of ‘Galega Vulgar’ olive oils. The Tukey’s post-hoc multi-comparison test was further used when a significant effect was detected. Principal component analysis (PCA), an unsupervised multivariate recognition tool, was used to verify if the quality and chemical data could be used to recognize the geographical origin of the studied olive, which was evaluated using the 3D plot of the first three principal components (PCs), computed using the mean values of the recorded oils duplicate analysis. Linear discriminant analysis (LDA) coupled with the meta-heuristic simulated annealing (SA) variable selection algorithm was also applied as described by Lamas et al. (2021) to evaluate the potential use of the FTIR spectra to assess the geographical origin of the studied oils. 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). This latter variant uses 25 % of the dataset for validation and the other 75 % of the data were used for training purposes. The predictive capability was checked by calculating the percentage of correct classifications 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.

3. Results and discussion

3.1. Physicochemical profiles of ’galega vulgar’ monovarietal olive oils

The quality parameters of the 35 olive oils from cv. ’Galega Vulgar’ (Table 1), indicated that all olive oils fulfilled the legal limits established by the European Community Regulation EEC/2568/91 of 11 July and subsequent amendments for the classification as Extra Virgin Olive Oil (EVOO) category.

Although the geographical origin significantly influenced the quality parameters (i.e., FA, PV, K_{232} and K_{270}) (Table 1) the observed ranges were well below the legal limit for the EVOO category (≤0.8 % oleic acid, ≤ 20 mEq O_2 kg^{-1}, ≤ 2.5 and, ≤ 0.22, respectively). In fact, the FA varied between 0.04 and 0.19 %, PV ranged from 2.5 to 8.8 mEq O_2 kg^{-1} of olive oil, K_{232} varied between 1.42 and 1.83 and, K_{270} ranged from 0.10 to 0.16. Moreover, regarding the organoleptic assessment, all oils were classified as EVOO, in agreement with the legal regulations for EVOO category classification.

Also, the geographical origin significantly influenced the fatty acids’ relative abundance of the studied ‘Galega Vulgar’ oils (Table 2). Oleic acid ($C_{18:1}$), palmitic acid ($C_{16:0}$) followed by linoleic acid ($C_{18:2}$) were the most abundant fatty acids in all evaluated olive oils. Olive oils from Covilhã showed the highest levels of MUFA, with a mean content of approximately 77 %. In turn, OO from Elvas and Alijó had the lowest MUFA levels (~69 and 70 %, respectively). These findings can be attributed to the fact that oils from Covilhã were extracted from olives grown at higher altitudes and subjected to lower mean temperatures, which could favour the MUFA abundance, as discussed by Borges et al. (2017). Indeed, a positive linear correlation could be found between the MUFA abundance and the olive groves’ altitudes (*R*-Pearson = +0.6528). The tocopherols contents were significantly affected by the geographical origin. Table 3 shows that α-tocopherol was the major vitamin found in the ’Galega Vulgar’ olive oils, representing approximately 95 % of the Vitamin E content that varied between 225 mg/kg (Mirandela) and 374 mg/kg (Alijó). Interestingly, the extreme concentrations were observed for oils obtained from the two locations from the north of Portugal, indicative of a possible major contribution from other factors than the geographical origin, probably related with agricultural practices, plant stress or even the altitude of the olive grove. In fact, negative linear correlations could be established between the amounts of α- or β-tocopherol in the oils and the olive groves’ altitudes (*R*-Pearson = –0.5860 and –0.7997, respectively). The values obtained are in accordance with those of Peres et al. (2016), which also reported a similar variation for α-tocopherol (from 285 to 342 mg/kg for this same cultivar). The present study also revealed that the contents of both hydroxytyrosol and tyrosol derivatives were significantly affected by the olive oil geographical origin. As usual, the contents of hydroxytyrosol derivatives were greater than those of tyrosol derivatives, varying the former between 87 mg/kg (Elvas) and 325 mg/kg (Covilhã) and the latter from 37 mg/kg (Mirandela) to 121 mg/kg (Penamacor). All the evaluated ’Galega Vulgar’ oils fulfilled the polyphenolic-related health claim regarding the protection of blood lipids from oxidative stress, which can only be supported if the oil contained a minimum of 250 mg of hydroxytyrosol and derivatives per kg of olive oil (Commission Regulation (EU) No 432/2012, 2012). Indeed, re-calculating the contents of hydroxytyrosol or tyrosol derivatives, shown in Table 3**,** by applying the correction factors regarding the mass differences between phenolic derivatives and simple compounds (Tsimidou et al., 2019), all studied oils had mean contents varying from 285 mg/kg (Mirandela) to 905 mg/kg (Penamacor), well above the minimum limit established in the health claim (250 mg/kg).

As geographical location influences the oils’ chemical composition, this could justify the high dependence of the OS of the studied OO with the geographical origin (Table 3), having the oils from Covilhã the highest values (31 h), while those from Alijó showed the lowest ones (13 h), and so, for similar storage conditions, the former oils would have a longer shelf-life. The origin dependence observed in this study is in agreement with the results of Serrano et al. (2021). Those authors reported different chemical compositions that are origin-dependent and exert a high influence on OS values and sensory properties of the studied EVOOs. The OS values found in this study are much lower than those reported by Peres et al. (2016) for ‘Galega Vulgar’ oils, with values ranging from 33 to 42 h. Nevertheless, those ‘Galega Vulgar’ oils were obtained from olives with a lower ripening index, contained higher

Table 1
Free acidity (% oleic acid), peroxide value (mEq O_2/kg), specific extinction coefficients (K_{232} and K_{270}) of ’Galega Vulgar’ olive oils from seven Portuguese regions (n = 7 × 5) (mean ± standard deviation).

Quality parameters	Alijó	Castelo Branco	Covilhã	Elvas	Mirandela	Penamacor	Vila Velha de Rodão	*P*-value[#]
FA (%)	0.12 ± 0.05abc	0.09 ± 0.05bcd	0.12 ± 0.01abc	0.06 ± 0.03 cd	0.19 ± 0.12a	0.04 ± 0.00d	0.14 ± 0.02ab	< 0.0001
PV (mEq O_2 kg^{-1})	8.8 ± 1.7a	7.1 ± 1. 9a	4.5 ± 0.5b	7.7 ± 2.5a	2.5 ± 0.7b	8.4 ± 1.2a	4.5 ± 0.7b	< 0.0001
K_{232}	1.83 ± 0.23a	1.62 ± 0.11b	1.59 ± 0.01b	1.63 ± 0.12b	1.42 ± 0.02c	1.62 ± 0.05b	1.59 ± 0.02b	< 0.0001
K_{270}	0.14 ± 0.03bc	0.15 ± 0.04abc	0.18 ± 0.00a	0.12 ± 0.03 cd	0.10 ± 0.01d	0.16 ± 0.02ab	0.13 ± 0.01 cd	< 0.0001
Sensory evaluation	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	——

[#] *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

Fatty acids relative abundance (%) of 'Galega Vulgar' olive oils from seven Portuguese locations ($n = 7 \times 5$) (mean ± standard deviation).

Fatty Acid Profile (%)	Alijó	Castelo Branco	Covilhã	Elvas	Mirandela	Penamacor	Vila Velha de Rodão	*P*-value[#]
Myristic acid ($C_{14:0}$)	0.02 ± 0.00b	0.02 ± 0.00b	0.02 ± 0.00b	0.03 ± 0.01a	0.02 ± 0.00b	0.02 ± 0.00b	0.02 ± 0.00b	< 0.0001
Palmitic acid ($C_{16:0}$)	16.28 ± 2.56bc	14.60 ± 3.63c	14.41 ± 1.69c	19.85 ± 2.57a	17.28 ± 0.67abc	18.32 ± 1.57ab	19.02 ± 0.24ab	< 0.0001
Palmitoleic acid ($C_{16:1}$)	2.07 ± 0.74e	2.12 ± 1.02de	2.73 ± 0.04cde	3.42 ± 0.41ab	2.78 ± 0.11bcd	3.30 ± 0.06bc	3.98 ± 0.06a	< 0.0001
Heptadecanoic acid ($C_{17:0}$)	0.18 ± 0.07bc	0.19 ± 0.08abc	0.16 ± 0.06c	0.27 ± 0.07a	0.16 ± 0.01c	0.25 ± 0.06ab	0.12 ± 0.04c	< 0.0001
Heptadecenoic acid ($C_{17:1}$)	0.35 ± 0.19b	0.47 ± 0.20ab	0.45 ± 0.04ab	0.55 ± 0.08a	0.43 ± 0.02ab	0.56 ± 0.07a	0.39 ± 0.01b	< 0.0001
Stearic acid ($C_{18:0}$)	3.09 ± 0.37a	2.59 ± 0.58bc	2.31 ± 0.17bc	2.73 ± 0.28ab	2.18 ± 0.08 cd	2.31 ± 0.28c	1.78 ± 0.10d	< 0.0001
Oleic acid ($C_{18:1}$)	66.86 ± 2.95 cd	70.98 ± 2.68ab	73.45 ± 1.39a	65.03 ± 3.67d	71.25 ± 0.54ab	68.26 ± 2.19bc	68.32 ± 0.23bc	< 0.0001
Linoleic acid ($C_{18:2}$)	9.15 ± 3.64a	7.10 ± 1.90ab	4.98 ± 0.34bc	6.34 ± 0.53bc	4.42 ± 0.12c	5.28 ± 0.50bc	4.79 ± 0.06c	< 0.0001
Linolenic acid ($C_{18:3}$)	1.00 ± 0.10a	1.02 ± 0.25a	0.71 ± 0.05bc	0.87 ± 0.16ab	0.67 ± 0.02c	0.87 ± 0.08ab	0.87 ± 0.02ab	< 0.0001
Arachidic acid ($C_{20:0}$)	0.43 ± 0.02a	0.39 ± 0.07b	0.33 ± 0.02c	0.39 ± 0.03ab	0.33 ± 0.01c	0.36 ± 0.03bc	0.28 ± 0.01d	< 0.0001
Eicosenoic acid ($C_{20:1}$)	0.30 ± 0.03a	0.27 ± 0.05ab	0.24 ± 0.01b	0.25 ± 0.03b	0.26 ± 0.01b	0.26 ± 0.01b	0.24 ± 0.01b	< 0.0001
Behenic acid ($C_{22:0}$)	0.11 ± 0.01a	0.11 ± 0.03a	0.08 ± 0.01 cd	0.10 ± 0.01ab	0.09 ± 0.00bc	0.09 ± 0.01bc	0.07 ± 0.00d	< 0.0001
Lignoceric acid ($C_{24:0}$)	0.05 ± 0.01a	0.05 ± 0.01a	0.03 ± 0.00 cd	0.05 ± 0.01ab	0.04 ± 0.00bcd	0.04 ± 0.00bc	0.03 ± 0.00d	< 0.0001
ΣSFA	20.2 ± 2.7bc	18.0 ± 3.2c	17.3 ± 1.6c	23.4 ± 2.9a	20.1 ± 0.7bc	21.4 ± 1.8ab	21.3 ± 0.21ab	< 0.0001
ΣMUFA	69.6 ± 3.6 cd	73.8 ± 2.0ab	76.9 ± 1.4a	69.2 ± 3.3d	74.7 ± 0.6ab	72.4 ± 2.2bc	72.9 ± 0.2b	< 0.0001
ΣPUFA	10.2 ± 3.7a	8.1 ± 2.1ab	5.7 ± 0.4c	7.2 ± 0.7bc	5.1 ± 0.1c	6.1 ± 0.6bc	5.6 ± 0.1c	< 0.0001

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

Table 3

Oxidative stability (OS, h), tocopherols, hydroxytyrosol and tyrosol derivatives contents (expressed in mg/kg of olive oil) of 'Galega Vulgar' olive oils from seven Portuguese locations ($n = 7 \times 5$) (mean ± standard deviation).

Evaluated parameters	Alijó	Castelo Branco	Covilhã	Elvas	Mirandela	Penamacor	Vila Velha de Rodão	*P*-value[#]
α-tocopherol (mg/kg)	374 ± 41a	324 ± 65ab	277 ± 23bc	288 ± 49b	225 ± 9c	294 ± 35b	319 ± 32b	< 0.0001
β-tocopherol (mg/kg)	4.0 ± 0.4a	3.2 ± 0.7bc	2.9 ± 0.2cde	3.1 ± 0.4bcd	2.6 ± 0.1e	2.6 ± 0.2de	3.5 ± 0.2ab	< 0.0001
γ-tocopherol (mg/kg)	16 ± 10ab	15 ± 4ab	20 ± 2a	10 ± 3b	15 ± 1ab	13 ± 1b	20 ± 2a	< 0.0001
Hydroxytyrosol derivatives (mg/kg)	171 ± 39bc	212 ± 154b	325 ± 65a	188 ± 74bc	87 ± 50c	272 ± 38ab	179 ± 16bc	< 0.0001
Tyrosol derivatives (mg/kg)	75 ± 20bcd	55 ± 49 cd	113 ± 20ab	92 ± 38abc	37 ± 20d	121 ± 19a	75 ± 6 cd	< 0.0001
OS (h)	13 ± 2d	17 ± 7 cd	31 ± 1a	19 ± 4c	16 ± 4 cd	25 ± 1b	21 ± 2bc	< 0.0001

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

amounts of phenolic compounds, and lower abundance of unsaturated fatty acids. According to literature, OS can be correlated with the contents of fatty acid composition, phenolics, tocopherols, squalene, pigments, and sterols (Guclu et al., 2021). In this study, it was only possible to establish significant linear correlations between the OS of 'Galega Vulgar' oils and the contents of the hydroxytyrosol or tyrosol derivatives (*R*-Pearson = +0.8560 and + 0.7522, respectively), while no correlations were found with the contents of tocopherols or with the relative abundances of the fatty acids. Thus, it was confirmed that higher phenolic contents contribute to enhancing the oils' stability.

Lastly, based on the clear and significant effect of the geographical origin on the physicochemical composition of the 'Galega Vulgar' monovarietal olive oils produced in different Portuguese regions, it was evaluated the possibility of using the compiled information as possible geographical markers. Thus, a PCA was performed using the quality data, oxidative stability, fatty acids relative abundance, tocopherols contents, as well as the hydroxytyrosol and tyrosol derivatives contents, allowing the first three PCs to explain 64.2 % of the data variability. Fig. 1 shows that the evaluated physicochemical characteristics of the 'Galega Vulgar' olive oils allowed a preliminary unsupervised differentiation of the studied oils according to the Portuguese geographical origin, although not enabling a full grouping according to the broader geographical regions (i.e., north, centre or south). This finding highlighted the significant effect of each specific location on the composition of the olive oil, confirming the previous discussion (Tables 1 to 3). Nevertheless, it should be remarked that assessing all the above-mentioned parameters is a time-consuming and costly task. Although involving accurate analytical standard techniques, it results in time-consuming, high-cost, non-green and invasive/destructive procedures. Therefore, other analytical techniques should be considered to provide

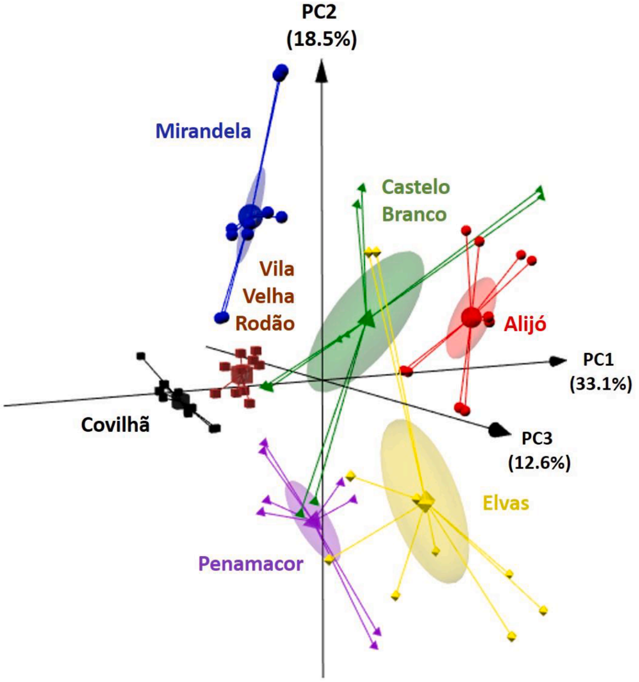

Fig. 1. 3D PCA plot: differentiation of 'Galega Vulgar' monovarietal olive oils according to the geographical origin based on quality data (FA, PV, K_{232} and K_{270}), oxidative stability, fatty acids relative abundance ($C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{20:1}$, $C_{22:0}$ and $C_{24:0}$), tocopherols contents (α-, β- and γ-), hydroxytyrosol and tyrosol derivatives contents.

the olive oil community with fast, green, non-destructive and cost-effective alternatives.

3.2. ATR-FTIR spectra of 'galega vulgar' monovarietal olive oils

ATR-FTIR spectroscopy was applied as a non-destructive tool, allowing the direct analysis of each olive oil sample, not requiring any pre-treatment. The FTIR vibration bands (Fig. 2) recorded for 'Galega Vulgar' oils are in agreement with the spectra previously reported in the literature, namely for olive oils from Turkish cultivars (Ayvalık/Edremit, Erkense, or Memecik) (Üçüncüoğlu & Küçük, 2019; Uncu et al., 2019) and Portuguese cultivars (e.g., 'Cobrançosa', 'Madural', 'Santulhana', or 'Verdeal Transmontana') (Machado et al., 2017; Lamas et al., 2021). Two absorption regions could be identified in all olive oils, independently of the geographical origin, namely between 3080 and 2750 cm^{-1} and 1850–600 cm^{-1}, showing typical bands that could be related with the known vibration modes of the molecular bonds (Üçüncüoğlu & Küçük, 2019; Uncu et al., 2019; Socaciu et al., 2020).

The raw spectra of the 'Galega Vulgar' oils showed typical bands related to the vibrations of $–CH_2$ at 2922, 2853, 1653, 1465, 1236 and 721 cm^{-1} (asymmetrical or symmetrical stretching, scissoring bending and bending rocking vibrations); of $–CH_3$ at 2951, 2853 and 1431 cm^{-1} (asymmetrical or symmetrical stretching and asymmetrical bending vibrations); of = CH at 3007 and 1377 cm^{-1} (stretching and bending vibrations); of $–C{=}O$ at 1744, 1236 and 1161–1051 cm^{-1} (stretching vibrations); and, of cis –HC = CH–at 914 cm^{-1} (bending out of plane vibrations). The ATR-FTIR raw spectra, 1st and 2nd derivatives (Fig. 2) of the studied oils showed minor spectral features differences with the geographical origin. These differences, namely of the transmittance intensities at specific wavelengths, can be hypothetically related to the different contents of fatty acids, phenolic and tocopherol compounds found on the studied oils (Tables 2 and 3). In fact, this hypothesis is supported by the literature, which shows that FTIR data, through the application of quantitative chemometric tools, can be correlated with the chemical composition of OO (Bendini et al., 2007; Maggio et al., 2009; Cerretani et al., 2010; Uncu et al., 2019; Tarhan, 2020).

Finally, the spectra recorded were further used together with LDA-SA to evaluate the possibility of using FTIR as a non-destructive approach for discriminating the geographical origin of the studied 'Galega Vulgar' monovarietal OO. The SA algorithm identified the most representative wavelengths from the two previous referred absorption regions (3080–2750 cm^{-1} and 1850–600 cm^{-1}). Raw transmittances, 1st or 2nd derivatives transformed values, could be used to establish LDA-SA classification models with similar predictive performances. Thus, in this study, only the raw transmittances were used, aiming to avoid applying any of the referred feature extraction techniques (i.e., derivation), making the methodology simpler and straightforward. Thus, a LDA-SA-FTIR model was established based on the transmittance data (%) at seven selected wavelengths (1468, 1215, 1132, 1096, 1028, 918, and 642 cm^{-1}), which first and second DFs explained 99.7 % of the raw data variability. The selected wavenumbers can be related with the asymmetric bending, bending, scissoring and stretching vibrations for 1488–924 cm^{-1} (–HC = CH–(cis), $–C{-}O–$,$–CH_2–$, $–C{-}H–$ of $–CH_2$ or terminal $–CH_3$) or bending and rocking vibrations for 793–650 cm^{-1} ($–(CH_2)_n–$,–HC = CH–(cis)). The multivariate model correctly classified 100 % of the oil samples for the training (Fig. 3) and LOO-CV internal validation procedure. Moreover, a mean correct sensitivity of 99 ± 3 % was achieved for the repeated K-fold-CV, being only two oils produced in two locations of the centre region of Portugal misclassified (one oil from Castelo Branco misclassified as being produced in Covilhã; and, one oil from Covilhã misclassified as Penamacor). Indeed, all the other OO were correctly classified, being not observed any misclassifications across oils belonged to the north, centre or south regions. According to the 1st DF of the LDA-SA-FTIR model, OO from Elvas (south of Portugal, inverted open triangle) were easily discriminated from all the others. On the other hand, oils from the north (sign symbols) and centre (filled symbols) regions were closer to each other, although it could be inferred that those originated from the centre region of Portugal (Castelo Branco, Covilhã, Penamacor and Vila Velha de Rodão) could be further grouped into a single cluster. Contrary, oils from the two locations of the north of Portugal (Alijó and Mirandela) were far way and in opposite positions regarding those from the centre region. Overall, these results showed the great effect that the geographical origin has on the FTIR spectra of the studied olive oils, even within a specific broader geographical region (north, centre or south of Portugal) and so, indirectly, confirming the origin impact on the physicochemical contents of the studied olive oils.

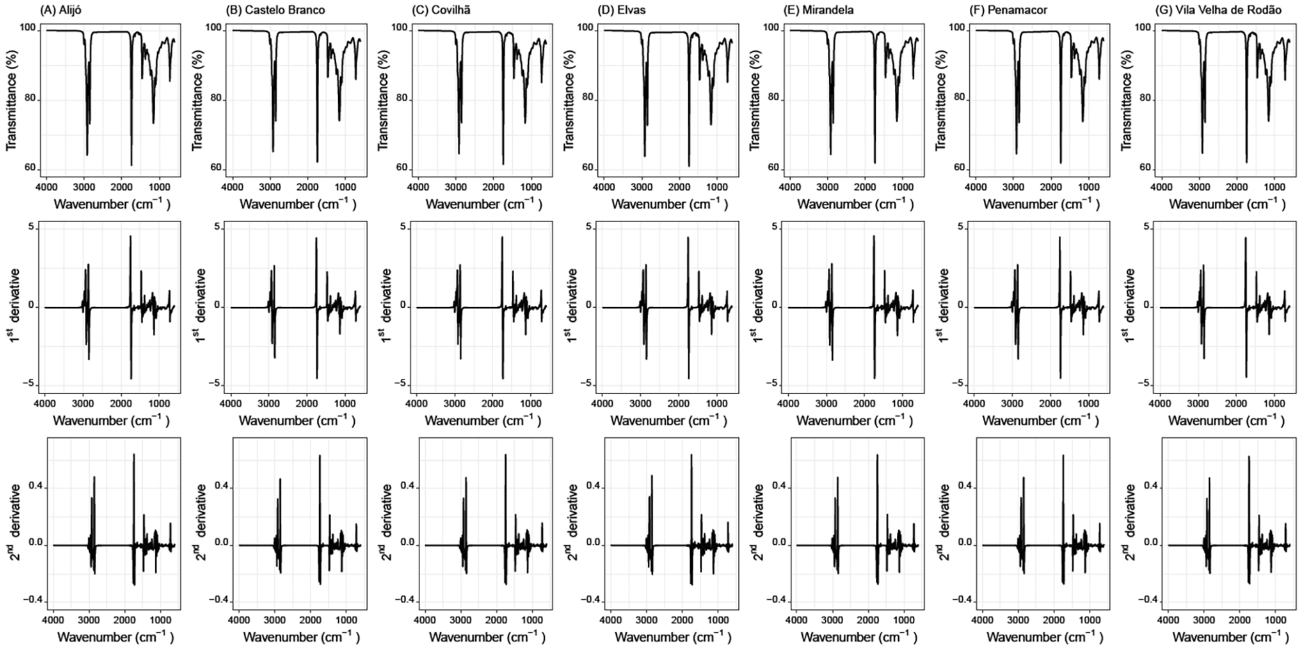

Fig. 2. ATR-FTIR spectra (wavenumbers ranging from 4000 to 500 cm^{-1}) and respective 1st and 2nd derivatives for 'Galega Vulgar' monovarietal olive oils from seven Portuguese geographical origins: (A) Alijó; (B) Castelo Branco; (C) Covilhã; (D) Elvas; (E) Mirandela; (F) Penamacor; and, (G) Vila Velha de Rodão.

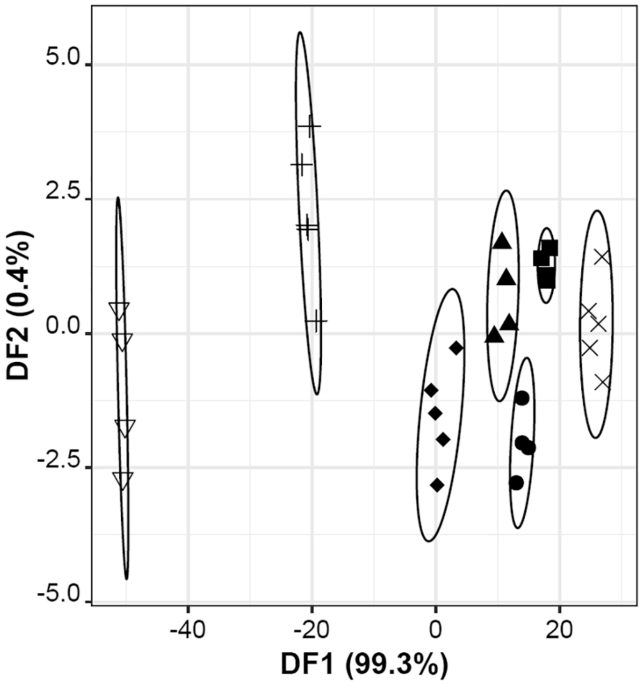

Fig. 3. Discrimination of 'Galega Vulgar' monovarietal olive oils according to the geographical origin: (+) Alijó; (■) Castelo Branco; (●) Covilhã; (▽) Elvas; (×) Mirandela; (▲) Penamacor; and, (◆) Vila Velha de Rodão. 2D-LDA-SA-FTIR model based on the transmittance (%) values recorded at seven wavenumbers (1468, 1215, 1132, 1096, 1028, 918, and 642 cm^{-1}), selected by the SA algorithm.

Nevertheless, the data supporting the chemical and spectroscopic separation are clearly diverse, with the first receiving equal contribution from identical chemical classes analysed separately, and therefore using a maximum discrimination potential even for minor components, while in the FTIR data the minor chemical classes (as phenolics and tocopherols) are shadowed by the fatty acids. Indeed, while in the chemical PCA, Castelo Branco samples presented a wide dispersion, consistent with the higher variability for tocopherols and phenolics (Table 3), in the spectroscopic LDA, these samples presented the smallest dispersion among the 7 studied regions. This indirect relationship can be supported, for example, by the linear correlations that can be established between the centroids of the 1st DF and the relative abundance of $C_{14:0}$ (R-Pearson = − 0.9114) and $C_{18:1}$ (R-Pearson = +0.8482).

Indeed, the LDA-SA-FTIR raw data model could be used as a predictive tool for discriminating 'Galega Vulgar' OO according to the seven Portuguese geographical origins studied, and in special regarding the three regions under study (north, centre and south of Portugal). Also, it should be referred that the developed FTIR model shows a predictive discrimination (LOO-CV and/or repeated K-fold-CV) similar or slightly better than the supervised classifications reported by Zaroual et al. (2021) with FTIR-LDA models developed for identifying the origin of Moroccan VOO among five studied regions (92 % of correct classifications), or by Uncu et al. (2020) for discriminating Turkish olive oils according to three geographical origins (70 to 100 % of correct classifications).

Thus, the results pointed out that the single use of FTIR can be foreseen as a feasible and accurate authentication tool, allowing to identify the geographical origin of 'Galega Vulgar' OO produced in three regions of Portugal (north, centre and south). This capability is of major relevance since the geographical origin of olive oil plays a key role in the purchasing decision by the consumers, which usually prefer oils produced in specific and traditional olive oil-producing regions. Furthermore, the FTIR-chemometric tool can be seen as a practical non-invasive/non-destructive alternative to the use of the time demanding and expensive standard analytical techniques that provide physico-chemical information commonly used as putative geographical origin authentication fingerprints (e.g., fatty acids, phenolic and tocopherol profiles).

4. Conclusion

The present work contributes to the knowledge and characterization of the olive oils of one of the cultivars with the greatest expression in Portugal. The study showed that the geographical origin significantly influenced the quality parameters of 'Galega Vulgar'. Nevertheless, since all oils could be classified as extra virgin, the effect of the geographical origin on the olive oil quality parameters could be considered negligible. Indeed, the quality parameters are much more related to the quality of the olive fruits, harvesting, transporting and storage practices before extraction, as well as to the olive oils extraction conditions. This study demonstrated that the geographical origin significantly influenced the chemical composition of the 'Galega Vulgar' olive oils, namely the relative abundance of the fatty acids as well as the tocopherols and phenols contents. The use of FTIR confirmed the influence of the region on the chemical composition of the oils, which can be envisaged as a viable and accurate authentication tool, allowing to identify the geographical origin of 'Galega Vulgar' olive oils produced from olives grown in seven locations of three regions of Portugal (north, centre and south).

CRediT authorship contribution statement

Nuno Rodrigues: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Fátima Peres:** Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **Susana Casal:** Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Arantzazu Santamaria-Echart:** Formal analysis, Writing – review & editing. **Filomena Barreiro:** Funding acquisition, Methodology, Writing – review & editing. **António M. Peres:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **José Alberto Pereira:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020), to the Associate Laboratory SusTEC (LA/P/0007/2020), and to REQUIMTE-LAQV (UIDB/50006/2020). Authors also thanks the financial support from the GreenHealth Project (NORTE-01-0145-FEDER-000042) co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (Northern Regional Operational Program 2014/2020). Nuno Rodrigues thanks the FCT- Foundation for Science and Technology, P.I., for the National funding through the institutional program contract for scientific employment.

References

Abdallah, M., Vergara-Barberán, M., Lerma-García, M. J., Herrero-Martínez, J. M., Simó-Alfonso, E. F., & Guerfel, M. (2016). Cultivar discrimination and prediction of mixtures of Tunisian extra virgin olive oils by FTIR. *European Journal of Lipid Science and Technology, 118*, 1236–1242. https://doi.org/10.1002/ejlt.201500041

Bendini, A., Cerretani, L., Di Virgilio, F., Belloni, P., Bonoli-Carbognin, M., & Lercker, G. (2007). Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. *Journal of Food Quality, 30*, 424–437. https://doi.org/10.1111/j.1745-4557.2007.00132.x

Bishop, C. M. (2006). *Pattern recognition and machine learning* (1st ed.). New York: Springer.

Borges, T. H., Pereira, J. A., Cabrera-Vique, C., Lara, L., Oliveira, A. F., & Seiquer, I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. *Food Chemistry, 215*, 454–462. https://doi.org/10.1016/j.foodchem.2016.07.162

Cerretani, L., Giuliani, A., Maggio, R. M., Bendini, A., Toschi, T. G., & Cichelli, A. (2010). Rapid FTIR determination of water, phenolics and antioxidant activity of olive oil. *European Journal of Lipid Science and Technology, 112*, 1150–1157. https://doi.org/10.1002/ejlt.201000356

Cordeiro, A., Sanchez-Sevilla, J., Alvarez-Tinaut, M., & Gomez-Jimenez, M. (2008). Genetic diversity assessment in Portugal accessions of *Olea europaea* by RAPD markers. *Biologia Plantarum, 52*, 642–647. https://doi.org/10.1007/s10535-008-0125-1

Cubero-Leon, E., Penalver, R., & Maquet, A. (2014). Review on metabolomics for food authentication. *Food Research International, 60*, 95–107. https://doi.org/10.1016/j.foodres.2013.11.041

EU Comission Delegated Regulation (EU) 2015/1830 of 8th July 2015, 2015. Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of the European Union, L266*, 9–13.

EU Commission Regulation (EU) No 432/2012, 2012. Establishing a List of Permitted Health Claims Made on Foods Other Than Those Referring to the Reduction of Disease Risk and to Children's Development and Health. *Official Journal of the European Union, L136*, 1-40.

Ghorbal, A. B., Leventdurur, S., Agirman, B., Boyaci-Gunduz, C. P., Kelebek, H., Carsanba, E., … Erten, H. (2018). Influence of geographic origin on agronomic traits and phenolic content of cv. Gemlik olive fruits. *Journal of Food Composition and Analysis, 74*, 1–9. https://doi.org/10.1016/j.jfca.2018.08.004

Guclu, G., Kelebek, H., & Selli, S. (2021). Antioxidant activity in olive oils. In: Academic Press. Olives and Olive Oil in Health and Disease Prevention, 2nd ed. pp.313-325.

INE. 2021-National Statistics Institute. Estatísticas Agrícolas-2020; Ano de Edição: Lisbon, Portugal, 2021; ISBN 978-989-25-0572-5.

IOC - International Olive Council. Guide for the Determination of the Characteristics of Oil-Olives; COI/OH/Doc. No 1 November 2011; IOC: Madrid, Spain, 2011; 39p.

ISO 9936 (2006) Animal and vegetable fats and oils - determination of tocopherol and tocotrienol contents by high-performance liquid chromatography.

Kalogeropoulos, N., & Tsimidou, M. Z. (2014). Antioxidants in Greek virgin olive oils. *Antioxidants, 3*, 387–413. https://doi.org/10.3390/antiox3020387

Lamas, S., Rodrigues, N., Fernandes, I. P., Barreiro, M. F., Pereira, J. A., & Peres, A. M. (2021). Fourier transform infrared spectroscopy-chemometric approach as a non-destructive olive-cultivar tool for discriminating Portuguese monovarietal olive oils. *European Food Research and Technology, 247*, 2473–2484. https://doi.org/10.1007/s00217-021-03809-8

Machado, M., Machado, N., Gouvinhas, I., Domínguez-Perles, R., & Barros, A. I. R. N. A. (2017). FTIR chemometrical approach for clonal assessment: Selection of Olea europaea L. optimal phenotypes from cv. Cobrançosa. *Journal of Chemometrics, 31*, e2860. https://doi.org/10.1002/cem.2860

Maggio, R. M., Kaufman, T. S., Carlo, M. D., Cerretani, L., Bendini, A., Cichelli, A., & Compagnone, D. (2009). Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. *Food Chemistry, 114*, 1549–1554. https://doi.org/10.1016/j.foodchem.2008.11.029

Mansour, A. B., Gargouri, B., Flamini, G., & Bouaziz, M. (2015). Effect of agricultural sites on differentiation between Chemlali and Neb Jmel olive oils. *Journal of Oleo Science, 64*, 381–392. https://doi.org/10.5650/jos.ess14204

Marx, Í. M. G., Casal, S., Rodrigues, N., Cruz, R., Peres, F., Veloso, A. C. A., … Peres, A. M. (2022). Impact of fresh olive leaves addition during the extraction of Arbequina virgin olive oils on the phenolic and volatile profiles. *Food Chemistry, 393*, Article 133327. https://doi.org/10.1016/j.foodchem.2022.133327

Marx, Í. M. G., Casal, S., Rodrigues, N., Veloso, A. C. A., Pereira, J. A., & Peres, A. M. (2021). Estimating hydroxytyrosol-tyrosol derivatives amounts in cv. Cobrançosa olive oils based on the electronic tongue analysis of olive paste extracts. *LWT – Food Science and Technology, 147*, Article 111542. https://doi.org/10.1016/j.lwt.2021.111542

Peres, F., Martins, L. L., Mourato, M., Vitorino, C., Antunes, P., & Ferreira-Dias, S. (2016). Phenolic compounds of 'Galega Vulgar' and 'Cobrançosa' olive oils along early ripening stages. *Food Chemistry, 211*, 51–58. https://doi.org/10.1016/j.foodchem.2016.05.022

Peres, F., Luz, J. P., Fragoso, P., Gouveia, C., Vitorino, C., Amaro, C., … Ferreira, A. (2010). Integrated production and quality of Galega olive oil. *IOBC WPRS Bulletin, 53*, 145–149.

Rallo, L., Díez, C. M., Morales-Sillero, A., Miho, H., Priego-Capote, F., & Rallo, P. (2018). Quality of olives: A focus on agricultural preharvest factors. *Scientia Horticulturae, 233*, 491–509. https://doi.org/10.1016/j.scienta.2017.12.034

Ramos-Román, M. J., Jiménez-Moreno, G., Anderson, R. S., García-Alix, A., Camuera, J., Mesa-Fernández, J. M., & Manzano, S. (2019). Climate controlled historic olive tree occurrences and olive oil production in southern Spain. *Global and Planetary Change, 182*, Article 102996. https://doi.org/10.1016/j.gloplacha.2019.102996

Revelou, P. K., Pappa, C., Kakouri E., Kanakis., C. D., Papadopoulos, G. K, Pappas, C. S., & Tarantilis, P. A. (2021). Discrimination of botanical origin of olive oil from selected Greek cultivars by SPME-GC-MS and ATR-FTIR spectroscopy combined with chemometrics. *Journal of the Science of Food and Agriculture, 101*, 2994-3002. doi: 10.1002/jsfa.10932.

Rodrigues, N., Casal, S., Pinho, T., Cruz, R., Baptista, P., Martín, H., Asensio-S.-Manzanera, M. C., Peres, A. M., & Pereira, J. A. (2021). Olive oil characteristics of eleven cultivars produced in a high-density grove in Valladolid province (Spain). *European Food Research and Technology, 247*, 3113–3122. https://doi.org/10.1007/s00217-021-03858-z

Rodrigues, N., Peres, A. M., Baptista, P., & Pereira, J. A. (2022). Olive oil sensory analysis as a tool to preserve and valorize the heritage of centenarian olive trees. *Plants, 11*, 257. https://doi.org/10.3390/plants11030257

Romero, C., & Brenes, M. (2012). Analysis of total contents of hydroxytyrosol and tyrosol in olive oils. *Journal of Agricultural and Food Chemistry, 60*, 9017–9022. https://doi.org/10.1021/jf3026666

Serrano, A., De la Rosa, R., Sánchez-Ortiz, A., Cano, J., Pérez, A. G., Sanz, C., … León, L. (2021). Chemical components influencing oxidative stability and sensorial properties of extra virgin olive oil and effect of genotype and location on their expression. *LWT - Food Science and Technology, 136*, Article 110257. https://doi.org/10.1016/j.lwt.2020.110257

Socaciu, C., Fetea, F., Ranga, F., Bunea, A., Dulf, F., Socaci, S., & Pintea, A. (2020). Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) coupled with chemometrics, to control the botanical authenticity and quality of cold-pressed functional oils commercialized in Romania. *Applied Sciences, 10*, 8695. https://doi.org/10.3390/app10238695

Tarhan, İ. (2020). A comparative study of ATR-FTIR, UV-visible and fluorescence spectroscopy combined with chemometrics for quantification of squalene in extra virgin olive oils. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 241*, Article 118714. https://doi.org/10.1016/j.saa.2020.118714

Tarhan, İ., Ismail, A. A., & Kara, H. (2017). Quantitative determination of free fatty acids in extra virgin olive oils by multivariate methods and Fourier transform infrared spectroscopy considering different absorption modes. *International Journal of Food Properties, 20*, S790–S797. https://doi.org/10.1080/10942912.2017.1312437

Tsimidou, M. Z., Nenadis, N., Mastralexi, A., Servili, M., Butinar, B., Vichi, S., … Toschi, T. G. (2019). Toward a harmonized and standardized protocol for the determination of total hydroxytyrosol and tyrosol content in virgin olive oil (VOO). The pros of a fit for the purpose ultra high-performance liquid chromatography (UHPLC) procedure. *Molecules, 24*, 2429. https://doi.org/10.3390/molecules24132429

Üçüncüoğlu, D., & Küçük, V. A. (2019). A multivariate data approach for FTIR-ATR monitoring of virgin olive oils: Lipid structure during autoxidation. *Oilseeds fats Crops. Lipids, 26*, Article 42. https://doi.org/10.1051/ocl/2019043

Üçüncüoğlu, D., & Sivri-Özay, D. (2020). Geographical origin impact on volatile composition and some quality parameters of virgin olive oils extracted from the "Ayvalık" variety. *Heliyon, 6*, e04919. https://doi.org/10.1016/j.heliyon.2020.e04919

Uncu, O., Ozen, B., & Tokatli, F. (2019). Use of FTIR and UV–visible spectroscopy in determination of chemical characteristics of olive oils. *Talanta, 201*, 65–73. https://doi.org/10.1016/j.talanta.2019.03.116

Uncu, O., Ozen, B., & Tokatli, F. (2020). Authentication of Turkish olive oils by using detailed pigment profile and spectroscopic techniques. *Journal of the Science of Food and Agriculture, 100*, 2153–2165. https://doi.org/10.1002/jsfa.10239

Youssef, O., Guido, F., Manel, I., Youssef, N. B., Pier Luigi, C. P., Mohamed, H., … Zarrouk Mokhtar, Z. (2011). Volatile compounds and compositional quality of virgin olive oil from Oueslati variety: Influence of geographical origin. *Food Chemistry, 124*, 1770–1776. https://doi.org/10.1016/j.foodchem.2010.08.023

Zaroual, H., El Hadrami, E. M. E., & Karoui, R. (2021). Preliminary study on the potential application of Fourier-transform mid-infrared for the evaluation of overall quality and authenticity of Moroccan virgin olive oil. *Journal of the Science of Food and Agriculture, 101*, 2901–2911. https://doi.org/10.1002/jsfa.10922