

Can the region of production affect the quality and composition of olive oil? Comparative study on two olive cultivars

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ABSTRACT

Portugal is a notable olive oil-producing country, gaining prominence in the last decade. This study examined how geographic origin influences olive oil quality by analyzing samples from three key regions (Alentejo, Beira Interior, and Trás-os-Montes) and two major cultivars: Cobrançosa and Galega. All oils met the extra-virgin classification criteria, with free acidity consistently below 0.30%, and peroxide values ranging from 2.3 to 6.7 mEq O₂/kg. Alentejo oils were sensorially more intense, showing higher bitterness and pungency (up to 6.1 and 6.8, respectively). Significant chemical differences were also observed: Trás-os-Montes oils presented the highest monounsaturated fatty acid content (up to 76% MUFA), whereas Alentejo showed the greatest concentrations of hydroxytyrosol-based compounds (up to 450 mg/kg) and strong antioxidant activity (DPPH inhibition up to 80%). Beira Interior oils exhibited the highest α -tocopherol levels (up to 428 mg/kg). Heatmaps and principal component analysis (PCA) revealed clear chemical differences based on origin, demonstrating that environmental factors significantly influence composition, oxidative stability, and antioxidant potential. These results highlight the role of terroir in shaping both the sensory and chemical properties of Portuguese olive oils.

1. Introduction

The olive tree (*Olea europaea* L.) is an emblematic evergreen species of the *Oleaceae* family (Besnard et al., 2018), and one of the earliest domesticated fruit tree (Laaribi et al., 2017). Archaeological and genetic evidence indicates that its cultivation began in region of Palestine around 7000 years ago, later expanding throughout North Africa and the Mediterranean Basin Torres et al. (2017); Barazani et al. (2023). Over the last century, its cultivation has extended to non-traditional olive-growing regions with climatic conditions similar to those in the Mediterranean, supporting the global expansion of olive oil production and consumption (Torres et al., 2017). Currently, olive groves occupy about 11.14 million hectares worldwide, yielding approximately 17.30 million tons of olives (FAOSTAT, 2023). In Portugal, the olive tree is cultivated across the country, with production concentrated mainly in Alentejo (south), followed by the Trás-os-Montes region (north), Beira Interior (center interior) and Ribatejo (center) (INE, 2023).

Among the most representative Portuguese cultivars, Galega and Cobrançosa stand out for their wide adaptability to different environmental conditions and for their relevance to high-quality olive oil

production. Cobrançosa is characterized by high productivity and intense sensory characteristics, while Galega is particularly appreciated for its balanced and harmonious flavour profiles (Peres et al., 2016). However, beyond genetic factors, the chemical composition and sensory quality of olive oil are strongly modulated by geographical origin, reflecting the combined influence of local climate, soil properties, parent material, cultivation practices, and the technological expertise involved in oil extraction (Damak et al., 2021). Other aspects such as altitude, pedoclimatic conditions, temperature patterns, sunlight exposure, precipitation regimes and agronomic techniques (e.g. fertilization and irrigation) (Lechhab et al., 2022), further contribute to significant compositional variability among oils.

Environmental conditions play a central role in regulating the plant's secondary metabolism, determining the biosynthesis of key bioactive compounds such as phenols, tocopherols and volatile constituents (Rodrigues et al., 2023). For instance, areas with pronounced diurnal temperature variations tend to promote phenolic accumulation, enhancing oxidative stability and antioxidant capacity (Pant et al., 2021). Similarly, soils with different physicochemical characteristics such as pH, texture and organic matter content can influence the

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absorption of essential nutrients and, consequently, the enzymatic activity involved in lipid synthesis (Campillo-Cora et al., 2025). Among compositional parameters, fatty acid distribution has consistently proven to be one of the most informative markers for discriminating olive oils from different regions (Hlima et al., 2017). Furthermore, previous studies suggest that environmental and soil conditions often exert a stronger influence on certain sensory-related compounds than the cultivar itself (Boukachabine et al., 2011; Bajoub et al., 2016). Variations in latitude also determine factors such as temperature, solar radiation, and photoperiod, which can significantly influence the biosynthesis of key chemical compounds in virgin olive oil, thereby reinforcing the importance of geographical origin Romero et al. (2015). Likewise, irrigation regimes particularly full irrigation can buffer the effects of environmental stress, including those associated with latitudinal climatic variability (Romero et al., 2015). Altitude is also an important differentiating factor: olive groves located at higher altitudes are often associated with a cooler climate and greater abiotic stress, which can induce changes in the fatty acid profile, particularly an increase in the oleic acid content, favoring the balance between oxidative stability and the sensory properties of the oil (Rodrigues et al., 2023).

All these natural and human induced variations result in olive oils with different chemical compositions between regions, even when obtained from the same cultivar and harvested at the same stage of ripeness. Therefore, the regional characterization of olive oils becomes fundamental not only for valorising local production, but also for improving the understanding of how environmental variables interact with the agronomic performance of specific cultivars. In this context, the present study aims to investigate the influence of territorial characteristics on the physicochemical properties and antioxidant activity of olive oils extracted from the Cobrançosa and Galega cultivars, two of the most important Portuguese varieties in olive oil production.

2. Material and methods

2.1. Tree selection and fruit harvesting

For this study, two olive groves of each cultivar, Cobrançosa and Galega were selected in each of the three Portuguese regions under evaluation (Alentejo, Beira Interior, and Trás-os-Montes). All selected groves followed comparable agricultural practices regarding soil management, fertilization, water availability, and phytosanitary treatments, ensuring the minimization of agronomic variability. Specifically, the soils in these groves were tilled in spring and autumn for weed control, and fertilization was applied in April using a 7:14:14 compound fertilizer containing 7 % nitrogen (5 % ammoniacal N and 2 % urea N), 14 % P₂O₅ (11 % water-soluble), and 14 % K₂O. The olive trees were approximately 20 to 30 years old. All groves were managed organically and under rainfed conditions, with no irrigation systems in place.

The climatic conditions of the regions differ markedly, particularly during the ripening period (September-October). The Alentejo region, characterized by a hot and dry Mediterranean climate, records average temperatures of 23.3 °C in August (min 15.9 °C; max 32.3 °C), which gradually decline to 17.8 °C in October (min 12.3 °C; max 23.2 °C) (IPMA, 2025). Beira Interior, located in the central inland area, exhibits a more continental climate, with an August average of 24.4 °C (min 17.2 °C; max 31.6 °C) decreasing to 16.3 °C in October (min 11.6 °C; max 21.0 °C) (IPMA, 2025). In Trás-os-Montes, a northeastern region with pronounced thermal amplitude, average temperatures drop from 21.1 °C in August (min 13.7 °C; max 28.5 °C) to 12.8 °C in October (min 7.5 °C; max 18.1 °C), marking the transition into cooler winter conditions (IPMA, 2025).

The olive groves were distributed as follows: Alentejo region (cv Cobrançosa located at 38°11'33.4"N 7°39'08.9"W and 38°10'41.3"N 7°38'30.3"W; cv Galega located at 38°11'30.7"N 7°38'16.7"W and 38°11'34.0"N 7°38'17.3"W). Beira Interior region (cv Cobrançosa located at 40°51'51.1"N 7°05'04.8"W and 39°54'52.5"N 7°36'18.1"W; cv

Galega located at 39°54'54.6"N 7°40'42.3"W and 39°49'34.039"N 7°27'30.834"W). Trás-os-Montes region (cv Cobrançosa located at 41°32'23.5"N 7°23'40.6"W and 41°34'24.456"N 7°5'23.399"W; cv Galega located at 41°30'19.8"N 6°58'15.1"W and 41°26'18.337"N 7°16'44.501"W). During the 2022 harvest season, five trees were selected in each grove, and 5 kg of olives were manually collected at a uniform maturity index (MI = 3.5–4.5), according to the standards of the International Olive Council (IOC, 2011). Following harvesting, the fruits were immediately transported to the laboratory of the Escola Superior Agrária of the Instituto Politécnico de Bragança (IPB), for olive oil extraction.

2.2. Oil extraction

Within 24 h of harvesting, the oils were extracted in an Abencor pilot plant (Comercial Abengoa SA, Seville, Spain). The process began by grinding the olives in an MC2 MM100 hammer mill (Seville, Spain), equipped with a 5.5 mm diameter sieve and a 1.5 kW single-phase motor. Next, the resulting olive paste (700 g) underwent temperature-controlled malaxation (at 25 °C.) in the MC2 Thermo-Mixer TB-100 (Seville, Spain), a piece of equipment with eight work stations and individual mixing jars, each with temperature control and specific blades to ensure efficient homogenization. The heating time was 30 min at a rotation speed of 50 rpm. The paste was then centrifuged in the MC2 CF-100 Centrifuge Machine (Seville, Spain), equipped with a stainless-steel drum rotating at 3500 rpm, driven by a 1.5 kW three-phase motor and an automatic timer. After centrifugation, the samples were filtered with Whatman No 4 paper over anhydrous sodium sulphate to remove solid particles and residual water. Finally, the extracted oil was stored in 125 mL amber glass bottles and kept in a dark place at room temperature (20–25 °C) for further analysis. The time interval between extraction and analysis was approximately one month.

2.3. Physicochemical quality parameters

All the olive oil samples were analyzed according to the methodologies established by Commission Implementing Regulation (EU) 2022/2105 of 29 July 2022. The following physicochemical parameters were assessed: free acidity (FA, expressed as % oleic acid), peroxide value (PV, in mEq O₂ kg⁻¹) and the specific extinction coefficients at 232 nm and 268 nm (K₂₃₂ and K₂₆₈, respectively). All analyses were performed in duplicate. Additionally, all the oils underwent sensory evaluation to classify them according to their quality category. Sensory analysis was conducted by a sensory panel composed of eight trained members from the Escola Superior Agrária of the Instituto Politécnico de Bragança. Evaluations were conducted in a tasting room using a test form established by Commission Implementing Regulation (EU) 2022/2105 of 29 July 2022. The test form used for descriptive sensory analysis included the presence or absence of sensory defects, as well as the sensation of fruitiness (green or ripe), bitterness, and spiciness. An unstructured intensity scale ranging from 0 (no sensation perceived) to 10 (maximum intensity of the sensation perceived) was used. According to current legislation, only olive oils that are free of defects and exhibit balanced positive attributes can be classified as extra virgin olive oil, the highest quality category. Olive oils with minor defects, while still maintaining acceptable characteristics, are classified as virgin olive oil, while those with more pronounced sensory defects cannot be marketed as virgin olive oil and must be refined.

2.4. Fatty acid composition

The relative proportions of the fatty acids were determined in accordance with the guidelines established by the Commission Implementing Regulation (EU) 2022/2105 of 29 July 2022 for extra-virgin olive oil (EVOO). The process involved cold transesterification using methanolic potassium hydroxide and heptane. The analyses were

carried out on a Chrompack CP 9001 chromatograph (Burladingen, Germany), equipped with a flame ionization detector (FID) maintained at 270 °C and an automatic sampler (CP-9050). The sample (1 µL in a 1:50 dilution) was injected at 250 °C and separated on a fused silica capillary column (Select FAME: 50 m length × 0.25 mm i.d., Palo Alto, CA, USA) with a temperature gradient from 140 to 220 °C (2 °C/min). Helium was used as the carrier gas under an internal pressure of 160 kPa. A standard mixture of certified fatty acid methyl esters (Supelco 37 Component FAME Mix – CRM47885, TraceCERT®, in dichloromethane (varied conc.), Merck KGaA, Darmstadt, Germany), was used to calibrate the FID detector and identify the chromatographic peaks. All analyses were performed in duplicate.

2.5. Tocopherols composition

The concentration of α -, β - and γ -tocopherol was determined by high-performance liquid chromatography (HPLC), according to the ISO, 9936:2016 standard, with minor adaptations described by Rodrigues et al. (2021). Briefly, an exact mass amount of sample was weighed and dissolved in hexane after the addition of the internal standard (20 µg of internal standard (from a tocopherol solution at 1 mg/mL in hexane) was added). The quantification of each tocopherol (α -, β - and γ -) was expressed in milligrams per kilogram (mg/kg) of oil, using individual calibration curves based on analytical standards (Sigma-Aldrich, Germany) and the internal standard, tocol (Matreya Inc., Pleasant Gap, PA, USA). The HPLC analyses were carried out on a Jasco system (Japan) equipped with a fluorescence detector (FLD). The separation was conducted on a Supelcosil™ LC-SI silica column (7.5 cm × 3 mm; particle size: 3 µm; Supelco, Bellefonte, PA, USA) at 23 °C, with elution using a mixture of 1,4-dioxane (Sigma-Aldrich-Allentown, PA, USA) in n-hexane (2.5 %, v/v) at a flow rate of 0.75 mL/min (isocratic conditions). Data analysis was carried out using the ChromNAV Control Center software from the JASCO Chromatography Data Station (Jasco, Tokyo, Japan). The tocopherols were detected by fluorescence at an excitation wavelength of 290 nm and emission wavelength of 330 nm. All analyses were performed in duplicate.

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

The hydroxytyrosol and tyrosol contents were determined after acid hydrolysis, as described by Marx et al. (2021). Syringic acid (0.6 µg from a 0.15 mg/mL solution in methanol:water (80:20, v/v)) (Sigma-Aldrich) was used as an internal standard and added before the hydrolysis process, which took place in a solution of 2 mol/L hydrochloric acid in methanol:water (80:20, v/v). The reaction was carried out at 25 °C for 6 h, with periodic stirring in a vortex, followed by extraction of the soluble compounds in acetonitrile:water (50:50, v/v). The filtered solution was injected into a Jasco HPLC-DAD system (Japan), equipped with a column oven (ECOM Eco2000, Czech Republic) and a diode array detector (DAD, model MD-4010). The compounds were separated on a Gemini-NX C18 LC column (150 × 4.6 mm, 5 µm, 110 Å, Phenomenex) operating at 35 °C under an isocratic system of water/acetonitrile (86:14, v/v), with 0.1 % of formic acid, at a flow rate of 2 mL/min for 6 min. Calibration curves for hydroxytyrosol and tyrosol were established under the same hydrolysis conditions, all using syringic acid as internal standard, extracted at 280 nm. Results are expressed as mg/kg of oil, after the application of the correction factor of 2.2 for hydroxytyrosol and the correction factor of 2.5 for tyrosol, to estimate their original molecular forms (Tsimidou et al., 2019). All analyses were performed in duplicate.

2.7. Oxidative stability (Rancimat)

Oxidative stability (OS) was determined according to the method described by Rodrigues et al. (2021), using a Rancimat 743 apparatus

(Metrohm CH, Switzerland). To evaluate the oxidation induction time, 3.0 g of olive oil were heated to 120.0 ± 1.6 °C under an air flow of 20 L/h (filtered, clean and dry). The volatile compounds generated during oxidation were collected in water (60 mL of distilled water), and the continuous increase in conductivity (mS/cm) was monitored. The time taken to reach the inflection point of the conductivity curve, expressed in hours, was recorded as the OS value. All analyses were performed in duplicate.

2.8. Antioxidant activity of olive oils

The antioxidant activity was assessed using olive oil extracts obtained by methanol-water microextraction (Pizarro et al., 2013). 1 mL of methanol-water solution (80 % MeOH–H₂O) was added to 0.5 g of olive oil in a 2 mL Eppendorf tube. The mixture was shaken and centrifuged for 5 min at 13,200 rpm at a temperature of 20 °C, and the supernatant was transferred to a 5 mL volumetric flask. The process was repeated twice, combining the extracts and topping up to 3 mL with the same solution. Each sample was extracted in duplicate. The antioxidant capacity was determined by measuring the inhibition of the DPPH radical at 517 nm using a UV–VIS spectrophotometer (UV-1280, Shimadzu). The control solution was prepared with methanol and DPPH (0.06 mM), and the absorbance was measured after 30 min in the dark. For the samples, 0.5 mL of the methanolic extract was added to 3.5 mL of DPPH solution, and the absorbance was recorded after 30 min in the dark. Elimination of the DPPH radical was expressed as the percentage reduction in activity. For total reduction capacity (TRC), 1500 µL of ultrapure water, 100 µL of the phenolic extract and 100 µL of the Folin-Ciocalteu reagent were mixed, reacting for 3 min. 300 µL of 20 % sodium carbonate was added, stirred and incubated for 60 min at 20–22 °C. The reading was taken at 765 nm (UV–VIS/UV-1280 Shimadzu) and the quantification was expressed in mg of gallic acid equivalents (GAE) per kg of olive oil, based on calibration curves constructed with five concentration levels of gallic acid (0.016–1.0 mg/mL). Linearity was confirmed by least-squares regression of absorbance versus concentration, yielding $R^2 \geq 0.999$.

2.9. Statistical analysis

A one-way ANOVA was carried out to assess the impact of geographical origin on quality parameters (free acidity, peroxide value, specific extinction coefficients and sensory analysis), chemical composition (fatty acids, tocopherols and phenolic compounds) and antioxidant activity (DPPH and TRC) of Galega and Cobrançosa olive oils harvested in Alentejo, Beira Interior and Trás-os-Montes. When a significant effect was found, Tukey's post-hoc test was used for multiple comparisons. A heatmap was used to visualize the correlations between variables, facilitating the identification of relationships between different parameters. This type of map, often combined with clustering algorithms, is useful for grouping similar data and visualizing these groupings. In this study, chemical markers were used to understand how they differentiate olive oils from different regions and cultivars. Principal component analysis (PCA) was applied to see if chemical and quality data could be used to identify the geographical origin of olive oils. This analysis was visualized using a 2D-biplot graph, which simplifies the interpretation of multivariate data by projecting it in two dimensions to facilitate visualization. The software used was R (version 4.4.1).

3. Results and discussion

3.1. Quality parameters

The present work compared oils from two varieties of olives (Cobrançosa and Galega) produced in three different regions (Alentejo, Beira Interior and Trás-os-Montes). Regarding quality parameters

Table 1

Mean values \pm standard deviation (SD) and range of quality parameters (free acidity (% oleic acid), peroxide value (mEq O₂/kg), specific extinction coefficients (K₂₃₂ and K₂₆₈) and commercial classification) of olive oils of the Cobrançosa and Galega cultivars from three different regions of Portugal (Alentejo, Beira Interior and Trás-os-Montes).

Quality parameters		Region of Portugal			P-value
		Alentejo	Beira Interior	Trás-os-Montes	
cv. Cobrançosa	Free acidity	0.17 \pm 0.00b	0.26 \pm 0.03a	0.17 \pm 0.00b	<0.0001
		0.17–0.17	0.23–0.28	0.17–0.17	
	Peroxide value	4.6 \pm 0.5a	2.5 \pm 0.1b	2.8 \pm 0.1b	<0.0001
		3.3–5.0	2.3–2.7	2.5–2.8	
	K ₂₃₂	1.89 \pm 0.06a	1.56 \pm 0.10b	1.36 \pm 0.06b	<0.0001
		1.75–1.95	1.41–1.80	1.27–1.49	
	K ₂₆₈	0.16 \pm 0.01a	0.14 \pm 0.02b	0.13 \pm 0.00b	<0.0001
		0.15–0.17	0.11–0.17	0.13–0.14	
	Green fruitiness	5.2 \pm 0.3a,b	5.2 \pm 0.3a	5.0 \pm 0.6b	0.0122
		4.8–5.7	4.8–5.6	4.3–5.7	
Bitterness	6.1 \pm 0.3a	5.7 \pm 0.2b	5.4 \pm 0.4b	<0.0001	
	5.7–6.4	5.5–6.0	4.9–6.0		
Pungency	6.8 \pm 0.4a	6.2 \pm 0.2b	6.1 \pm 0.3b	<0.0001	
	6.2–7.1	6.0–6.5	5.7–6.5		
Comercial grade	EVOO	EVOO	EVOO	-	
cv. Galega	Free acidity	0.24 \pm 0.03a	0.17 \pm 0.01b	0.18 \pm 0.02b	<0.0001
		0.23–0.28	0.17–0.20	0.17–0.23	
	Peroxide value	5.5 \pm 1.2a	2.6 \pm 0.3b	2.7 \pm 0.1b	<0.0001
		4.1–6.7	2.3–3.3	2.5–2.8	
	K ₂₃₂	1.72 \pm 0.12a	1.29 \pm 0.11b	1.32 \pm 0.13b	<0.0001
		1.48–1.83	1.16–1.48	1.14–1.52	
	K ₂₆₈	0.14 \pm 0.01	0.11 \pm 0.04	0.12 \pm 0.03	0.0534
		0.12–0.16	0.06–0.15	0.08–0.15	
	Green fruitiness	4.2 \pm 0.4a	3.7 \pm 0.1b	5.0 \pm 0.7a	0.0012
		3.8–4.7	3.6–3.9	4.3–5.8	
Bitterness	5.6 \pm 0.2a	3.9 \pm 0.4c	4.4 \pm 0.5b	<0.0001	
	5.3–6.0	3.3–4.4	3.7–5.0		
Pungency	6.3 \pm 0.2a	4.4 \pm 0.5c	5.1 \pm 0.6b	<0.0001	
	5.9–6.6	3.7–4.9	4.3–5.8		
Comercial grade	EVOO	EVOO	EVOO	-	

Analysis of variance between regions for each cultivar was applied (p-value indicated) where equal letters in the lines do not indicate a significant difference in the means according to Tukey's test ($p < 0.05$).

(Table 1), all analyzed oils met the legal limits for the extra-virgin olive oil (EVOO) category, as specified in the [Commission Delegated Regulation \(EU\) 2022/2104](#) of 29 July 2022. The obtained values for acidity, peroxide values and specific extinction coefficients were low for all olive oils, and despite being statistically different (P-value < 0.05) between regions, the differences were reduced, not supporting a significant influence of both region factor and cultivar on these parameters. These results are within expectations since these parameters depend mainly on agronomic aspects, which affect the quality of the fruit, harvesting practices, transport and storage of the fruits before extraction and the technological process of extracting olive oil (Méndez et al., 2007; Yousfi et al., 2012), being used to establish the commercial category.

Regarding sensory characteristics, since the fruits were at a similar maturation stage and underwent similar treatment before and during the extraction process, the differences observed within the cultivar could be attributed to the possible effect of the geographic region (Table 1). Although the results for green fruitiness showed some cultivar-dependent variation, olive oils from Alentejo region consistently exhibited significantly higher bitterness and pungency compared to those from Beira Interior and Trás-os-Montes, regardless of the cultivar (Table 1). These findings are of particular interest, considering that sensory intensities such as bitterness and pungency are generally associated with higher phenolic contents, which tend to be more prominent in olive oils produced under cooler climate conditions, such as those in the Trás-os-Montes region. Therefore, the greater expression of bitterness and pungency observed in the warmer Alentejo region was not initially expected. Nevertheless, factors such as harvest timing, olive maturity index, or water stress, and other aspects related to the terroir of the region may have contributed to increased phenolic biosynthesis in this specific crop year (Giuffrè, 2018; Servili et al., 2009; Salvador et al., 2001).

3.2. Olive oil composition and oxidative stability

Regarding the fatty acid composition, the most representative results obtained for the olive oils of the Cobrançosa and Galega varieties in the three analyzed regions (Alentejo, Beira Interior, and Trás-os-Montes) are presented in Table 2. In this context, more than the compliance of the fatty acid profile with the criteria established for extra virgin olive oil, according to [Commission Delegated Regulation \(EU\) 2022/2104](#) of 29 July 2022, it is important to highlight the variations and trends recorded in the majority fatty acids between the different regions under study.

Among the predominant fatty acids, oleic acid (C_{18:1}) stands out, the main representative of monounsaturated acids. There was a variation between 67.9 % and 72.9 %, with olive oils from Beira Interior and Trás-os-Montes having the highest values and those from Alentejo having the lowest levels. Significant differences (P-value < 0.05) were observed in this parameter. The second most common fatty acid identified was linoleic acid (C_{18:2}), a polyunsaturated fatty acid, varying between 4.0 % and 10.5 %, with the highest levels found in olive oils from the Alentejo region and the lowest in the Trás-os-Montes region. Among the saturated fatty acids, palmitic acid (C_{16:0}) was the most representative, ranging from 13.8 % to 18.3 %, with slightly higher values observed in olive oils from Alentejo region. Additionally, linolenic acid (C_{18:3}), another polyunsaturated fatty acid, was present in lower amounts, ranging from 0.7 to 1.1 %. No significant differences were observed for eicosenoic in the olive oils of the Cobrançosa cultivar, presenting a P-value of 0.0602. These compositional values varied according to both cultivar and region. The Cobrançosa cv showed the greatest effect of region. The effect of region on fatty acid composition is observed due to the presence of environmental factors that alter the composition of olive oils. Temperature is found to be one of the primary factors influencing the composition of olive oils. Regarding monounsaturated fatty acids

Table 2

Mean values \pm standard deviation (SD), (range), of fatty acids relative abundance (%) of olive oils of the Cobrançosa and Galega cultivars from three different regions of Portugal (Alentejo, Beira Interior and Trás-os-Montes).

Fatty acid composition		Region of Portugal			P-value
		Alentejo	Beira Interior	Trás-os-Montes	
cv. Cobrançosa	Palmitic acid (C _{16:0})	15.3 \pm 0.4a	13.9 \pm 0.4b	13.7 \pm 0.6b	<0.0001
		14.7–15.8	13.4–14.5	12.8–14.6	
	Palmitoleic acid (C _{16:1})	1.2 \pm 0.2a	1.0 \pm 0.0b	1.1 \pm 0.2a,b	0.0464
		0.9–1.4	0.9–1.1	0.9–1.3	
	Stearic acid (C _{18:0})	4.0 \pm 0.4a,b	3.7 \pm 0.1b	4.1 \pm 0.5a	0.0454
		3.5–4.4	3.5–3.9	3.6–4.9	
	Oleic acid (C _{18:1})	67.9 \pm 1.4c	72.0 \pm 0.6a	70.3 \pm 0.8b	<0.0001
		65.0–69.6	71.1–72.9	69.2–71.8	
	Linoleic acid (C _{18:2})	9.1 \pm 1.1a	7.2 \pm 0.3b	8.5 \pm 0.6a	<0.0001
		8.0–11.6	6.6–7.7	7.5–9.2	
	Linolenic acid (C _{18:3})	1.1 \pm 0.0a	0.9 \pm 0.1b	0.9 \pm 0.1b	<0.0001
		1.0–1.2	0.8–1.0	0.8–1.0	
	Arachidic acid (C _{20:0})	0.4 \pm 0.1a	0.5 \pm 0.0b	0.5 \pm 0.0a,b	0.0414
		0.4–0.6	0.4–0.5	0.5–0.6	
Eicosenoic acid (C _{20:1})	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.0602	
	0.2–0.3	0.2–0.2	0.2–0.3		
Σ SFA	20.2 \pm 0.3a	18.4 \pm 0.3b	18.7 \pm 0.2b	<0.0001	
	19.5–20.5	17.8–19.0	18.4–18.9		
Σ MUFA	69.6 \pm 1.3c	73.4 \pm 0.5a	71.8 \pm 0.7b	<0.0001	
	66.8–71.3	72.7–74.4	70.8–73.1		
Σ PUFA	10.2 \pm 1.1a	8.1 \pm 0.3b	9.4 \pm 0.6a	<0.0001	
	9.1–12.6	7.5–8.4	8.3–10.2		
cv. Galega	Palmitic acid (C _{16:0})	18.6 \pm 0.4a	17.0 \pm 0.5b	16.4 \pm 0.5c	<0.0001
		18.2–19.3	16.2–17.8	15.4–17.2	
	Palmitoleic acid (C _{16:1})	3.2 \pm 0.2a	2.4 \pm 0.2b	2.5 \pm 0.3b	<0.0001
		2.9–3.5	2.1–2.7	2.2–3.1	
	Stearic acid (C _{18:0})	2.4 \pm 0.1a	1.7 \pm 0.1b	1.8 \pm 0.2b	<0.0001
		2.2–2.5	1.7–1.8	1.5–2.3	
	Oleic acid (C _{18:1})	68.7 \pm 0.6c	71.5 \pm 0.9b	72.9 \pm 0.7a	<0.0001
		67.3–69.3	70.2–72.7	71.8–74.3	
	Linoleic acid (C _{18:2})	4.8 \pm 0.2a	4.9 \pm 0.2a	4.4 \pm 0.4b	0.0042
		2.9–3.5	4.5–5.2	3.9–5.4	
	Linolenic acid (C _{18:3})	0.8 \pm 0.0b	1.0 \pm 0.0a	0.7 \pm 0.1c	<0.0001
		0.8–0.9	0.9–1.0	0.6–0.8	
	Arachidic acid (C _{20:0})	0.4 \pm 0.0a	0.4 \pm 0.0b	0.3 \pm 0.0c	<0.0001
		0.4–0.4	0.4–0.4	0.3–0.4	
Eicosenoic acid (C _{20:1})	0.3 \pm 0.0c	0.3 \pm 0.0a	0.3 \pm 0.0b	<0.0001	
	0.2–0.3	0.3–0.4	0.3–0.3		
Σ SFA	21.8 \pm 0.3a	19.5 \pm 0.3b	18.8 \pm 0.5c	<0.0001	
	21.4–22.4	17.8–19.0	17.8–19.5		
Σ MUFA	72.5 \pm 0.4c	74.6 \pm 0.7b	76.0 \pm 0.7a	<0.0001	
	71.5–73.0	73.4–75.7	74.6–77.3		
Σ PUFA	5.6 \pm 0.1a	5.6 \pm 0.2a	5.1 \pm 0.5b	<0.0001	
	5.5–5.9	5.4–6.2	4.6–6.1		

Analysis of variance was applied (p-value indicated) where equal letters in the lines do not indicate a significant difference in the means according to Tukey's test ($p < 0.05$).

(MUFAs), the results obtained reveal significant differences in levels between the studied regions. The olive oils from Trás-os-Montes had the highest levels of MUFA, with an average content of 76 % (Galega cv) and 71.8 % (Cobrançosa cv). In contrast, the olive oils from Beira Interior and Alentejo had the lowest values. This phenomenon is essentially linked to climatic conditions. According to [Borges et al., 2017](#), in a study characterizing virgin olive oils of the Arbequina cultivar produced in different regions of Brazil and Spain, it was found that monounsaturated fatty acids tend to have higher levels in colder regions. The same was observed by [Rodrigues et al., 2023](#) where, in a study of Galega olive oils obtained in seven Portuguese regions, they found that geographical origin significantly affected the chemical composition of olive oils, especially in colder regions.

Regarding tocopherols ([Table 3](#)), α -tocopherol was the primary vitamin found in the samples studied, accounting for approximately 90 % of the total vitamin E content, as expected. The region significantly affected the amount of this vitamin, and significant differences were observed (P -value < 0.05). In the Cobrançosa cultivar, α -tocopherol showed the highest concentration in the Alentejo region, with 432 mg/kg, while in the Galega cultivar the highest concentration was observed

in Beira Interior, with 428 mg/kg. The values observed were higher than those obtained by [Peres et al., 2016](#), in their study of the phenolic compounds in olive oils from the 'Galega Vulgar' and 'Cobrançosa' cultivars throughout the initial stages of ripening, where the α -tocopherol values were 371 mg/kg for the Cobrançosa cultivar and 342 mg/kg for the Galega cultivar. Interestingly, the extreme concentrations observed in the oils of the two cultivars did not follow the same trend, indicating a possible contribution from factors other than geographical origin, probably related to agricultural practices, plant stress or even the altitude of the olive grove and the cultivar itself ([Beltrán et al., 2005](#)).

The determination of hydroxytyrosol and tyrosol, quantified after acid hydrolysis ([Table 3](#)), showed that their levels were significantly affected by the geographical origin of the oil. Among all the regions studied, the Alentejo had the highest hydroxytyrosol levels, with an average of 437 mg/kg for the Cobrançosa cultivar and 450 mg/kg for the Galega cultivar. The same trend was observed for tyrosol-based compounds, whose content ranged from 275 mg/kg in the Cobrançosa cultivar to 342 mg/kg in the Galega cultivar. The Trás-os-Montes region had the lowest concentrations of hydroxytyrosol and tyrosol. The values obtained for the Cobrançosa cv. were much higher than those found by

Table 3

Mean values \pm standard deviation (SD), (range), of tocopherols, hydroxytyrosol and tyrosol derived compounds (expressed in mg/kg of olive oil), oxidative stability (OS, h), DPPH (inhibition %) and total reducing capacity (mg GAE per kg of oil) of olive oils of the Cobrançosa and Galega cultivars from three different regions of Portugal (Alentejo, Beira Interior and Trás-os-Montes).

Evaluated parameters		Region of Portugal			P-value
		Alentejo	Beira Interior	Trás-os-Montes	
cv. Cobrançosa	α -tocopherol	432 \pm 32a	269 \pm 21b	246 \pm 18b	<0.0001
		384–481	234–300	229–288	
	β -tocopherol	3.3 \pm 0.3a	2.1 \pm 0.3b	2.2 \pm 0.3b	<0.0001
		2.9–3.9	1.7–2.7	1.7–2.8	
	γ -tocopherol	3.0 \pm 0.4c	6.5 \pm 0.7b	9.7 \pm 1.4a	<0.0001
		2.4–3.5	5.3–7.4	7.9–12.3	
	Σ tocopherols	438 \pm 32a	277 \pm 21b	258 \pm 19b	<0.0001
		389–489	243–308	241–303	
	Hydroxytyrosol based-compounds	437 \pm 130a	352 \pm 74a,b	262 \pm 101b	0.0034
		233–582	253–441	128–408	
	Tyrosol based-compounds	275 \pm 124	232 \pm 59	177 \pm 63	0.0577
		94–445	145–324	103–297	
	OS	21.8 \pm 2.0	22.5 \pm 1.8	25.0 \pm 8.8	0.362
17.3–24.6		21.0–26.1	13.5–35.7		
DPPH	79.3 \pm 2.1a	56.2 \pm 13.7b	35.7 \pm 7.4c	<0.0001	
	73.9–80.9	41.1–74.4	26.4–46.0		
Total reducing capacity	902 \pm 67a	804 \pm 142a	529 \pm 99b	<0.0001	
	768–979	619–1037	401–676		
cv. Galega	α -tocopherol	375.6 \pm 24.8b	427.7 \pm 18.4a	248.7 \pm 71.9c	<0.0001
		347.5–418.9	400.4–454.8	168.0–383.4	
	β -tocopherol	3.6 \pm 0.3a	3.9 \pm 0.3a	2.0 \pm 0.8b	<0.0001
		3.2–4.0	3.4–4.3	1.2–3.5	
	γ -tocopherol	6.4 \pm 0.7c	10.4 \pm 1.0b	15.0 \pm 5.3a	<0.0001
		5.6–7.9	9.0–12.1	7.3–23.0	
	Σ tocopherols	385.5 \pm 25.5b	442.0 \pm 18.0a	265.7 \pm 74.4c	<0.0001
		356.3–430.8	413.6–468.5	180.9–394.2	
	Hydroxytyrosol based-compounds	449.6 \pm 82.1a	380.0 \pm 64.8a	197.6 \pm 73.9b	<0.0001
		341.2–605.8	238.9–454.6	103.8–334.3	
	Tyrosol based-compounds	342.8 \pm 80.0a	303.2 \pm 52.0a	112.1 \pm 32.7b	<0.0001
		229.3–450.8	193.3–361.3	82.2–191.0	
	OS	32.5 \pm 3.6a	18.4 \pm 2.3c	26.2 \pm 5.5b	<0.0001
23.1–35.6		15.8–22.4	19.7–33.8		
DPPH	76.5 \pm 2.5a	31.9 \pm 7.2c	55.1 \pm 4.0b	<0.0001	
	71.7–80.4	23.1–44.2	49.7–61.0		
Total reducing capacity	1025.2 \pm 79.0a	263.4 \pm 37.7b	308.1 \pm 159.1b	<0.0001	
	920.5–1182.9	204.8–324.9	137.0–495.7		

Analysis of variance between regions was applied (p-value indicated) where equal letters in the lines do not indicate a significant difference in the means according to Tukey's test ($p < 0.05$).

Marx et al., 2021, who estimated the amounts of hydroxytyrosol and tyrosol derivatives in olive oils from the Cobrançosa cultivar using electronic tongue analysis of olive paste extracts. The same was observed for the Galega cv. when compared with the values obtained by Rodrigues et al., 2023, in their study on the geographical discrimination of olive oils from the 'Galega Vulgar' cultivar. These concentrations are higher than those reported in studies from other regions. For example, Rozanska et al. (2020) found hydroxytyrosol levels ranging from 22.8 to 208.1 mg/kg, and tyrosol levels between 5.4 and 124.2 mg/kg in Italian olive oils. In contrast, Gómez-Rico et al. (2008) reported hydroxytyrosol concentrations between 219 and 349 mg/kg in olive oils from Arbequina cultivar, 206 to 271 mg/kg in olive oils from Cornicabra cultivar, and 321 to 377 mg/kg in olive oils from Picual cultivar, from the region of Spain. The concentrations obtained confirm the alignment with the health claim related to polyphenols regarding their protection of blood lipids against oxidative stress. This claim can only be supported if the oil contains a minimum of 250 mg of hydroxytyrosol and its derivatives per kg of oil, as established by Commission Regulation (EU) No 432/2012.

When determining the oxidative stability, the oils from the Galega cv. showed greater stability, with 32.5 h of resistance. In the olive oils of the Cobrançosa cv. no significant differences were observed between the regions, presenting an average value of 23.1 h of resistance. It was found that geographical location does not directly affect these results, but other factors, such as water stress, temperature, or other environmental elements, do. The values obtained for both cultivars are in line with those reported by Peres et al., 2016 for the same harvest period, who

found a stability of 23 h for the Cobrançosa cv. and 33 h for the Galega cv. With regard to antioxidant activity, the same trend was observed as for oxidative stability, with the Alentejo region showing the highest antioxidant activity for both olive oils. In the context of this study, the conditions influencing the olive oils composition include regional differences in temperature regimes (e.g., higher average temperatures in Alentejo and markedly cooler conditions in Trás-os-Montes), altitude gradients (with Trás-os-Montes representing the highest-altitude groves), and climatic characteristics related to water availability and continentality, particularly relevant in Beira Interior. These environmental factors, combined with similar agronomic practices across all groves (soil management, fertilization, irrigation regime and phytosanitary treatments), help explain the observed regional variability in fatty acids, tocopherols and phenolic compounds.

3.3. Regional discrimination based on quality parameters and chemical composition of Cobrançosa and Galega olive oils

A heatmap was used to check the correlations between variables, helping to identify relationships between different parameters. Figs. 1 and 2 show the statistical analysis of the parameters evaluated in the olive oils of the Cobrançosa and Galega cultivars from three different regions namely Alentejo, Beira Interior and Trás-os-Montes. This figure aims to illustrate how the region influences the composition and quality of the oils, considering physical-chemical, sensory, and antioxidant parameters. The arrangement of colors on the heatmap enables us to

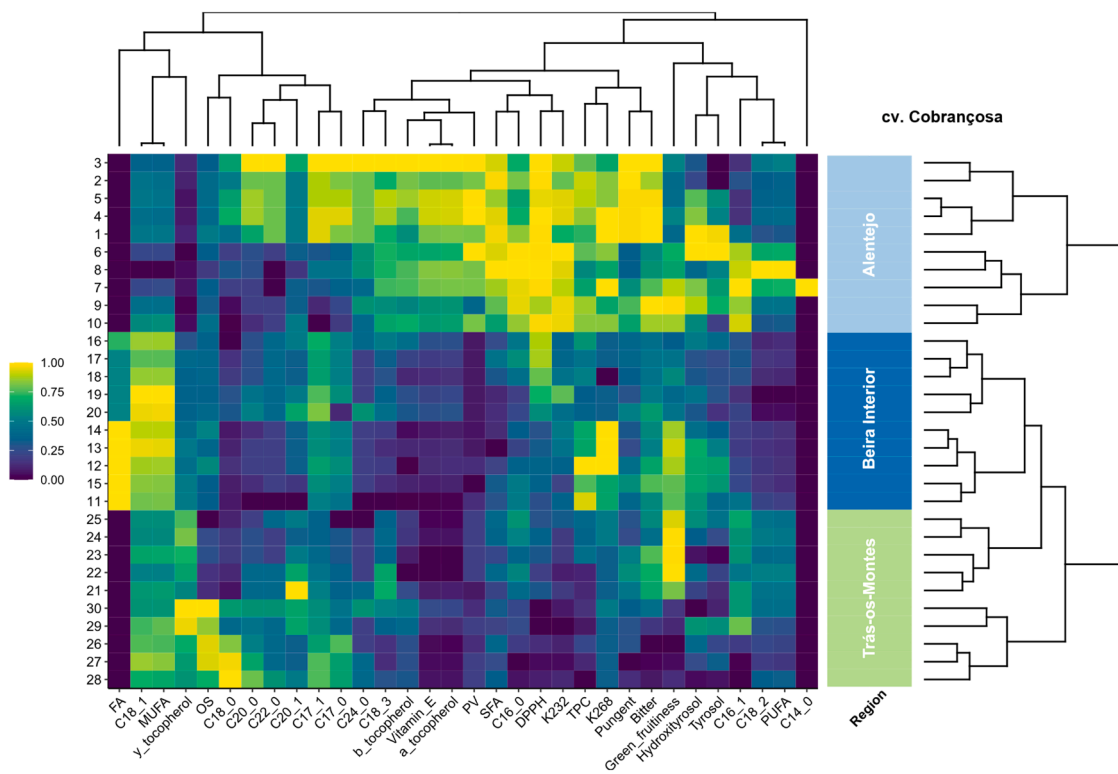


Fig. 1. Heatmap representing the variability of data in different regions (Alentejo, Beira Interior and Trás-os-Montes) for the cv. Cobrançosa.

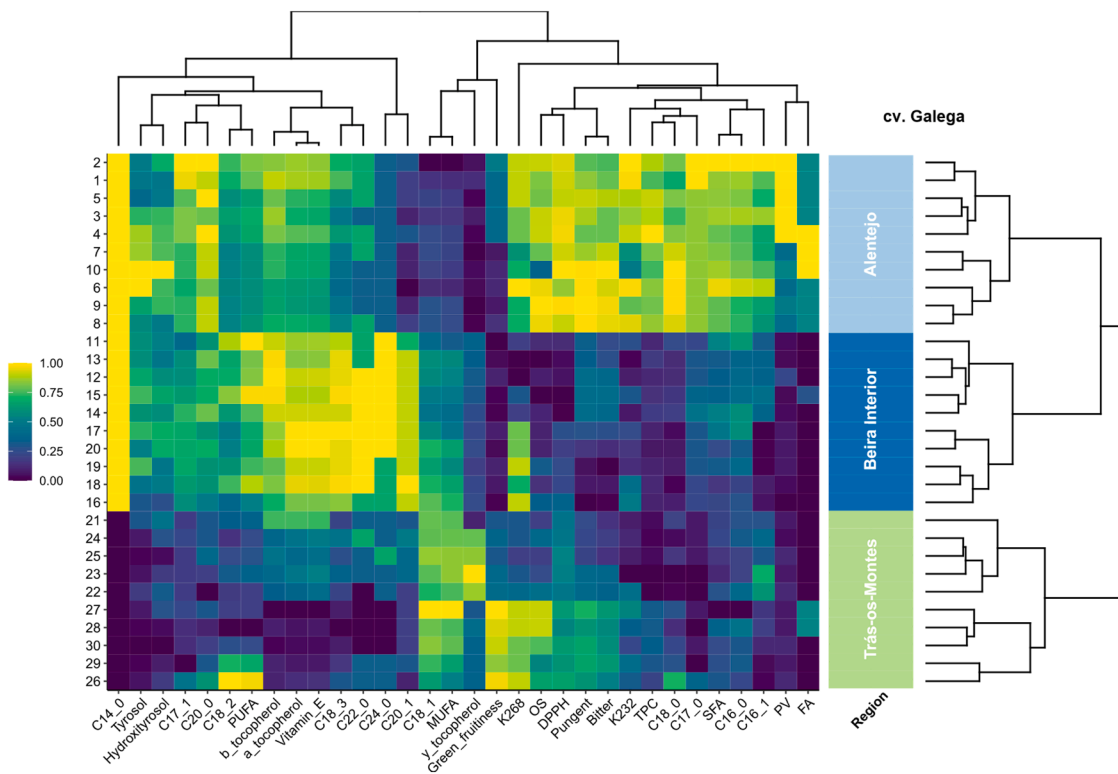


Fig. 2. Heatmap representing the variability of data in different regions (Alentejo, Beira Interior and Trás-os-Montes) for the cv. Galega.

identify distinct patterns between the olive oils from different regions. The observed variability indicates that the oils have distinct profiles, suggesting that their geographical origin has a direct influence on their composition. In addition, the hierarchical grouping at the top of the

image reinforces this distinction, as it shows that the samples are organized differently according to their region of origin.

For the Galega cultivar (Fig. 1), it was found that the olive oils from the Alentejo have a different profile to those from Beira Interior and

Trás-os-Montes regions, with some more expressive characteristics, as shown by the yellow areas on the heatmap. This region stands out for its oxidative stability, bitter and pungent sensations, antioxidant activity measured by the DPPH method, total reducing capacity and the presence of saturated fatty acids. Olive oils from Beira Interior, on the other hand, differ in their content of tocopherols, hydroxytyrosol and tyrosol, as well as containing a greater amount of polyunsaturated fatty acids. Meanwhile, the olive oils from Trás-os-Montes region show distinct patterns, differing from the other regions mainly in their green fruitiness and the predominance of monounsaturated fatty acids. These results demonstrate that each region imparts a distinct chemical and sensory profile to olive oils.

In the Cobrançosa cultivar (Fig. 2), the differentiation from Alentejo olive oils is based on bitter and pungent sensations, antioxidant activity (DPPH), total reducing capacity, the presence of hydroxytyrosol and tyrosol, and the composition of saturated and polyunsaturated fatty acids. The tocopherol content also contributes significantly to distinguishing this region. Olive oils from Beira Interior and Trás-os-Montes regions, on the other hand, show distinct patterns, differing from the other regions mainly in their green fruitiness and the composition of monounsaturated fatty acids. The regional discrimination observed in the Galega and Cobrançosa cultivars indicates that geographical origin has a significant impact on the composition and quality of olive oils, reinforcing the importance of environmental, soil, and climatic conditions in defining their sensory and chemical characteristics.

The Principal Component Analysis (PCA) (Fig. 3) shows the distribution of the samples and the relationship between the variables analyzed, including fatty acids, tocopherols, oxidative stability, antioxidant activity measured by the DPPH assay and total reducing capacity. Fig. 3a shows a clear differentiation between the olive oils of the Galega cultivar from the three regions studied (Alentejo, Beira Interior and Trás-os-Montes) with 64.5 % of the samples correctly classified. The olive oil samples from Alentejo are grouped together on the right-hand side of the biplot and are well separated from the others, indicating that these oils have distinct chemical profiles. Olive oils from the Beira Interior and Trás-os-Montes regions, on the other hand, appear more distributed on the left-hand side of the graph but still form two distinct groupings, with olive oils from Beira Interior positioned at the top and those from Trás-os-Montes at the bottom. This separation suggests that, despite some similarities, there are marked chemical differences between the samples from the three regions. Among the different analyses carried out, fatty acids such as C_{18:3}, C_{20:1}, C_{22:0} and C_{24:0} are more associated with olive oils from Beira Interior region. The tocopherols appear to be distributed in different directions, indicating variations in their concentration between the samples. Oxidative stability and total reducing capacity exhibit a strong correlation and are more closely associated with olive oils from the Alentejo, suggesting that these oils possess greater resistance to oxidation and a higher phenolic content. Likewise, the antioxidant activity measured by the DPPH assay is also strongly correlated with this group, reinforcing the idea that olive oils

from the Alentejo have greater antioxidant potential. In Fig. 3b, the PCA biplot of the Cobrançosa cultivar reveals a clear separation between the oils from the three analyzed regions, with 84.3 % separation. The Alentejo olive oils are grouped together on the right-hand side of the graph, represented by the blue-shaded area, suggesting that these oils have a distinct chemical profile, separate from the other regions. The olive oils from Beira Interior region appear in the central area, with a partial overlap with those olive oils from Trás-os-Montes region, which indicates that, although they show some differences, they share certain chemical characteristics. The variables analyzed indicate that the fatty acids, C_{16:0}, C_{16:1}, C_{18:2}, C_{18:3}, and C_{24:0} show a strong association with olive oils from Alentejo region. Specifically, higher levels of palmitic (C_{16:0}), palmitoleic acid (C_{16:1}), linoleic acid (C_{18:2}), and linolenic acid (C_{18:3}) were observed in olive oils from region compared to Beira Interior and Trás-os-Montes. In contrast, lower levels of lignoceric acid (C_{24:0}) were detected.

On the other hand, parameters such as oxidative stability, fatty acids, C_{18:1} and MUFA are more correlated with olive oils from Trás-os-Montes and Beira Interior regions. The separation of the groups in the biplot confirms that geographical origin has a significant influence on the chemical composition of olive oils from the Cobrançosa cultivar. The oils from Alentejo exhibit a more homogeneous and distinct profile compared to the other regions, while those from Beira Interior display an intermediate variation. Fig. 3c shows a 66.1 % separation between the two cultivars Galega and Cobrançosa, based on the chemical composition of their compounds, including fatty acids, tocopherols, total reducing capacity, oxidative stability and antioxidant activity (DPPH). The oils from the Galega cultivar are grouped predominantly in the yellow region on the left of the graph, while the samples from the Cobrançosa cultivar are concentrated in the blue region on the right. This separation indicates that each cultivar has a distinct chemical profile, with differences in its composition of fatty acids and bioactive compounds. The vectors of the chemical variables suggest that Galega oils are more associated with certain fatty acids, such as C_{16:0}, C_{16:1}, C_{18:1} and total MUFA, while Cobrançosa oils show a greater correlation with fruitiness, bitter and pungent sensations and with the fatty acids C_{18:0}, C_{18:2}, C_{20:0} and total PUFA. The vectors of the variables point in different directions, indicating how they influence the separation of the samples. The well-defined separation between the two groups reinforces that the composition of the oils is strongly influenced by the cultivar, and it is possible to distinguish between Galega and Cobrançosa based on their chemical profiles. However, it's important to note that these findings are based on olives harvested in a single crop year, and therefore may be influenced by specific climatic or agronomic conditions of that season. This study presents some limitations that should be acknowledged. First, the work was conducted over a single harvest year, which may restrict the generalization of the results, as interannual climatic variability can significantly influence olive oil composition. Additionally, only two cultivars although highly representative of Portuguese olive production were evaluated, limiting the extrapolation of

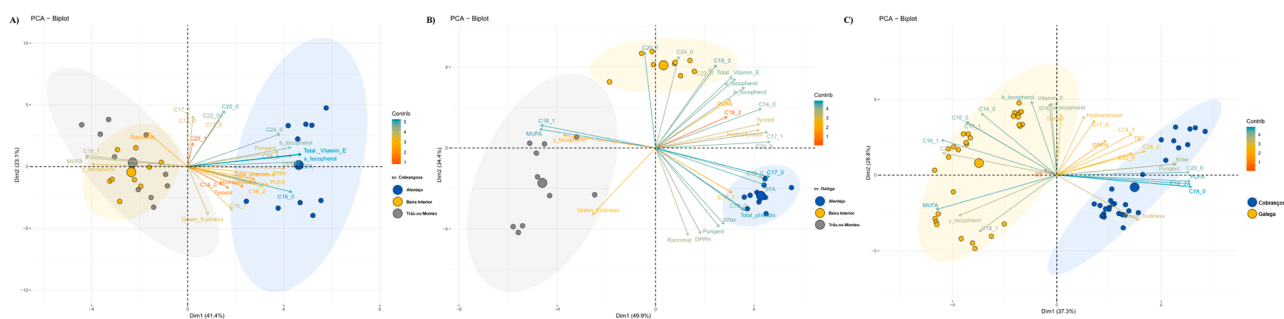


Fig. 3. Visualization of the Principal Component Analysis (PCA), highlighting the relationship between variables and sample groupings in particular: A) variability of cv Cobrançosa olive oils between the regions of Alentejo, Beira Interior and Trás-os-Montes; B) variability of cv Galega olive oils between the regions of Alentejo, Beira Interior and Trás-os-Montes; C) variability of Cobrançosa cv and Galega cv olive oil.

the findings to other genetic backgrounds. Furthermore, while the study characterized regional climatic conditions, a detailed physicochemical characterization of soils was not performed, which could further clarify the specific contribution of edaphic factors to the observed differences among regions. Future studies including multi-year assessments, additional cultivars, and comprehensive soil analyses would help strengthen the understanding of how environmental and agronomic variables shape olive oil quality.

4. Conclusion

This study highlights the influence of geographical origin and cultivar on the composition and quality of olive oils. Samples of the Cobrançosa and Galega cultivars, produced in Alentejo, Beira Interior and Trás-os-Montes, showed differences in physical-chemical parameters, sensory attributes and antioxidant properties. All oils met the standards for extra virgin olive oil (EVOO). However, quality parameters varied between regions, being more influenced by agronomic and technological processes than by geographical factors. The fatty acid composition revealed significant variations: Trás-os-Montes stood out for its higher content of monounsaturated fatty acids (MUFA), reflecting a fruitier and healthier profile; Alentejo presented higher levels of linoleic acid, as well as the phenolic substances hydroxytyrosol and tyrosol, which points to its potential in the production of olive oils with high antioxidant power; Beira Interior stood out for its higher concentration of bioactive compounds, especially α -tocopherol, evidencing its relevance in the nutritional value of olive oils. Statistical analysis confirmed that the geographical origin significantly impacts the chemical and sensory profiles of olive oils. Alentejo was associated with high oxidative stability and strong bitterness; Beira Interior stood out for its content of antioxidant compounds, while Trás-os-Montes presented a fruity profile rich in MUFA. Based on these findings, it was possible to identify specific quality markers for each region: Alentejo oils were characterized by high hydroxytyrosol-derived compounds and intense bitterness and pungency; Beira Interior oils showed the highest α -tocopherol levels; and Trás-os-Montes oils exhibited the greatest MUFA content and pronounced green fruitiness, reinforcing the distinct identity of each geographical origin.

Ethical statement

This study did not involve any animal experiments or medical procedures on human participants. The sensory evaluation was conducted using olive oil samples extracted from Galega and Cobrançosa cultivars and followed standard sensory analysis procedures in accordance with international guidelines.

As the study did not include the development or testing of new food products and posed no risk to participants, formal approval from an ethics committee was not required. All assessors participated voluntarily and were informed about the nature of the evaluation.

CRediT authorship contribution statement

Pedrina Rocha: Writing – original draft, Methodology, Investigation, Formal analysis. **Sandra Lamas:** Methodology, Investigation, Formal analysis. **Susana Casal:** Writing – review & editing, Investigation, Formal analysis. **Luísa L. Martins:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Nuno Rodrigues:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Data availability

Data will be made available on request.

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