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Characterisation of Olive Oils from the Douro Valley, Portugal: Study of the Volatile Fraction and Its Relationship with Sensory Characteristics

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Featured Application: The findings of the present study may support the creation of databases of olive oils from the Douro region (Portugal), boost their competitiveness in the global market, and encourage their worldwide exploitation.

Abstract: In recent years, the demand for olive oils from the Douro region (Portugal) has increased in line with wine. Thus, it is essential to characterise these olive oils to evaluate them. Therefore, this work describes the sensory and volatile profiles of olive oils produced in the Douro region. These were mainly identified as ripe fruity. Among the olfactory and gustatory sensations, the attributes of dried fruits, tomato, apple, tomato leaves, banana, and lavender stood out. Regarding volatile compounds, the following were detected in all samples: (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol acetate, 1-hexanol, and 2-methyl-4-pentanal. Moreover, it was impossible to separate the olive oils by sub-regions. In conclusion, studying the volatile fraction and their relationship with sensory attributes are essential to guarantee the genuineness and identity of these olive oils with a view to their future appreciation.

Keywords: Douro region; olive oils; valorisation; volatiles; sensory analysis

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1. Introduction

The Douro region, Portugal, is known worldwide for its wine production. However, other products, such as olive oil, demonstrate different characteristics compared with other areas of the country and even the world. This differentiation results from the soil and climate conditions that give its products a specific terroir. Volatile compounds are responsible for the aromatic profile of olive oils. This way, the functional groups belong to, and their proportions will influence the perceived aromas [1]. The primary step in the formation of volatile compounds occurs during milling, in which the rupture of the olive skin promotes the release of enzymes, which, in contact with triglycerides and phospholipids, cause their hydrolysis into free fatty acids, triggering a chain of enzymatic reactions, called the lipoxygenase (LOX) pathway. This pathway leads to the production of 13-hydroperoxides from the degradation of linoleic and linolenic fatty acids. These fatty acids, through hydroperoxide lyases, give rise to C6 aldehydes (hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal). The aldehydes are then reduced to alcohols (hexanol, (*E*)-2-hexenol, (*Z*)-3-hexenol) via alcohol dehydrogenase. These alcohols serve as a substrate for the alcohol acetyltransferase to produce esters (hexyl acetate and 3-hexenyl acetate) [2,3]. Although the LOX pathway is the main pathway for forming volatile compounds, others contribute to volatile compound profile in olive oils. Although less significant, amino acid fermentation, fungal enzymatic activities, or oxidative processes can be involved. However, it has been observed that an increase in volatile compounds produced by less significant pathways negatively influences the aroma of oils, with the appearance of undesirable smells [4]. Many volatile compounds in virgin olive oils are C5 and C6 compounds. In particular, 3-methylbutan-1-ol, hexanal, hexan-1-ol, and (*E*)-2-hexanal were found in most virgin olive oils in Europe [5]. The volatile compound profile is not significantly influenced by variations in the fatty acid profile. On the contrary, factors such as cultivar [1,6,7], geographic region [8], climate [8], fruit maturation stage [8–11], and extraction conditions [5,12–17] are those that most influence olive oils' volatile profile. Volatile compounds that originate in the LOX pathway, such as hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and pentanal, are mainly influenced by the maximum temperature at which the cultures are subjected. However, this influence does not prove to be direct, depending on other factors such as the cultivar. Also related to temperature is the altitude at which the crops are located, showing that olive oils produced in mountainous areas with lower temperatures are more bitter, spicy, and fruity compared with olive oils produced in places with lower altitudes and temperatures. Identifying the origin of the olive oils becomes difficult because variables such as the stage of maturation of the olive and the cultivar alter the chemical profile of the olive oils [8]. The different stages that intervene throughout the process of obtaining the olive oil can also influence the profile of volatile compounds, contributing to a set of characteristic olfactory and gustatory sensations. Several authors have described the relationship between the aromas perceived in olive oils and the volatile compounds identified [5,6,8,18,19]. However, aromas and sensory attributes are not associated only with a specific volatile compound but with the interaction of several compounds simultaneously [5]. Thus, this work aimed to determine the relationship between the perceived sensory attributes and the volatile compounds identified in the olive oils produced in Portugal's Douro Valley region.

2. Materials and Methods

2.1. Sampling

A total of 134 olive oil samples were collected in three distinct sub-regions of the Douro region, 32 from the Baixo Corgo, 57 from the Cima Corgo, and 45 from the Douro Superior. All samples were collected from local producers. In general, the trees presented more than 50 years old and are cultivated according to the Integrated Protection Guidelines. In the Douro region, olive trees grow in small areas or on the border of the vineyards, which is considered the main crop. The trees are non-irrigated. Except for one treatment with copper products against olive diseases at the beginning of spring, no other phytosanitary treatments were made. No detailed information about each olive oil is available regarding the olive cultivars. In the region, there is a great diversity of trees belonging to different cultivars, some of which are unknown and uncharacterized. Nevertheless, according to the producers, Galega, Madural, Verdeal, Cobrançosa, Negrinha, and Cordovil are the main cultivars. The pedoclimatic conditions were very similar in all the regions and are characterized by schist soils with some inclusions of a granitic nature and are distributed in two fundamental groups: anthrosols (≈30% of the area) and less intervened soils present in the region, such as leptosols, cambisols, fluvisols, and regosols. The ripening stage at the harvest was from 2.5 to 4.0, determined according to the International Olive Council guidelines. All the olive oils were extracted in one two phases industrial extraction line, with a total of 5000 kg of paste per hour. For each producer, three dark glass bottles of 500 mL were collected, one for the sensory analysis, one for the physicochemical analysis, and the third to reserve for results confirmation if needed.

From all samples, only fifty-seven samples of olive oil were considered extra virgin. A sensory analysis was conducted to confirm that it was indeed extra virgin olive oil. Additionally, quality parameters were also determined. In more detail, all olive oils presented characteristics to be classified as extra virgin olive oils, namely no sensory defects and a fruity intensity higher than zero. The values of free acidity varied from 0.28 to 0.34, expressed in grams of oleic acid per 100 g of olive oil. The peroxide values ranged from

5.40 to 6.23 mEq of O_2/kg of olive oil, and the values of K_{232} (1.83 to 1.98) and K_{270} (0.12 to 0.13) were also inside the values of the extra virgin category.

2.2. Sensory Analysis

Following the EU regulation, a sensory panel with eight trained members (five men and three women, aged from 25 to 52 years old, with an average age of 37 years) evaluated all olive oil samples. The panel, from the Agriculture School of the Polytechnic Institute of Bragança, is a well-trained panel with more than five years of experience in sensory analysis of olive oil and table olives and is very familiar with the olive oil sensory lexicon and assessment scales. A proof sheet prepared following the International Olive Council (IOC) [20] was used, with some modifications [21]. Descriptive sensory analysis was evaluated on a scale from 0 to 10, unstructured, where olfactory and gustatory sensations (fruity sensations, herbaceous sensations, and fruit sensations) were assessed. Olfactory-gustatory sensations such as complexity and persistence were also evaluated.

2.3. Volatile Compounds

The determination of the profile in volatile compounds was carried out by HS-SPME (headspace solid-phase microextraction) and GC/MS (gas chromatography coupled to a mass spectrometer) [22]. In short, 3 g of olive oil were measured in a 50 mL glass vial. Five µL of internal standard (4-methyl-2-pentanol) (Sigma-Aldrich, St. Louis, MO, USA) at 0.125 mg/mL was added. The vial was placed in a water bath at 40 °C and shaken at 300 rpm for 5 min to release the volatile compounds. Then, under the same temperature and agitation conditions, the SPME fibre (divinylbenzene/carbonex/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm) (Supelco, Bellefonte, PA, USA) was exposed for 30 min for adsorption of the volatile compounds present in the headspace. Volatile compounds were removed from the fibre by thermal desorption (220 °C) for 1 min in the chromatograph injection port. Nevertheless, the fibre was kept in the injector for 10 min for cleaning and conditioning.

The gas chromatograph used was a Shimadzu GC-2010 Plus, equipped with a Shimadzu GC/MS-QP2010 SE mass spectrometer. A TRB-5MS column (30 m × 0.25 mm × 0.25 µm) (Teknokroma, Barcelona, Spain) was used. The injector was at a temperature of 220 °C, and manual injection was performed in splitless mode. The mobile phase consisted of helium 5.0 (Linde, Lisboa, Portugal) at a linear velocity of 30 cm/s and a 24.4 mL/min flow rate. The oven temperature was 40 °C for 1 min, followed by an increase of 2 °C/min until reaching 220 °C. The ionisation source was maintained at 250 °C with an energy of 70 eV and a current of 0.1 kV. All mass spectra were obtained by electronic ionisation in the m/z range 35–500. Compounds were identified by comparing mass spectra and Kovat's Indexes, using the NIST 69, PubChem, and ChemSpider databases. The areas of the compounds were determined by integration of TIC (total ion chromatogram). The semi-quantitation of volatile compounds was performed from the relative area of each peak. Then, each relative area was converted into a mass equivalent to the mass of the added internal standard. Each sample was evaluated in triplicate.

2.4. Statistical Analysis

Data were statistically analysed using Rstudio version 1.2.5001 and respective packages to verify the prerequisites of data homogeneity and normality. The normality and homogeneity of the variances were assessed by the Shapiro–Wilk and Levene tests, respectively. The data was found to be normal. A Pearson correlation analysis was also performed to verify the existence of significant correlations (p-value < 0.05) between volatile compounds and olfactory sensory attributes.

3. Results and Discussion

3.1. Volatile Compounds

Regarding the volatile compounds identified in the olive oils, the compounds were grouped by class. Table 1 describes the volatile compounds detected in the extra virgin olive oils collected in the Douro Valley. The percentage of samples with a specific compound and the average concentration in those samples of that particular compound were determined. Figure 1 shows the percentages of compound classes identified in these same olive oils. Despite being identified, all compounds reported in less than 5% of the samples were not considered in this study, as they were not guaranteed to be characteristic of the olive oils. Thus, 62 volatile compounds were identified and quantified: 15 hydrocarbons, 14 terpenes, 11 alcohols, 9 aldehydes, 5 ketones, 5 esters, 2 ethers, and 1 nitrile. The group of hydrocarbons included compounds such as alkanes, alkenes, cycloalkanes, and aromatic hydrocarbons.

Table 1. Volatile compounds identified in Douro extra virgin olive oils.

Volatile Compounds	% of Samples	Concentration (μg/g)	Identification [a]
		Alcohols	
1-Heptanol	7	0.3 ± 0.2 (0.2–0.7)	MS/IK.DB
1-Hexanol	89	44.4 ± 71.8 (1.4–516.9)	MS/IK.DB
2-Ethyl-1-hexanol	7	0.7 ± 0.3 (0.4–1.2)	MS/IK.DB
1-Nonanol	91	0.7 ± 0.5 (0.1–2.5)	MS/IK.DB
4,8-Dimethyl-1-nonanol	30	0.4 ± 0.2 (0.2–0.8)	MS/IK.DB
1-Octanol	96	0.8 ± 0.5 (0.3–2.9)	MS/IK.DB
(*E*)-2-Hepten-1-ol	28	1.3 ± 0.5 (0.6–2.3)	MS/IK.DB
(*E*)-2-Hexen-1-ol	19	105.4 ± 148.4 (1.9–532.5)	MS/IK.DB
(*Z*)-2-Penten-1-ol	100	4.6 ± 3.8 (1.3–27.0)	MS/IK.DB
(*Z*)-3-Hexen-1-ol	16	156.8 ± 108.6 (62.9–439.9)	MS/IK.DB
Phenylethyl Alcohol	95	0.6 ± 0.3 (0.3–1.7)	MS/IK.DB
		Aldehydes	
(*E*)-4-Oxohex-2-enal	96	6.0 ± 4.9 (0.6–22.9)	MS/IK.DB
(*E*,*E*)-2,4-Heptadienal	32	1.2 ± 0.3 (0.7–1.8)	MS/IK.DB
(*E*,*E*)-2,4-Hexadienal	96	4.3 ± 2.0 (1.3–8.9)	MS/IK.DB
(*Z*)-2-Heptenal	30	0.9 ± 0.6 (0.3–2.6)	MS/IK.DB

Table 1. *Cont.*

Volatile Compounds	% of Samples	Concentration (µg/g)	Identification [a]
(*E*)-2-Hexenal	100	258.9 ± 187.4 (5.3–781.1)	MS/IK.DB
(*E*)-2-Tridecenal	53	0.3 ± 0.2 (0.1–1.0)	MS/IK.DB
2-Methyl-4-pentenal	100	37.7 ± 21.7 (2.8–94.4)	MS/IK.DB
Nonanal	100	11.5 ± 6.9 (1.2–32.8)	MS/IK.DB
Octanal	5	2.8 ± 0.3 (2.5–3.2)	MS/IK.DB
Esters			
(*Z*)-3-Hexen-1-ol acetate	100	45.0 ± 78.2 (2.3–582.5)	MS/IK.DB
Acetic acid, hexyl ester	96	5.6 ± 4.9 (0.6–26.5)	MS/IK.DB
(*E*)-Hex-3-enyl butyrate	7	0.3 ± 0.1 (0.1–0.4)	MS/IK.DB
Methyl salicylate	60	0.3 ± 0.2 (0.1–0.9)	MS/IK.DB
Prop-2-yn-1-yl 2-methylbutanoate	91	3.3 ± 1.7 (0.9–9.6)	MS
Ethers			
(*Z*)-1-Methoxy-3-hexene	91	7.0 ± 4.4 (1.4–18.6)	MS
1-Methoxy-hexane	63	3.4 ± 1.4 (1.5–6.5)	MS/IK.BD
Hydrocarbons			
1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha., 3a.beta.,4.alpha.,5.alpha., 7a.beta.,8s*)]	70	0.8 ± 0.7 (0.1–3.3)	MS/IK.DB
4,8-Dimethyl-1,7-nonadiene	30	4.1 ± 1.6 (1.6–8.7)	MS
1-Undecene	5	1.3 ± 0.4 (0.7–1.7)	MS/IK.DB
3-Ethyl-1,5-octadiene	100	16.0 ± 14.3 (1.5–56.5)	MS/IK.DB
(*E*)-5-Octadecene	100	11.8 ± 10.8 (1.4–43.0)	MS
1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	25	0.4 ± 0.3 (0.1–1.0)	MS/IK.DB
1,3,5-Tris(methylene)-cycloheptane	95	0.8 ± 0.5 (0.2–2.4)	MS
1,1-Dimethyl-2-(1-methyl-2-propenyl)-cyclopropane,	11	2.9 ± 1.6 (0.3–5.3)	MS/IK.DB

Table 1. *Cont.*

Volatile Compounds	% of Samples	Concentration (μg/g)	Identification [a]
Dodecane	67	0.4 ± 0.5 (0.1–3.2)	MS/IK.DB
(1S-Z)-Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	67	0.6 ± 0.3 (0.2–1.6)	MS/IK.DB
2,2,6-Trimethyl-octane	5	0.5 ± 0.3 (0.2–0.9)	MS/IK.DB
o-Cymene	9	1.0 ± 0.6 (0.4–1.9)	MS/IK.DB
Tetradecane	5	0.3 ± 0.1 (0.2–0.5)	MS/IK.DB
3,6-Diethyl-3,6-dimethyltricyclo[3.1.0.0^{2,4}] hexane	21	0.2 ± 0.1 (0.1–0.4)	MS
Undecane	39	0.5 ± 0.1 (0.3–0.8)	MS/IK.DB
Ketones			
3,3,6-Trimethyl-1,5-heptadien-4-one	72	1.3 ± 0.6 (0.3–3.5)	MS/IK.DB
1-Penten-3-one	68	3.9 ± 3.3 (0.9–16.8)	MS/IK.DB
2-Heptanone	5	0.6 ± 0.1 (0.4–0.8)	MS/IK.DB
3-Pentanone	56	5.5 ± 2.7 (1.8–17.0)	MS/IK.DB
6-Methyl-5-hepten-2-one	95	1.6 ± 0.8 (0.2–4.2)	MS/IK.DB
Nitriles			
Neryl nitrile	100	16.7 ± 9.9 (1.2–48.2)	MS
Terpenes			
(+)-Eremophilene	93	0.8 ± 0.6 (0.1–2.6)	MS/IK.DB
(*E*)-α-Bergamotene	32	0.4 ± 0.4 (0.1–1.9)	MS/IK.DB
(*E*)-β-Ocimene	51	0.3 ± 0.1 (0.1–0.7)	MS/IK.DB
(*Z*)-3,7-Dimethyl-1,3,6-octatriene	100	6.7 ± 3.7 (1.2–18.1)	MS/IK.DB
2,6-Dimethyl-2,4,6-octatriene	98	0.5 ± 0.3 (0.1–1.5)	MS/IK.DB
α-Bergamotene	47	0.6 ± 0.4 (0.2–1.8)	MS/IK.DB
α-Copaene	100	4.5 ± 3.5 (0.8–19.7)	MS/IK.DB
α-Cubebene	75	0.7 ± 0.4 (0.2–1.8)	MS/IK.DB

Table 1. *Cont.*

Volatile Compounds	% of Samples	Concentration (µg/g)	Identification [a]
α-Farnesene	100	3.0 ± 2.2 (0.4–11.3)	MS/IK.DB
α-Muurolene	70	0.6 ± 0.6 (0.1–2.7)	MS/IK.DB
α-Pinene	26	3.2 ± 4.1 (0.3–15.2)	MS/IK.DB
Caryophyllene	60	0.3 ± 0.2 (0.1–1.0)	MS/IK.DB
D-Limonene	39	1.8 ± 2.8 (0.3–11.9)	MS/IK.DB
β-Myrcene	14	1.3 ± 1.3 (0.4–4.5)	MS/IK.DB

[a] MS—Identification performed by mass spectrum confirmation; IK.DB—Identification performed by confirmation of the Kovat's Index indicated in public databases. The results are presented as mean ± standard deviation. The values in brackets represent the range (minimum–maximum).

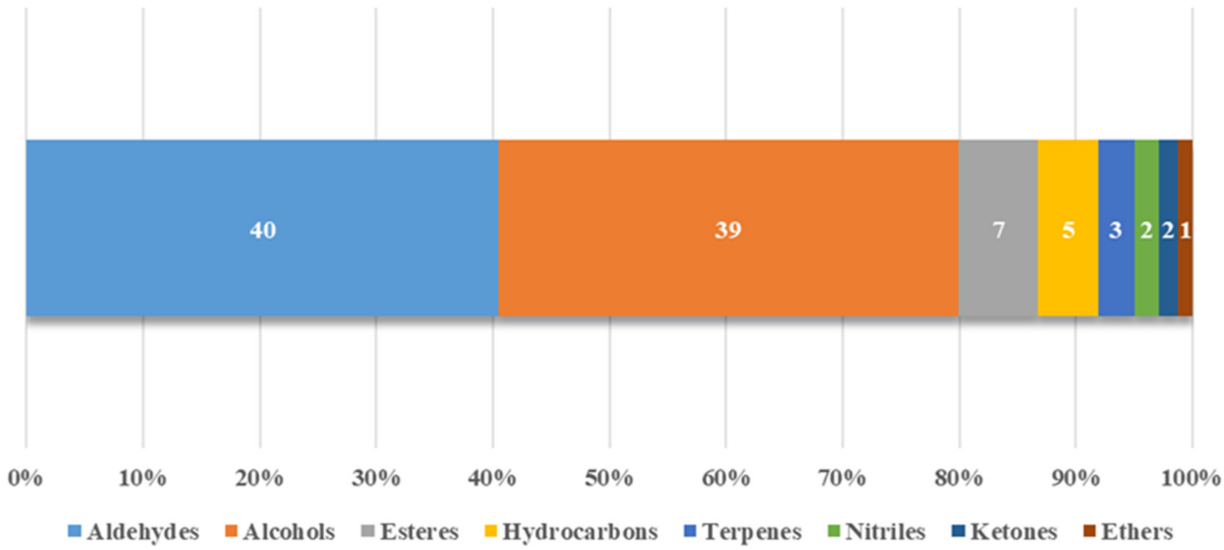


Figure 1. Volatile profile (%) of extra virgin olive oils considering the main classes of the compounds determined.

Although hydrocarbons were present in greater numbers, these were not the compounds that most contributed to the volatile profile (Figure 1). Aldehydes were the compounds that presented the highest concentrations, representing 40% of the concentration of volatile compounds, corresponding approximately to 324 µg/g. Identically to the previous class, and very close to it, the second class that most contributed to the total concentration of volatile compounds were alcohols, representing 39%, corresponding to an average concentration of 316 µg/g.

The major compounds detected in the Douro olive oils were the following: (*E*)-2-hexenal at 259 ± 187 µg/g, (*Z*)-3-hexen-1-ol at 157 ± 109 µg/g, (*E*)-2-hexen-1-ol at 105 ± 148 µg/g, (*Z*)-hexen-1-ol acetate at 45.0 ± 78.2 µg/g, 1-hexanol at 44.4 ± 71.8 µg/g, and 2-methyl-4-pentenal at 37.7 ± 21.7 µg/g. These compounds were identified in a significant number of samples except for (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol, which were identified only in 19 and 16% of samples, respectively. This may be because these compounds have a retention time close to that of (*E*)-2-hexenal, making their presence and identification difficult in certain situations. The six compounds mentioned above were identified as the major compounds in oils produced in the Douro Valley [23]. These major compounds originate from the LOX pathway. In more detail, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol come from linolenic acid (C18:3), whereas 1-hexanol comes from linoleic acid (C18:2) [24]. (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol are associated with green aromas such as cut grass, bitter almond, banana, and apple [7,25–31], whereas 2-methyl-4-pentenal appears associated with the sensations of bitterness, pungency [30], and the aroma of dry leaves [31]. It should be noted that compounds such as (*E*)-5-octadecene, 3-ethyl-1,5-octadiene, (*Z*)-2-penten-1-ol, nonanal, neryl nitrile, α-copaene, α-farnesene, and 3,7-dimethyl-(*Z*)-1,3,6-octatriene, despite having a reduced concentration, were present in all the samples studied. These compounds are associated with green aromas, almonds, banana, and cut grass for (*Z*)-2-penten-1-ol [30,32,33], spicy and wood for α-copaene [34,35], and leaves and grass for α-farnesene [36]. Contrary to this, nonanal is associated with rancid aroma [37,38] and was quantified in all samples (11.5 ± 6.9 µg/g). However, the rancid defect was not detected during the sensory analysis. This fact indicates that the average concentration determined corresponds to a low value, not perceptible to the human nose. Authors state that the detection limit of nonanal is 13.5 µg/g [37,39], above the average concentration detected in the olive oils studied. The process of rancidity of the olive oil begins immediately after the olive oil extraction process [32], which explains the presence of this compound in all the samples studied.

3.2. Sensory Analysis

Regarding the sensory analysis, the results are described in Table 2. Starting with the olfactory sensations of the 57 samples, 65% of the olive oils were classified as ripe fruity (median intensity of 5.1, which varied between 2.6 and 8.0) and 35% of the samples were classified as green fruity (median intensity of 4.4, in a range of 1.8 to 7.1). Olive oil is considered green fruity when sensory attributes refer to green sensations of herbs or fruits, whereas ripe fruity refers to sensations of ripe fruits and dried herbs [40].

Table 2. Positive attributes detected in Douro extra virgin olive oils.

Attributes	% Incidence of Attribute	Median	Min–Max
	Olfactory		
Fruity			
Ripe Fruity	65	5.1	2.6–8.0
Green Fruity	35	4.4	1.8–7.1
Positive attributes			
Lavender	16	2.5	1.1–3.4
Apricot	4	3.9	3.2–4.6
Banana	54	2.6	1.4–5.1
Cinnamon	2	2.9	2.9–2.9
Cherry	9	2.9	2.2–3.3
Cabbage	30	3.7	1.1–6.2
Dry grass	33	3.2	0.6–4.8
Olive leaves	4	2.8	2.7–2.9
Dried fruits	98	3.0	1.1–5.6
Kiwi	5	3.5	0.8–4.3
Apple	77	3.5	1.3–6.5
Pistachio	4	2.5	1.7–3.2

Table 2. *Cont.*

Attributes	% Incidence of Attribute	Median	Min–Max
Tomato leaves	56	3.7	1.2–5.6
Fresh grass	37	3.7	0.9–5.4
Resinous	2	2.5	2.5–2.5
Rosemary	32	2.8	1.5–5.3
Tomato	84	3.7	1.2–6.0
Harmony		7.0	4.6–8.9
	Gustatory		
Fruity			
Ripe Fruity	63	5.3	1.1–7.8
Green Fruity	39	4.2	1.9–7.5
Positive attributes			
Lavender	12	2.7	1.6–3.4
Apricot	4	2.5	1.9–3.0
Banana	81	2.7	0.7–6.0
Vanilla	2	4.2	4.2–4.2
Cherry	16	2.8	1.6–4.7
Cabbage	30	3.7	1.4–7.3
Dry grass	33	3.5	0.7–6.1
Wildflowers	2	1.8	1.8–1.8
Fig leaves	2	4.2	4.2–4.2
Olive leaves	9	2.6	1.2–4.4
Dried fruits	100	3.0	0.8–5.6
Kiwi	5	2.0	0.6–3.7
Apple	74	3.5	1.1–6.3
Pistachio	4	2.1	1.8–2.4
Tomato leaves	58	3.5	1.1–4.8
Fresh grass	42	2.8	0.6–5.7
Resinous	2	3.1	3.1–3.1
Rosemary	37	2.8	1.3–5.1
Tomato	82	4.0	1.2–5.8
Sweet		4.2	1.3–6.4
Bitter		1.9	0.9–5.0
Pungent		2.4	0.7–5.8

Seventeen positive olfactory and nineteen gustatory attributes were determined, most of which were present in less than 50% of the samples, such as lavender, apricot, cinnamon, cherry, cabbage, dry grass, olive leaves, kiwi, pistachio, fresh grass, resinous, and rosemary in the olfactory sensations, and lavender, apricot, vanilla, cherry, cabbage, dried grass, wildflowers, fig and olive leaves, kiwi, pistachio, fresh grass, resinous, and rosemary for taste sensations. The perceived attributes generally agree with authors who have studied olive oils from the region [41]. Furthermore, the samples showed a median for harmony of 7.0, ranging between 4.6 and 8.9. This parameter measures the balance between all the evaluated sensations [42]. Regarding gustatory sensations, 63% of the olive oils were classified as ripe fruity, with a median intensity of 5.3, ranging from 1.1 to 7.8, and 39% were classified as green fruity, with a median intensity of 4.2, which ranged between 1.9 and 7.5. The main positive attributes detected were dried fruits in 100% of the samples, 82% tomato, 81% banana, 74% apple, 58% tomato leaves, 42% fresh grass, 37% rosemary, 33% dry grass, 30% cabbage, 16% cherry, and 12% lavender. In the perceived basic sensations, the samples presented the following medians for the parameters: (i) sweetness: median of 4.2, varying between 1.3 and 6.4; (ii) bitterness: median of 1.9, varying between 0.9 and 5.0; and (iii) pungency: median of 2.4, ranging from 0.7 to 5.8. Bitter and pungent sensations are related to the content of phenolic compounds [43] that activate the trigeminal nerve endings associated with the taste receptors in the taste buds. This sensation can be prolonged over time, evaluating the persistence of these attributes [44]. The harmony, complexity, and persistence parameters had medians of 6.7, 6.3, and 7.4, respectively, suggesting that the Douro olive oils present a good balance of complexity and persistence.

3.3. Relationship between Volatile Compounds and Sensory Attributes

The sensory attributes have a direct relationship with volatile compounds. A Pearson correlation test was performed to verify the existence of significant correlations between the volatile compounds and the perceived olfactory sensory attributes. The most common positive attribute detected in the olive oils at the olfactory level was dried fruits, present in 98% of the samples, with a median intensity of 3.0. This attribute showed a positive correlation greater than 0.36 with (*E*)-2-hexenal, prop-2-yn-1-yl 2-methylbutanoate, and (*Z*)-3,7-dimethyl-1,3,6-octatriene. Other authors have already described the relationship between (*E*)-2-hexenal and nut aroma [8,36,45]. The tomato attribute showed a very significant negative correlation (p-value < 0.001) with 1-nonanol (−0.457), a compound described as mainly associated with fatty sensations [26]. Other authors have shown that 1-nonanol may be associated with the profile in polyphenols, showing a positive correlation with tyrosol and caffeic acid [46]. The attribute of cherry showed a very significant positive correlation with octanal (0.573). However, to our knowledge, this relationship has not yet been reported by other authors, with octanal being mainly associated with almond aromas [30] and citrus and fatty sensations [47]. The rosemary attribute showed very significant positive correlations (0.522) with the (*E*,*E*)-2,4-heptadienal and (*E*)-2-hexenal. This result is identical to that reported by other authors [32], who reported a relationship between (*E*)-2-hexenal and wildflower aroma. The attribute, resinous sensation, showed a significant positive correlation with 1-heptanol and α-pinene, 0.871 and 0.471, respectively. The relationship between resinous sensations and α-pinene has been mentioned in the bibliography [34]. Other attributes showed less significant positive and negative correlations (p-values between 0.001 and 0.05), as shown in Figure 2.

3.4. Assessment of the Possible Separation of Olive Oils into Sub-Regions, Considering Their Volatile Profile and Sensory Characteristics

Currently, the Douro Demarcated Region for wine production is divided into three sub-regions, Baixo Corgo, Cima Corgo, and Douro Superior. The wines produced in each of the sub-regions differ. This fact is due to the regions presenting different characteristics, such as temperature, rainfall, and soil, influencing the properties of the wines [48,49]. To assess whether or not these differences detected in wine were also observed in olive oils, the olive oil characteristics of the referred subregions were evaluated. Since olive trees in the Douro region are mainly found on vineyard borders and are subject to the same edaphoclimatic conditions as the vineyards, the sensory profiles (olfactory and gustatory) and the profiles in volatile compounds of the samples were subjected to hierarchical cluster analysis methods. Thus, this task's main objective was to verify if the olive oils from a given region clustered together or not (Figure 3), demonstrating if the same behaviour as the wines was observed in the olive oils.

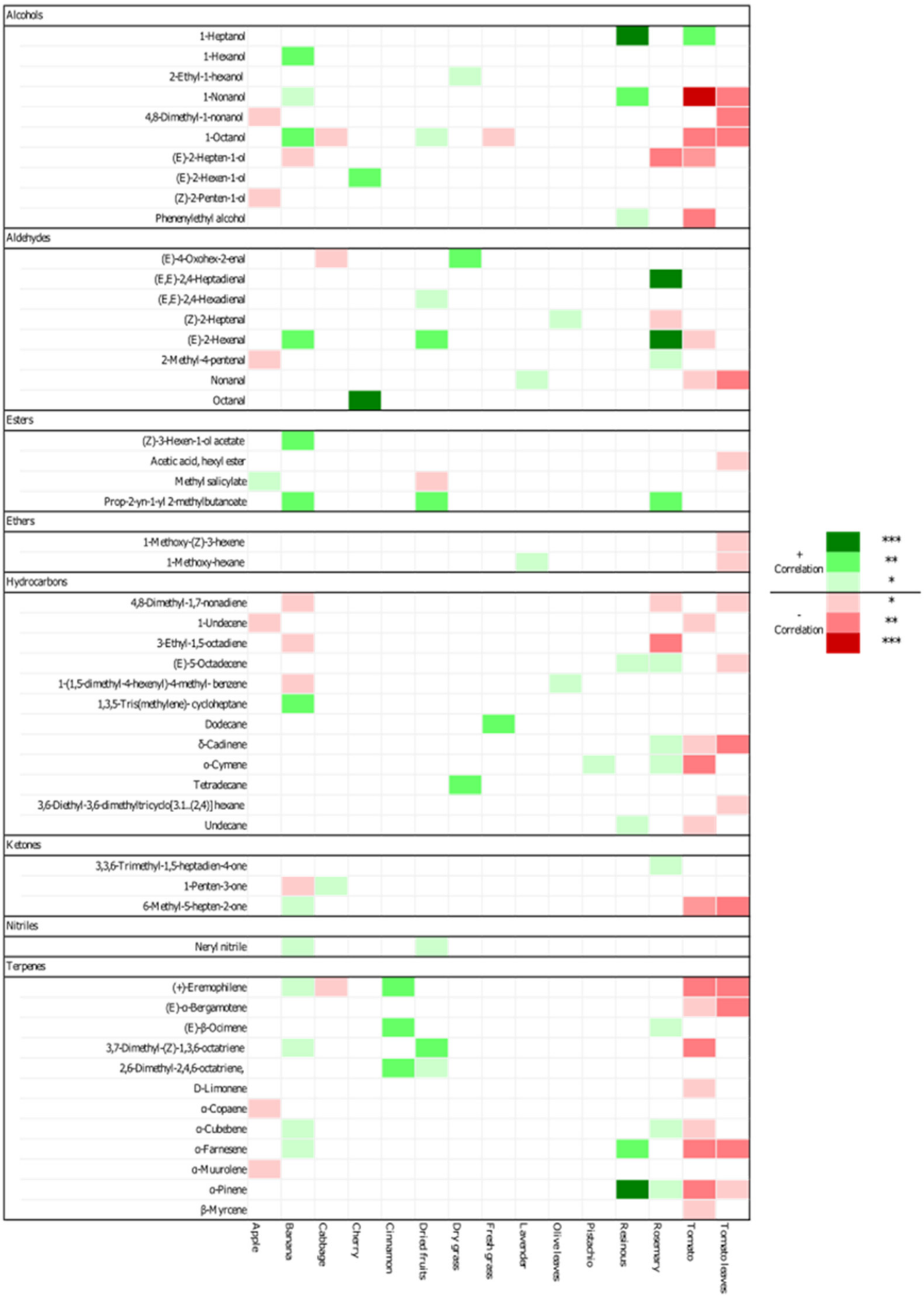
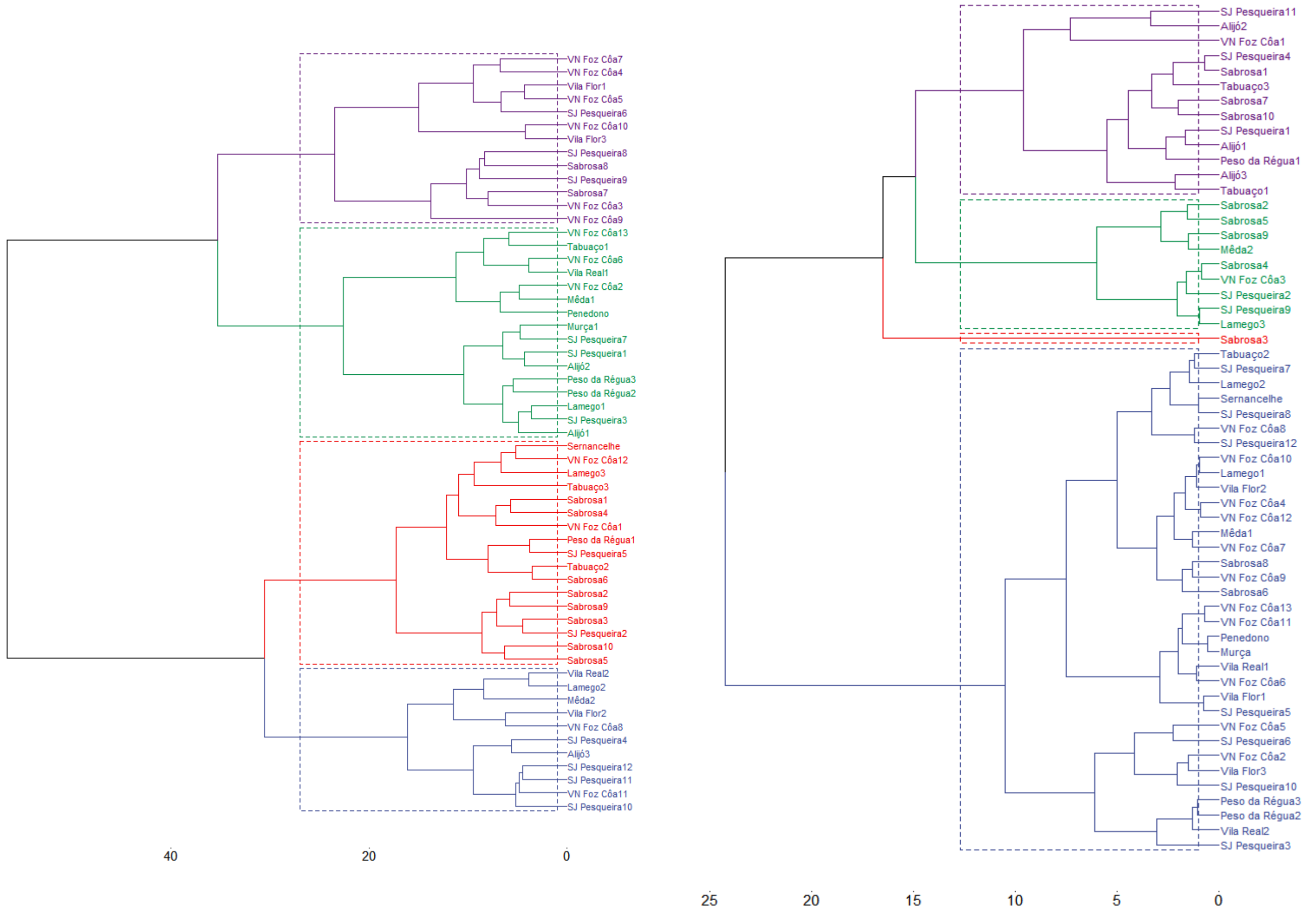


Figure 2. Pearson's correlation test (only results with a p-value < 0.05 are shown). Note: * $0.01 < p$-value < 0.05; ** $0.001 < p$-value ≤ 0.01; *** p-value ≤ 0.001.

The results did not show a clear trend of possible groupings of samples from a given region, with olive oils from the same municipalities present in all groups. This situation is acceptable because, although the volatile and sensory profiles may be influenced by soil and climate conditions, these are mainly influenced by the cultivars used and their maturation state. These facts give rise to a profile in fatty acids and different enzyme concentrations, producing volatile compounds that differ from cultivar to cultivar, thus leading to different volatile compounds and different sensory profiles. Other factors affecting the sensory profile are the olive harvesting and olive oil extraction processes that affect the volatile profile and the concentrations of biophenols, thus influencing the sensations of bitterness and pungency [50].



(**a**) (**b**)

Figure 3. Dendrograms obtained from olfactory and gustatory sensations (**a**) and volatile profile (**b**) evaluated in olive oils from the Douro region.

4. Conclusions

In conclusion, this study came to fill a gap regarding the characterisation of the profile of volatile compounds of olive oils from the Douro region. It was possible to identify the volatile compounds characteristic of extra virgin olive oils from this region. In sensory terms, most of the extra virgin olive oils (approximately 64%) were classified as ripe fruity. Among the olfactory and gustatory sensations, the attributes of dried fruits, tomato, apple, tomato leaves, banana, and lavender stood out. Regarding volatile compounds, the following compounds were detected in all samples: (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol acetate, 1-hexanol, and 2-methyl-4-pentanal. The study of the sensory profile demonstrated the potential of this region in the production of differentiated oils with unusual sensory attributes. Considering the results obtained in the sensory analysis, it was impossible to separate the olive oils by municipalities or sub-regions. Thus, the volatile fraction study of the olive oils from the Douro region and their relationship with the sensory attributes are essential to guarantee their genuineness and identity regarding their future appreciation. It is necessary to characterise the genetic material present in the Douro region in future work.

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