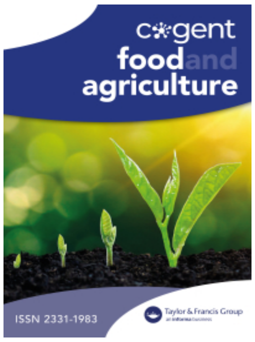

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Nutritional and health claims compliance of olive oils produced from centenarian olive trees

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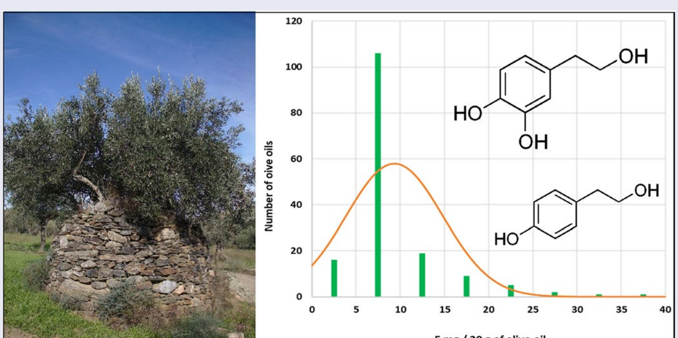
ABSTRACT

This work examined whether centenarian trees could be a source of virgin olive oil (VOO) that accomplishes nutritional and health claims regarding unsaturated fatty acids (UFA), tocopherols and phenolic compounds (tyrosol and hydroxytyrosol derivatives). A total of 159 VOOs from centenarian trees of Côa Valley-Portugal were analysed to assess their compliance with those claims. All VOOs met the criteria for 'high source of UFA' (≥70% of total FA). The oils' vitamin E richness ensured compliance with the claim (≥36 mg/kg of oil), with some samples surpassing 484 mg/kg. Regarding polyphenols, 88.6% of the oils fulfilled the requirements (≥5 mg/20g of oil) for health claims. Among these, 75% fell within 5–10 mg/20g, with up to 32 mg/20g. These findings underscore the significant nutritional and health potential inherent to oils extracted from centenarian trees, emphasizing the importance of preserving these specimens for their genetic diversity and valuable properties.

GRAPHICAL ABSTRACT



Centenarian olive trees *vs.* olive oil health claims

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1. Introduction

In the Mediterranean agroecosystem, olive tree has a paramount position, presenting a great source of natural resources and genetic and cultural heritage. The olive trees can produce different products some of them with a huge relevance in terms of nutrition and health. Among these, its virgin oil stands out as one of the earliest recognized foods known for its health-promoting attributes, primarily linked to the degree of unsaturated fatty acids and later to its minor compounds such us tocopherols and phenolic acids (Olmo-Cunillera et al., 2022; Pastor et al., 2021). Olive oil richness in monounsaturated fatty acids, particularly oleic acid, contributes to both cardiovascular health advantages to consumers and to the stability of

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the product, highlighting its organoleptic properties (Guasch-Ferré et al., 2020; Hijawi, 2021; Martínez-González et al., 2022). Regarding tocopherols, commonly recognized as vitamin E, they exert a pivotal influence on both the olive oil nutritional properties and its oxidative stability. Concerning olive oil tocopherols, α-tocopherol is the most abundant, constituting up to 90% of the overall content (Jimenez-Lopez et al., 2020). It is well known the protecting action of these compounds against free radicals on body tissues and contribute to the prevention of degenerative neuropathies while serving as antioxidants to olive oil itself (Baccouri et al., 2023; Reyes-Goya et al., 2024). Phenolic compounds derived from hydroxytyrosol and tyrosol represent the major virgin olive oils phenolic class that are associated with the oil's antioxidant activity and its flavor conferring pungency and bitterness (Baccouri et al., 2023; Passeri et al., 2023). Additionally, these phenolics due to its antioxidants and anti-inflammatory action protecting human body from oxidative damages (Guasch-Ferré et al., 2020; Riolo et al., 2022; Tarabanis et al., 2023), while also reducing risk factors for cardiovascular disease and age-related illnesses (Roselli et al., 2020).

Several factors influence olive oil composition, including its bioactive compounds, leading to substantial variations among different oil samples. These differences are frequently associated with genetic diversity and involve a multifaceted interplay of factors, encompassing adaptation and resistance to local agro-climatic conditions, cultivation practices, as well as harvesting periods and extraction conditions (Banco et al., 2023; Figueiredo-González et al., 2022; Passeri et al., 2023). An example is the adverse impact of temperatures exceeding 25 °C, occurring between the flowering and harvest stages, on the proportion of oleic acid in the extracted oils (García-Inza et al., 2018). Furthermore, yearly climatic oscillations exert stress on the olive trees, leading to significant variations in the tocopherols and phenolic acids contents (Rodrigues et al., 2020a). The olive's maturity index is also relevant, with phenolic concentrations being enhanced with lower maturation indices in opposition to monosaturated acids (Rodrigues et al., 2020b; Wani et al., 2018). Depending on their composition, olive oils can be commercialized with nutritional declaration and/or health claims, which can be used by producers as a commercial advantage. The European Food Safety Authority (EFSA) officially recognized some beneficial actions linked with the consumption of olive oil, approving the use of claims for extra virgin olive oil (EVOO) related to its content of unsaturated fatty acids; hydroxytyrosol, tyrosol and its derivatives and also tocopherols. The use of the *'high concentration of monounsaturated fatty acids'* requires at least 70% of these fatty acids in the olive oil (European Commission Regulation (EC) N° 432/2012). These regulations also point out that replacing saturated fats with unsaturated ones helps to maintain normal blood cholesterol levels, which is associated with cardiovascular health benefits (Riolo et al., 2022; Tarabanis et al., 2023; Visioli & Poli, 2020). For phenols, hydroxytyrosol, tyrosol and their derivatives are known to '*contribute to the protection of blood lipids against undesirable oxidation*' and can receive this health claim if the total content equals or is greater than 5 mg per 20 g of olive oil (European Commission Regulation (EC) N° 432/2012). For vitamin E, naturally present in olive oil, its amounts support nutritional and health claims related to the protection of cells against oxidation. For '*high vitamin E content*' claim, the oil must contain at least 3.6 mg of vitamin E per 100 g of olive oil (European Commission Regulation (EC) N° 432/2012). In recent years, various studies have reported that olive oils obtained from olives produced by centenarian trees are different from those obtained from younger trees (El Chami et al., 2023; Korkmaz, 2023; Rodrigues et al., 2020a, 2022; Sasaki & Isoda, 2022). Also, the same authors refer the urgent need to protect and preserve the unexplored genetic variability of centenarian specimens. Considering this frame, the present study aimed to evaluate the composition of 159 olive oils that were extracted from olives collected in centenarian trees, cultivated in the Côa Valley region (northeast of Portugal), namely the fatty acid profiles, phenolic acid levels and amounts of tocopherols, to check whether these ancient specimens can produce olive oils with nutritional/health claims. This information will be critical to complete genetic studies and to select and preserve unique specimens for the future.

2. Materials and methods

2.1. Sampling

Overall, 159 olive oils were obtained from fruits harvested from 112 centenarian trees located in the Côa Valley region of Portugal. The samples were collected over three consecutive harvest seasons

(2020–2022). These trees are spread across eight traditional olive groves situated in different sub-regions of the valley: Chãos de Freire—Barca D'Alva, Srª do Campo—Almendra, Adro da Igreja—Almendra, Vale das Quelhas—Muxagata, Costa—Muxagata, Salgueiro—Vila Nova de Foz Côa, Entrada da Costa—Pocinho, and Vale Verde—Pocinho (Figure 1). The regional climate is marked by pronounced seasonal temperature fluctuations and irregular rainfall throughout the year. Maximum temperatures occur between June and August, frequently exceeding 30 °C, while minimum winter temperatures can drop below 5 °C. Average seasonal temperatures typically range from around 5 °C in winter to 25 °C in summer. Rainfall is concentrated mainly in autumn and winter, with significant peaks in February, November, and December. In contrast, summer months are notably dry, with precipitation often approaching zero. This climate pattern is characteristic of the Mediterranean region, defined by hot, arid summers and mild to cool, rainy winters. Trees were chosen based on age-indicative criteria, such as the structure of the trunk and treetop, overall plant integrity, and information provided by the owners. Each grove comprises around 100 trees per hectare, planted with a spacing of 10 × 10 m. These trees rely solely on rainwater for irrigation, exhibit limited vegetative growth, and undergo minimal pruning annually. The soil consists of a thin and nutrient-poor layer of schist leptosol, with a gradient of approximately 20 to 30%, managed through seasonal tillage twice a year (spring and autumn) to control weed growth. Every year, in April, a compound fertilizer (7:14:14) was applied, containing 7% N (5% ammoniacal N and 2% urea N), 14% P_2O_5 (11% water-soluble), and 14% K_2O. Despite their age, the specific variety of these trees remains unidentified, lacking any available information on their genetic classification. Each selected tree was tagged and georeferenced for future identification purposes. The olives were handpicked from each identified tree, with the oil extracted separately. Harvest occurred from October to November, typically around 150 to 160 days post-flowering and took place during ripeness stages two (characterized by red spots on less than half of the olive) and three (marked by red or purple spots on over half of the olive), following the International Olive Council (IOC, 2011) guidelines. Around four to five kg of olives was collected from each tree to produce separate batches of olive oil.

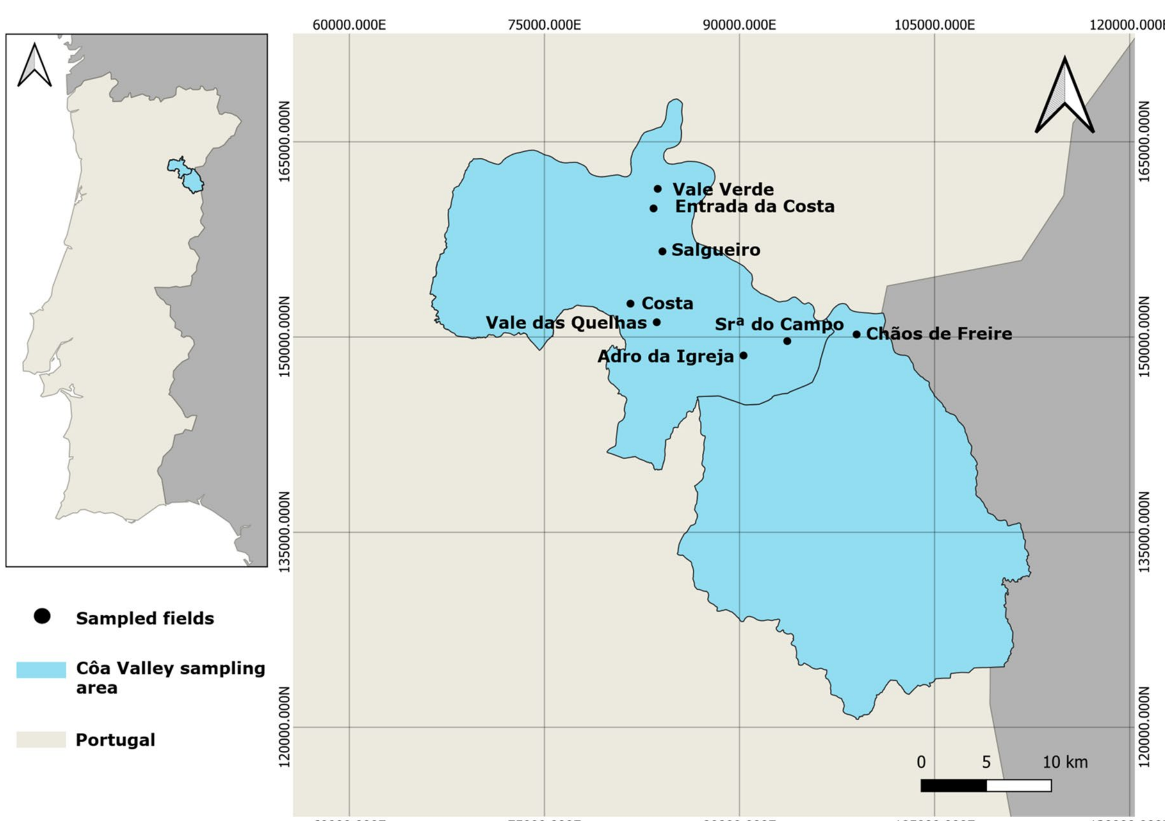

Figure 1. Map of the Côa Valley region showing the locations of the sampling groves. Map projected in the ETRS89/PT-TM06 system.

However, not all trees yielded enough olives to produce olive oil during the three seasons, causing some trees to be excluded from the study during certain harvest years. Consequently, only 159 olive oils were gathered over a three-year span from the 112 trees. Therefore, only some oils were obtained from the same tree in different crop years. After fruit harvesting, the oils were extracted, in the first 24h, using an Abencor pilot plant (Commercial Abengoa S.A., Seville, Spain), following standard methodology currently used in the IPB Laboratory and described by Rodrigues et al. (2022). Subsequently, the oils were filtered and packaged in amber glass bottles to minimize light-induced oxidation and stored refrigerated at 4 °C. All analyses were performed within 1 to 3 months after extraction to ensure sample integrity.

2.2. Fatty acids profile

Fatty acid profile of each olive oil sample was established following the guidelines provided by European Community Regulation (Commission Implementing Regulation (EU) 2022/2105 of 29 July 2022) for EVOO. The cold transesterification method using methanolic potassium hydroxide and heptane was used. A Chrompack CP 9001 chromatograph (Burladingen, Germany) equipped with a FID detector at 250 °C and an automatic sampler (CP-9050). Injection (1 μL at a 1:50 ratio) was performed at 230 °C, and separation was accomplished using a fused silica capillary column (Select FAME: 50 m × 0.25 mm i.d. Varian, Palo Alto, CA, USA). Helium served as the carrier gas, maintaining an internal pressure of 160 kPa. To match the retention times of the peaks and calibrate de FID, a standard mixture of certified fatty acid methyl esters was obtained from Sigma (Barcelona, Spain).

2.3. Tocopherols contents

High-performance liquid chromatography (HPLC) was performed using a Jasco system (Japan) equipped with a fluorescence detector (FLD), to evaluate α-, β-, and γ-tocopherol levels, according to ISO 9936:2016 (ISO, 2016) with some modifications. Each individual tocopherol (α-, β-, and γ-tocopherols) was quantified in milligrams per kilogram (mg/kg) of olive oil, based on individual calibration curves with analytical standards (Sigma-Aldrich, Germany) and the internal standard, tocol (Matreya Inc., Pleasant Gap, PA, USA). The separation accomplished with a Supelcosil TM LC-SI silica column (7.5 cm × 3 mm; particle size: 3 μm; Supelco, Bellefonte, PA, USA) at 23 °C and elution with 1,4-dioxane (Sigma-Aldrich-Allentown, PA, USA) in n-hexane (2.5%, v/v) at 0.75 mL/min. Data analysis was performed by the ChromNAV Control Center program at the JASCO Chromatography Data Station (Jasco, Tokyo, Japan).

2.4. Hydroxytyrosol and tyrosol derivatives contents after acid hydrolysis

The tyrosol and hydroxytyrosol contents were quantified after acid hydrolysis, following Marx et al. (2021) using syringic acid (Sigma-Aldrich) as internal standard, added before hydrolysis (2 mol/L hydrochloric acid solution in methanol/water 80:20, v/v). Hydrolysis occurred at 25 °C for 6 h with periodic vortex mixing, followed by extraction of the soluble compounds with acetonitrile/water solution (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 the DAD (MD-4010). Compound separation was achieved using a C18 reverse-phase column at 35 °C (Kinetex C18; particle size: 2.6 μm; pore size: 100 Å; LC length: 100 mm; internal diameter: 3.00 mm, Phenomenex), with a mobile phase consisting of water and acetonitrile (both with 0.1% formic acid) at a flow rate of 0.8 mL/min for 20 minutes. Calibration curves for hydroxytyrosol and tyrosol were prepared in methanol/water (80:20, v/v) over a concentration range of 0.00031 to 0.0160 mg/mL (micrograms). The results are expressed as the sum of the individual amount (mg/kg of olive oil) of hydroxytyrosol or tyrosol. Because only the hydrolyzed forms of tyrosol and hydroxytyrosol are quantified, which leads to the loss of information about the molecular weight and structure of their original compounds, the native forms were estimated using correction factors reported in the literature: 2.2 for hydroxytyrosol and 2.5 for tyrosol (Mastralexi et al., 2014).

3. Results and discussion

3.1. Chemical composition

The present study evaluated glyceridic fatty acid profiles, free tocopherols, and both hydroxytyrosol and tyrosol contents after acid hydrolysis, of each one of the 159 independent olive oils, extracted from olives of centenarian trees, picked during three consecutive harvest years (2020–2022). Among the individual fatty acids assessed, not all samples from the different selected plants met the levels set by the European Community Regulation for EVOO (Commission Delegated Regulation (EU) N°. 2022/2104). The studied olive oils from the Côa Valley region presented a fatty acid profile summarized in Table 1. Oleic acid ($C_{18:1}$) predominated in all samples, averaging 71.4% with a range from 57.5% to 79.5%, falling within the reference values for EVOO category (Commission Delegated Regulation (EU) N°. 2022/2104). While, palmitic acid ($C_{16:0}$) reached the second position in abundance, averaging 13.0% and ranging from 9.4% to 17.7%, also within the reference values. Linoleic acid ($C_{18:2}$) ranked third in abundance, averaging 10.1% with values ranging from 2.2% to 18.8%. However, two samples (trees 141 and 57) fell below the threshold (2.5%) for the EVOO category. Palmitoleic acid ($C_{16:1}$) ranged from 0.09% to 2.1%, with only one sample (tree 54) showing a value below 0.3%, i.e. the minimum reference value allowed for EVOO classification. Linolenic acid ($C_{18:3}$) was found below the maximum reference value of 1.4% on all 159 oils studied, varying its abundance between 0.6% and 1.4%.

Globally, monounsaturated fatty acids (MUFA) ranged from 59.7% to 81.2%, while total polyunsaturated (PUFA) varied between 2.9% and 19.8%, and saturated fatty acids (SFA) were from 12.9% to 21.5%. These results align with previous studies, showing MUFA ranging from 60% to 80% (Gomez-del-Campo et al., 2023; Zago et al., 2019). Gomez-del-Campo et al. (2023) also reported PUFA values between 3.5% and 14.5%, with linoleic acid values between 3% and 14%. According to Baccouri et al. (2023), the relative abundance of fatty acids varies depending on the olive variety. Chaaibi oil showed a higher proportion of MUFAs (78.9%) attributed to its elevated oleic acid content, whereas Ajmi oil contained more PUFAs (21.5%) owing to its higher linoleic acid content. The average of oleic acid content, approximately 72%, found in this study aligns with the results of Peres et al. (2016) and Rodrigues et al. (2023) concerning olive oils produced from Portuguese olive varieties. This aligns similarly with the data collected by Cecchi et al. (2024) from 62 commercially available Portuguese olive oils of declared geographic origin.

Table 1. Minimum, maximum and average percentage fatty acid, in percentage, and α-, β- and γ-tocopherol, vitamin E, hydroxytyrosol and tyrosol contents after hydrolysis, in mg/kg oil, during the study years (2020–2022).

Fatty acid composition	Olive oil[a]	Minimum–maximum	Mean ± standard deviation
Myristic acid ($C_{14:0}$)	≤0.03	0.01–0.03	0.01 ± 0.00
Palmitic acid ($C_{16:0}$)	7.0–20.0	9.4–17.7	13.0 ± 1.4
Palmitoleic acid ($C_{16:1}$)	0.3–3.5	0.09–2.13	0.9 ± 0.3
Heptadecanoic ($C_{17:0}$)	≤0.4	0.03–0.26	0.09 ± 0.06
Heptadecenoic ($C_{17:1}$)	≤0.6	0.05–0.46	0.2 ± 0.1
Stearic acid ($C_{18:0}$)	0.5–5.0	1.63–3.79	2.6 ± 0.4
Oleic acid ($C_{18:1}$)	55.0–85.0	57.51–79.54	71.4 ± 5.1
Linoleic acid ($C_{18:2}$)	2.5–21.0	2.17–18.81	10.1 ± 4.7
Linolenic acid ($C_{18:3}$)	≤1.0*	0.61–1.36	0.9 ± 0.2
Arachidic acid ($C_{20:0}$)	≤0.6	0.29–0.50	0.37 ± 0.04
Eicosenoic acid ($C_{20:1}$)	≤0.5	0.20–0.39	0.29 ± 0.03
Behenic ($C_{22:0}$)	≤0.2	0.07–0.14	0.10 ± 0.01
Lignoceric acid ($C_{24:0}$)	≤0.2	0.02–0.09	0.05 ± 0.01
ΣMUFA	N.D[b]	59.67–81.15	72.7 ± 5.2
ΣPUFA	N.D[b]	2.90–19.78	11.0 ± 4.8
ΣSFA	N.D[b]	12.39–21.54	16.2 ± 1.4
α-Tocopherol	92–2083	121–474	266 ± 64
β-Tocopherol	0.8–1.9[c]	0.1–3.4	1.7 ± 0.6
γ-Tocopherol	0.7–2.2[c]	0.3–15.9	4.6 ± 2.8
Σ Tocopherols	93–2103	123–484	272 ± 65
Hydroxytyrosol	14.7–432[d]	40.0–982	294 ± 143
Tyrosol	16.1–136[d]	21.3–1277	174 ± 152
Hyd + Tyr (mg/20g)	≥5[e]	1.2–38	9.4 ± 5.5

[a]Reference values for olive oil according to the Commission Regulation (EEC) 2022/2104 of 29th July;
[b]Not defined by Commission Regulation (EEC) 2022/2104 of 29thJuly.
[c]Borges et al. (2017).
[d]Nenadis et al. (2019).
[e]Commission Regulation (EU) no 432/2012 of 16 May 2012.

The elevated oleic acid levels observed are consistent with findings from research on centenarian olive trees, which reported oleic acid abundances reaching up to 78% of the total fatty acids (Figueiredo-González et al., 2022; Serreta-Oliván et al., 2023; Squeo et al., 2021). Passeri et al. (2023) when studying centenarian olive trees, observed deviations in the fatty acid composition of Maltese genotypes, highlighting a disparity in comparison to prior studies. Unlike previous findings, significant imbalances were noted in the primary fatty acids of Maltese samples, with oleic acid levels falling below 55% in numerous samples and some of them exceeding 20% in palmitic acid abundance. Furthermore, deviations were also observed in linoleic and palmitoleic acid levels. Particularly notable were the high percentages of linolenic acid ($C_{18:3}$), which surpassed 3% and peaked at 7%, in contrast to the 1.4% recorded in this study. Additionally, high concentrations of palmitoleic acid ($C_{16:1}$), reaching up to 5.05%, were reported, exceeding the allowable 3.5%, whereas the present study found oils with palmitoleic acid levels below the 0.3% threshold. Regarding the vitamin E content, α-tocopherol, β-tocopherol, and γ-tocopherol were identified and quantified. The detailed findings for each tocopherol, along with the total vitamin E levels, are presented in Table 1. The α-tocopherol emerged as the most abundant, averaging 266 mg/kg, varying from 121 to 474 mg/kg and corresponding to 97.7% of total vitamin E mass on average Following was γ-tocopherol, averaging 4.6 mg/kg, ranging from 0.30 to 15.9 mg/kg, while β-tocopherol averaged 1.7 mg/kg (range from 0.11 to 3.38 mg/kg). The overall vitamin E levels were notably high, averaging 272 mg/kg across the samples, with a range of 123 mg/kg to 484 mg/kg. The levels are slightly greater than those presented by Cecchi et al. (2024), with 1000 samples ranging from 100 to 300 mg/kg, and Faci et al. (2021), who supported reference values for olive oil within the ranges of 93–210 mg/kg for Vitamin E. Our results are similar to those of Serrano et al. (2021) for Picual and Arbequina cultivars, with average Vitamin E values of 248 mg/kg and a range from 155 mg/kg to 383 mg/kg, closer to the ones observed in the present study. Previous results on Portuguese cultivars showed values consistent with the present ones, with values falling within this study's range and occasionally higher, depending on the variety, ranging from 124 mg/kg to 630 mg/kg for the Verdeal and Lentisca varieties, respectively (Rodrigues et al., 2020a,b). Baccouri et al. (2023) similarly noted a prevalence of α-tocopherol, albeit at lower levels compared to this study, ranging from 87 to 302 mg/kg depending on the variety. They also observed β-tocopherol as a less abundant compound, with concentrations lower than 5 mg/kg, consistent with the present study's findings. However, γ-tocopherol was found in quantities ranging from 2.6 to 9.0 mg/kg, showing no significant variation across varieties, whereas in this study, it reached values of up to 15.9 mg/kg. Faci et al. (2021) also reported higher γ-tocopherol values compared to β-tocopherol for Algerian varieties, reaching up to 20 mg/kg, depending on the tree's altitude. Table 1 presents the contents of total hydroxytyrosol and tyrosol derivatives in the studied oils, estimated after acid hydrolysis. On average, hydroxytyrosol derivatives were at 294 mg/kg, ranging from 40 to 982 mg/kg, while tyrosol derivatives averaged 174 mg/kg with a variation from 21 to 1277 mg/kg. Passeri et al. (2023) also observed this variability in their study of oils obtained from centenarian Maltese olive trees, where hydroxytyrosol and tyrosol exhibited the greatest diversity among different genotypes, ranging from 125 to 1807 mg/kg and 15 to 374 mg/kg, respectively. Rodrigues et al. (2023) observed smaller variations when analysing the Portuguese variety Galega Vulgar, with hydroxytyrosol and tyrosol ranging from 87 to 325 mg/kg and 37 to 121 mg/kg, respectively. Zago et al. (2019) identified significantly lower values, with average hydroxytyrosol at 14 mg/kg and tyrosol at 5.4 mg/kg, while Gomez-del-Campo et al. (2023) reported higher values, with a maximum of 18.3 mg/kg for hydroxytyrosol and 24.8 mg/kg for tyrosol. In a recent study by Cecchi et al. (2024), a wide range of total hydrophilic phenolic compound contents were reported among 1000 commercial EVOO. These values spanned from below 50 mg/kg to exceeding 1000 mg/kg. However, the majority of the oils analysed exhibited contents falling within the range of 150–650 mg/kg.

3.2. Nutritional and health claims

3.2.1. Content in unsaturated fats

As per Commission Delegated Regulation (EU) N°. 116/2010, olive oil with an abundance of unsaturated fats (MUFA plus PUFA) exceeding 70% of total fatty acids may be labelled as *'high in unsaturated fats'*. Such oils may also carry a health claim stating that *'replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels'*. Furthermore, if the abundance of MUFA alone exceeds 45% of total fatty acids, the nutritional claim 'high in monounsaturated fatty acids' may also be applicable. Figure 2 displays a histogram illustrating the distribution of the olive oils derived from centenarian trees across specific ranges of total unsaturated levels (MUFA plus PUFA), indicating that all samples surpass the minimum level required to satisfy the nutritional/health claims. Additionally, the theoretical normal distribution curve of total unsaturated fatty acids is plotted, derived from the average and standard deviation of MUFA plus PUFA contents of the oils studied. The histogram reveals an almost normal distribution of experimental total unsaturated fat, with a slight left tail indicating negative skewness, signifying that the highest values are shifted towards the left: mode > median > mean. As indicated in Table 1, all olive oils examined exhibited a monounsaturated fatty acid (MUFA) level exceeding 59%, surpassing the minimum threshold (45%) necessary for the *'high in monounsaturated fatty acids'* nutritional claim. Furthermore, all oils demonstrated a proportion of unsaturated fatty acids exceeding 70% (with the minimum level exceeding 78%, as depicted in Figure 2), thereby qualifying for the additional nutritional claim of *'high in unsaturated fats'*. Moreover, they meet the health claim stating that *'replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels'*. It is worth noting that while olive oil typically comprises 85% unsaturated fat, contributing 85% of the product's energy (Commission Delegated Regulation (EU) N°. 116/2010), among the 159 olive oils analysed, 17 oils exhibited an average unsaturated fat content surpassing 85%. These oils were derived from 16 centenarian trees. Additionally, it is noteworthy that 68.5% of the 159 oils studied displayed a relative abundance ranging between 83 and 85% of unsaturated fat.

Unsaturated fatty acids amounts observed in this study (78–88%) are consistent with the values observed for EVOO and literature as example Marino et al. (2019) and El Chami et al. (2023), who reported ranges between 80–85%. In contrast, Adakalic and Lazovic (2018) reported lower values for oils produced from Montenegro's centenarian olive trees, ranging from 70% to 75%. Similarly, Hijawi (2021) and Passeri et al. (2023) also noted that oils from centenarian trees had unsaturated fatty acid percentages below 70%. It is significant to highlight that 52.8% of the oils evaluated (84 out of 159 oils) exhibited a relative abundance of oleic acid ($C_{18:1}$) equal to or exceeding 70%, thereby meeting the minimum requirement for the *'high in unsaturated fats'* nutritional claim on their own. Finally, an additional health claim exists and is related to the content of linolenic acid ($C_{18:3}$) and its ability to *'maintain normal blood cholesterol levels'*, which can be used in olive oil if it contains at least 0.3 g of linolenic acid for 100 g of product and 100 kcal, being required to inform consumers that 2 g of this acid should be ingested per day to obtain this beneficial effect (Commission Delegated Regulation (EU) N° 1924/2006; Regulation (EU) No 432/2012; Commission Delegated Regulation (EU) N° 1226/2014; Mancebo-Campos et al., 2023).

3.2.2. Content in vitamin E

Olive oils containing vitamin E concentrations exceeding 36 mg/kg are eligible for a nutritional claim of 'high vitamin E content' and a health claim stating that *'vitamin E contributes to the protection of blood lipids against undesirable oxidation'* (Commission Delegated Regulation (EU) N° 1924/2006). The average vitamin E concentration of the studied oils, is depicted in Figure 3 alongside the respective theoretical

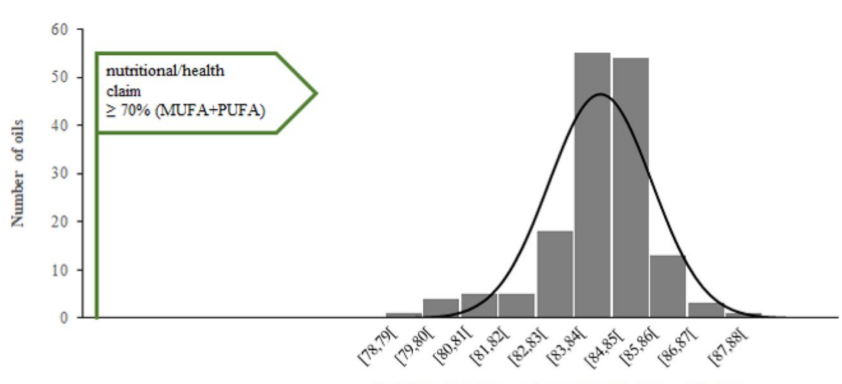

Figure 2. Total content is expressed as a percentage and related to health claims of at least 70% of total fatty acids in olive oils obtained from olives of centenarian trees (Côa Valley, 2020–22). Covers MUFA and PUFA during the study years (2020–2022).

normal distribution curve representing the number of trees per vitamin E concentration. Both Table 1 and Figure 3 unequivocally show that all oils qualify for both claims, as their vitamin E content surpasses 100 mg/kg, nearly three times the minimum required concentration. Furthermore, it is noteworthy that approximately 56% of the oils evaluated exhibited vitamin E concentrations ranging between 250 and 350 mg/kg. Notably, two oils derived from trees numbered 32 and 127 exhibited vitamin E contents falling within the 400–500 mg/kg range, representing 12–13 times the minimum level mandated by the claims. Figure 3 also indicates that the concentration of vitamin E in the studied oils conforms to a normal distribution (mode ≈ median ≈ mean). The findings of the current study align with those documented in the literature. Previous studies have reported vitamin E contents in olive oils varying from 100 to 200 mg/kg for samples from Brazil and Morocco (Gagour et al., 2024; Zago et al., 2019). Conversely, Squeo et al. (2021) found that olive oils derived from centenarian trees of Puglia, Italy, exhibited vitamin E concentrations within the range of 200–300 mg/kg. Pérez et al. (2019) reported significant variations in tocopherol concentrations (ranging from 89 to 1410 mg/kg) in oils extracted from olives of Istarska Bjelica and Dokkar cultivars. Recently Cecchi et al. (2024), analyzed 1000 olive oils from various geographical regions (Australia, Greece, Italy, Portugal, Spain, and Tunisia) proceed from two crop years (2013 and 2014), tocopherol concentrations were found to range from less than 100 mg/kg to greater than 500 mg/kg. However, the majority of oils (approximately 97–98%) exhibited concentrations between 100 and 400 mg/kg, consistent with the findings of the present study.

3.2.3. Content in hydroxytyrosol and tyrosol

As per Commission Delegated Regulation (EU) N°. 432/2012, olive oils containing a minimum of 5 mg of hydroxytyrosol and its derivatives per 20 g of oil are eligible for the health claim stating *'the polyphenols present in olive oil contribute to the protection of blood lipids against undesirable oxidation'*. However, it must be clarified that this beneficial effect applies solely to a daily intake of 20 g of olive oil. Figure 4 illustrates the distribution of oils based on the average concentration of hydroxytyrosol and tyrosol in mg/20g of olive oil, offering a visual depiction of the number of samples that may qualify for the related health claim. Additionally, the figure depicts the theoretical normal distribution curve, demonstrating that the experimental data conforms to a positively skewed distribution, characterized by a tail on the right side of the distribution (mode < median < mean). However, not all samples meet the criteria for such claims. Out of the 159 olive oil samples analysed, only 90% contained more than 5 mg/20 g of oil, meeting the requirements for the polyphenol-related health claim. It is noteworthy that four of the oils examined exhibited levels of hydroxytyrosol and its derivatives surpassing the threshold by 5–7 times, highlighting the high benefits potential for health associated with the consumption of these particular oils.

The findings align closely with the range of 4–31 mg/20g reported in Iranian olive oils by Shavakhi et al. (2021), and surpass the values documented by Tsimidou et al. (2019), which ranged from 0 to

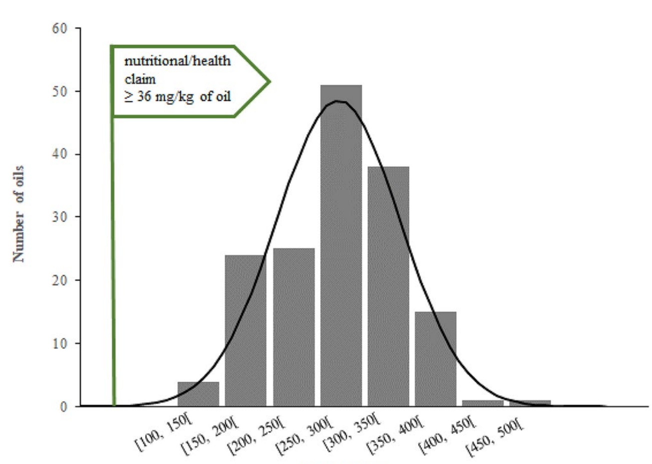

Figure 3. Total tocopherol content expressed as vitamin E found in olive oils produced from centenarian trees (Côa Valley, 2020–22). The content is related to health claims of a minimum value of 36 mg/kg of olive oil.

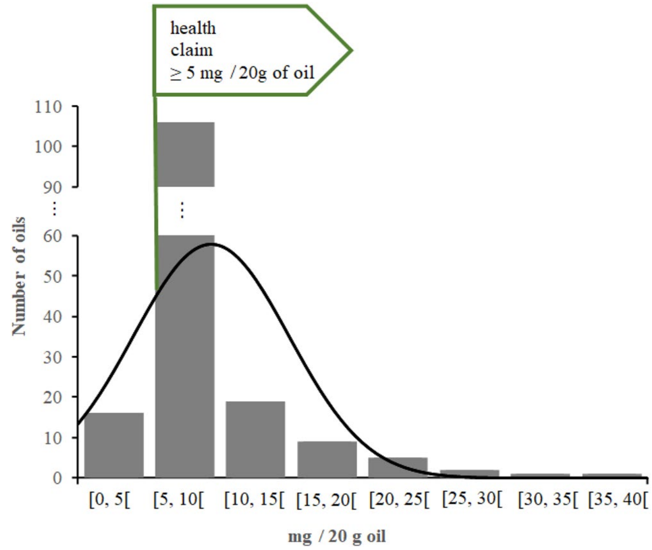

Figure 4. Total content of hydroxytyrosol and tyrosol after acid hydrolysis found in olive oils produced from centenarian trees (Côa Valley, 2020–22) related to health claims of a minimum value of 5 mg of hydroxytyrosol and its derivatives per 20 g of oil.

12 mg/20g. These results confirm the viability of centenarian trees as a potential source of olive oils eligible for the polyphenolic related health claim. The genetic diversity observed in centenarian olive groves, coupled with various intrinsic and extrinsic factors, bestows upon these oils a sense of uniqueness and distinction (Baccouri et al., 2023; Cecchi et al., 2020; Lechhab et al., 2022; Rodrigues et al., 2020a,b).

4. Conclusions

In the present work the potential of olive oils obtained from centenarian olive trees to meet the regulations for nutritional and health claims in terms of unsaturated fatty acids, tocopherols, hydroxytyrosol and their derivatives was confirmed. For the claim of high content of unsaturated fatty acids, all the samples complied with the regulations for olive oils with more than 70% of these fatty acids. As for vitamin E, all the samples allowed the claim, as their concentrations exceeded the required 36 mg/kg. It is noteworthy that 44.29% of the samples registered vitamin E levels between 200–300 mg/kg of oil, while 36.43% showed values in the 300–400 mg/kg range, reaching maximum values of 483.88 mg/kg. With regard to phenolic acids, 89% of the samples met the requirements for health claims related to polyphenols for olive oils with more than 5 mg/20g. Around 75% were in the 5–10 mg/20g range, and some samples had concentrations above 15%, with maximum values reaching 31.8 mg/20g. The composition found in the oils analyzed in this study reveals a remarkable potential for nutritional and health claims and highlights the importance of preserving these centenarian specimens.

Ethical statement

In which concerns the sensory panel, according to the institutional ethical commission, ethical permission was not required. Moreover, all panellists gave their consent.

Author contribution

CRediT: **Leticia Bortoluzzi**: Formal analysis, Investigation, Methodology, Writing – original draft; **Susana Casal**: Formal analysis, Investigation, Writing – review & editing; **Rebeca Cruz**: Formal analysis, Investigation, Methodology; **António M. Peres**: Investigation, Software, Writing – review & editing; **Paula Baptista**: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing; **Nuno Rodrigues**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

The authors report there are no competing interests to declare.

Data availability

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

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