Food Chemistry 525 (2026) 150369

Contents lists available at ScienceDirect

Food Chemistry

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

Impact of meteorological conditions on fatty acid composition and isotopic signatures in Slovenian extra virgin olive oil: A long-term study

Neri Bonciani [a,1], Marjeta Mencin [b,1], Doris Potočnik [b], Claudio Natali [a,c], José Manuel Moreno-Rojas [d], Milena Bučar-Miklavčič [e], Nives Ogrinc [b,f,2,*]

[a] Department of Earth Sciences, University of Florence, Via La Pira 4, 50121 Firenze, Italy
[b] Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[c] Institute of Environmental Geology and Geoengineering, Area della Ricerca di Roma 1- Montelibretti, Via Salaria km 29,300 - 00015, Monterotondo, RM, Italy
[d] Department of Food Science and Health, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Alameda del Obispo, Avda. Menéndez-Pidal, s/n. 14004 Córdoba, Spain
[e] Science and Research Centre Koper, Institute for Oliveculture, Garibaldijeva 1, 6000 Koper, Slovenia
[f] Research and Innovation Center Prekmurje, Jožef Stefan Institute, Lendavska ulica 5a, 9000 Murska Sobota, Slovenia

ARTICLE INFO

Keywords:
Olive oil
Fatty acid composition
Stable isotopes
Climate
Quality
Mediterranean region
Multivariate analysis

ABSTRACT

The olive tree (*Olea europaea* L.), a key crop in Mediterranean regions, is increasingly affected by climate variability. Meteorological factors such as temperature, rainfall, and humidity can influence the biosynthesis of fatty acids (FAs) in olives, potentially altering their concentrations and affecting the oil's compliance with established quality standards. In this study, we analyzed the FA composition of authentic Slovenian extra virgin olive oils (EVOOs) from 1993 to 2019. We also measured the $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ of bulk oil and the $\delta^{13}C$ of individual FAs for selected harvest years (2006–2008, 2019) to explore their relationships with changing meteorological conditions. The study was further extended to EVOO samples from other Mediterranean countries (Croatia, Italy, Spain, and Montenegro) to investigate the influence of regional meteorological parameters on the stable isotope composition of EVOOs. Our findings indicate that temperature significantly affects the FA composition of Slovenian EVOOs, with higher temperatures associated with lower oleic acid and higher linoleic and palmitoleic acid contents. Linear regression analysis indicated that $\delta^{18}O$ values of Slovenian samples primarily respond to the frequency of hot days and relative humidity, while $\delta^{2}H$ values demonstrate a strong relationship with temperature. Spanish samples, characterized by the highest average annual temperatures and the lowest rainfall, show significantly higher $\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$ values. This study provides insights into how climatic variability affects EVOO quality and supports efforts to ensure authenticity and traceability.

1. Introduction

Extra virgin olive oil (hereafter EVOO) is obtained from the fruits of the olive tree (*Olea europaea* L.), an important crop in Mediterranean agroecosystems that is increasingly exposed to climate variability. EVOO is a strategic product in the European Union (EU) market: Europe is the world's leading producer, exporter, and consumer of olive oil, and approximately 98% of the world's olive trees are located in the Mediterranean Basin (Bontempo et al., 2019). At the same time, olive oil is recognized as one of the most economically valuable and frequently adulterated food commodities worldwide (Commission Regulation, 2013; Everstine et al., 2024), which has led to the establishment of strict regulatory frameworks to protect consumers and ensure product authenticity.

In this context, the EU has issued two key regulations, Commission Regulation (EU) 2022/2104 (Commission Regulation, 2022a) and Commission Regulation (EU) 2022/2105 (Commission Regulation, 2022b), which define marketing standards, conformity criteria, and official analytical methods for olive oil quality and purity control. Among the important purity criteria is the fatty acid (FA) composition,

* Corresponding author at: Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
E-mail address: nives.ogrinc@ijs.si (N. Ogrinc).

[1] The authors equally contributed.

[2] Given her role as Regular Editor, prof. dr. Nives Ogrinc had no involvement in the peer-review of this article and has no access to information regarding its peer-review.

https://doi.org/10.1016/j.foodchem.2026.150369
Received 7 February 2026; Received in revised form 7 July 2026; Accepted 9 July 2026
Available online 10 July 2026

determined by gas chromatography, which is used to detect adulteration with some non-olive oils. According to these regulations, olive oil must comply with specific FA limits: oleic acid (C18:1) 55.00%–85.00%; myristic acid (C14:0) ≤ 0.03%; palmitic acid (C16:0) 7.00%–20.00%; palmitoleic acid (C16:1) 0.30%–3.50%; heptadecanoic acid (C17:0) ≤ 0.40%; heptadecenoic acid (C17:1) ≤ 0.60%; linoleic acid (C18:2) 2.50%–21.00%, linolenic acid (C18:3) ≤ 1.00%; arachidic acid (C20:0) ≤ 0.60%, eicosenoic acid (C20:1) ≤0.50%; behenic acid (C22:0) ≤ 0.20% and lignoceric acid (C24:0) ≤ 0.20% (IOC, 2022; IOC, 2025).

Despite the nutritional and economic importance of EVOO, its FA composition is not constant and can be influenced by numerous endogenous and exogenous factors, including cultivar (Massaccesi et al., 2026), fruit ripeness (Yamani et al., 2020), production origin (Zhang et al., 2022), altitude and latitude (Mikrou et al., 2023), as well as meteorological parameters, such as temperature, humidity, and precipitation (Bortoluzzi et al., 2026). Under ongoing global warming, these influences have become increasingly relevant (Lechhab et al., 2022). Several studies have demonstrated that warming temperatures can alter the FA profile of olive oil, potentially shifting it toward lower oleic acid and higher polyunsaturated fatty acid (PUFA) contents (Ben Hmida et al., 2022a; Contreras et al., 2023). In particular, García-Inza et al. (2014) reported a negative relationship between oleic acid concentration and increasing temperature during fruit development. Such climate-driven changes may challenge compliance with regulatory FA thresholds, complicating quality control and adulteration detection. Climate projections indicate that by the end of the twenty-first century, the Mediterranean Basin will experience significantly higher annual temperatures, particularly in southern Europe, as a consequence of rising greenhouse gas concentrations. This temperature shift is expected to intensify enzymatic processes in FA biosynthesis, potentially altering the FA composition of olive oil, with decreased oleic acid production and changes in the synthesis of linoleic and linolenic acids (IPCC, 2021; Ben Hmida et al., 2022b). Nevertheless, long-term studies evaluating how climate change affects EVOO FA composition and regulatory compliance remain scarce, highlighting a critical knowledge gap.

Beyond the FA profiling, the fact that climatic and environmental conditions leave measurable imprints on olive oil composition has prompted the use of stable isotope ratio analysis of light elements ($^{13}C/^{12}C$, $^{2}H/^{1}H$, and $^{18}O/^{16}O$, expressed as $\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$) measured by isotope ratio mass spectrometry (IRMS). This approach has become a powerful tool for tracing geographical origin and authenticity of olive oil and other food products (Bontempo et al., 2019; Camin et al., 2010; Paolini et al., 2017). The stable isotope ratios of $\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$ in olive oils can be influenced by meteorological conditions (e.g., relative humidity, temperature, precipitation) and geographical parameters (altitude, latitude, distance from the sea) and to a lesser extent, by plant cultivar (Iacumin et al., 2009). More recently, compound-specific isotopic analysis (CSIA) of individual FAs has proven particularly effective in this regard, as it provides a direct link between isotopic signatures and environmental factors (Bontempo et al., 2019). Adopting a stable isotope approach could therefore offer an alternative to monitor climate-driven changes in olive oil quality.

Given that no previous study has investigated how long-term climatic variability influences the FA content and isotopic composition of FAs in EVOOs, the objectives of this study were to: (i) evaluate the impact of climate on long-term relationships between FA composition and isotopic signatures ($\delta^{13}C$, $\delta^{18}O$, and $\delta^{2}H$) of Slovenian EVOOs as a case study and (ii) assess how region-specific meteorological conditions influence the isotopic compositions of EVOOs from different Mediterranean origins. Based on these aims, we hypothesized that long-term climatic variability has a measurable effect on both the FA composition and the isotopic signatures of EVOOs. We expected that interannual fluctuations in meteorological conditions would be reflected in the FA profile and isotopic composition of Slovenian EVOOs, while regional differences in meteorological conditions across Mediterranean areas would produce distinct isotopic patterns according to origin.

The results provide new insights into climate-induced changes in EVOO composition and demonstrate the value of stable isotope approaches in supporting quality control, authenticity, and traceability under changing climatic conditions.

2. Materials and methods

2.1. Sample collection

In this study, the Slovenian EVOO compositional database, compiled by the Institute for Oliveculture, Science and Research Centre (SCR) Koper and covering the period from 1993 to 2019, was used to evaluate changes in FA composition. The dataset includes 1643 authentic Slovenian olive oil samples from various olive varieties, collected directly during processing at olive oil mills with full traceability from olive orchard to final product. Although cultivar information was not explicitly included as a factor in the statistical analysis, the large and diverse dataset still provides a robust basis for assessing variability in fatty acid and isotope composition, with the understanding that some influence of cultivar may be present alongside climatic effects. All samples were final sale products; hence, the oil was cold-pressed in a two-phase system and filtered at local facilities in the same manner. In addition, 252 Slovenian EVOO samples from harvest years (2006–2008, 2019) were selected for the determination of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ in bulk oil and $\delta^{13}C$ in individual FAs.

To differentiate EVOOs based on geographical origin using isotopic compositions, 79 samples were analyzed: 46 EVOO from the Istria region in Slovenia (mainly Šmarje, Piran, Izola, Padna, Škofije, and Ankaran, Fig. 1a), and 33 from other countries (Fig. 1b), including five samples from Croatia (Krasica, Vodnjan), thirteen from Italy (mainly from Marche, but also from Molise, Sicily, Liguria, and Lombardia), ten from Spain (Jaén, Madrid, Córdoba), and five from Montenegro (Stari Bar, Valdanos, Ulcinj). All samples were produced in 2007. Although cultivar information was not available for all samples, the declared cultivars were as follows: for Slovenia and Croatia, mainly *Istrska belica*, *Leccino*, *Frantoio*, with *Karbonaca* and *Morasola,* typical of Croatia; for Italy: *Cerassuola*, *Taggiasca*, *Casaliva*, *Carboncella*, *Salvagno*, *Orbetana* and *Piantone di Falerone*; for Spain: *Picual*, *Manzanilla*, *Arbequina*, *Picuda*, *Hojiblanca* and for Montenegro, exclusively *Žutica* cultivar. As cultivar was not included as a controlled factor in the statistical analysis, its potential influence on isotopic composition cannot be fully separated from geographical effects, although it is assumed to be encompassed within the overall variability of the dataset.

2.2. Analytical procedures

2.2.1. Chemical analysis

2.2.1.1. Extraction and esterification of fatty acids (FAs) in olive oil. Olive oil consists predominantly of triglycerides, which were extracted by transesterification according to the COI/T.20/Doc. No 33/Rev.1–2017 by gas chromatography (GC) with FID detection (IOC, 2017). For the preparation of fatty acid methyl esters (FAMEs), approximately 0.1 g of EVOO was weighed into a 10 mL vial with 4 mL of hexane and 1 mL of 2 M sodium hydroxide in methanol, then shaken for 1 min at room temperature. The solution was left until the upper layer became clear. An aliquot of hexane (1.00 mL) containing FAMEs was transferred to a GC vial. To ensure data quality, a procedural blank was prepared with each batch of samples.

2.2.1.2. Gas chromatography of fatty acids (FAs). Samples were analyzed by gas chromatography with a flame ionization detector (GC-FID) using an Agilent 6890 N Network GC System (Agilent Technologies, Santa Clara, CA, USA). The separation was achieved on an Omegawax 320 capillary column (30 m × 0.32 mm × 0.25 μm; Supelco, Bellefonte,

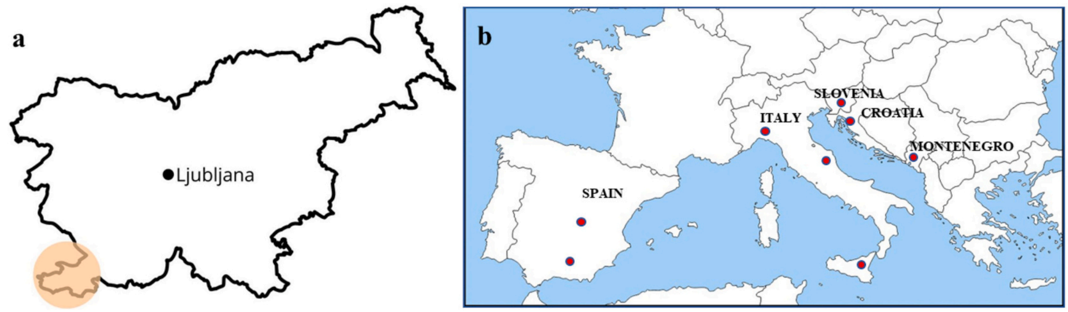

Fig. 1. a) Main olive oil producing area in Slovenia (Istria region); **b)** geographic distribution of samples: Italy has three main sampling sites, Spain two, while Slovenia, Croatia, and Montenegro each have one main locality.

PA, USA). The temperature program was as follows: 50 °C (held for 4 min), then to 170 °C at 30 °C/min, then to 200 °C at 2 °C/min, then to 210 °C at 1 °C/min. The carrier gas was helium at 1.2 mL/min. The injector was set to 240 °C and the detector to 270 °C. The individual FAs were identified by comparing their retention times with those of a standard Supelco 37-component FAME Mix (Supelco). The same set of FAs was consistently used across all analyses to ensure the validity and comparability of the results. All samples were injected in duplicate with a precision of ±5%. Results were expressed as the percentage of each FA with respect to the total FAs.

2.2.2. Stable isotope analysis

2.2.2.1. Determination of stable isotope composition of H, C and O in bulk olive oil. Stable isotope ratio measurements were performed using isotope ratio mass spectrometry (IRMS) and reported using the δ-notation in per mil (‰) relative to the Vienna-Pee Dee Belemnite (V-PDB) standard for C and Vienna-Standard Mean Ocean Water (V-SMOW) standard for H and O, following eq. (1) (Brand et al., 2014):

$$\delta^{i/j}E = \left(\frac{i/jR_{SA} - i/j\,R_{REF}}{i/j\,R_{REF}}\right) \bullet 1000 \qquad (1)$$

where i and j denote the highest and the lowest atomic mass number of element E, and R_{SA} and R_{REF} indicate the ratio between the heavier and the lighter isotopes ($^2H/^1H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$) in the sample (*SA*) and reference standard (*REF*), respectively.

To determine the bulk carbon isotope composition ($\delta^{13}C_{bulk}$) of the olive oil samples, approximately 0.3 mg of the sample was placed into a sealed tin capsule and loaded into the automatic sampler of an elemental analyzer. Analyses were conducted using a continuous-flow IRMS (Europa Scientific 20–20, Sercon Ltd., Crewe, UK) equipped with an ANCA-SL solid-liquid preparation module (Sercon, Crewe, UK). Analyses from 2006, 2007 and 2008 were normalized against the international reference materials: IAEA-CH-7 and IAEA-CH-3 with $\delta^{13}C$ values of −32.15 ± 0.05‰ and − 10.45 ± 0.03‰, respectively, while analyses from 2019 were normalized against international reference materials USGS61 and USGS62 with $\delta^{13}C$ values of −35.05 ± 0.04‰ and − 14.79 ± 0.04‰, respectively. For independent control, international reference material NBS 22a with $\delta^{13}C$ values of −29.72 ± 0.04‰ and USGS84 with $\delta^{13}C$ values of −28.80 ± 0.09‰. The analytical precision, expressed as the standard deviation of the control material, was ±0.2‰.

The $^2H/^1H$ and $^{18}O/^{16}O$ ratio in olive oil was determined directly by continuous flow IRMS using a total conversion elemental analyzer (TC/EA) coupled to a Delta PlusXP mass spectrometer (Thermo-Fisher, Rodano, Italy) and a Delta Plus XL stable isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany), respectively. The procedure followed that of Aramendía et al. (2007). Briefly, for hydrogen, 0.3 mg of the sample was placed in a silver capsule and introduced into the elemental analyzer. In contrast, for oxygen, around 0.25–0.3 mg of the sample was placed in a tin capsule. Normalization of the results was performed against two international reference materials, IAEA-CH-7 with δ^2H values of −100.3 ± 2‰, NBS 22 with δ^2H values of −120 ± 1‰ for hydrogen and laboratory reference material sucrose with a $\delta^{18}O$ value of +23‰ and olive oil with a $\delta^{18}O$ value of +25.5‰ for oxygen.

2.2.2.2. Compound-specific isotope analysis (CSIA) of $\delta^{13}C$ in fatty acids (FAs). The isotopic composition of individual FA was determined using an Agilent 6890 N GC-C system coupled to an IsoPrime GV IRMS (GV instruments Ltd., Manchester, UK). Separation of individual FAs was obtained using an INNOWAX capillary column (60 m × 0.32 mm × 0.50 μm) with a temperature program of 170 °C to 220 °C at 10 °C/min (held 25 min), then to 240 °C at 30 °C/min (held 5 min) with helium as the carrier gas (1.5 mL/min). The inlet temperature was 250 °C. Samples were injected in split mode (20:1). While samples from 2019 were determined using a VF-Wax capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J&W). The temperature program was as follows: 120 °C (held 1 min) to 190 °C at 50 °C/min, then to 200 °C at 1 °C/min (held 3 min), then to 210 °C at 2 °C/min (held 2 min). The injection was performed at 250 °C in splitless mode, and the oxidation reactor (Cu/O) in the 6890 N GC/C system was set to 900 °C. Helium was used as a carrier gas at a flow rate of 1.2 mL/min. Peak identification of individual fatty acids was performed by comparing their retention times with those of the standards: C16:0 (methyl palmitate, Sigma-Aldrich), C18:0 (methyl stearate, Sigma-Aldrich), C18:1 (methyl oleate, Sigma-Aldrich) and C18:2 (methyl linoleate, Sigma-Aldrich), which were analyzed at each measurement sequence. To ensure data quality, stability was checked before each run, with an accepted value of ≤0.08‰. Each sample was injected in duplicate. The results were normalized against international reference materials: Icosanoic acid methyl ester #X (C21:0) with $\delta^{13}C$ values of −6.91 ± 0.04‰, USGS 70 (Icosanoic Acid Methyl Esters) with $\delta^{13}C$ values of −30.53 ± 0.04‰. For independent control laboratory reference material C19:0 FAME (methyl nanodecanoate) with $\delta^{13}C$ values of −29.8 ± 0.1‰ (previously determined on EA-IRMS) was used. Reproducibility based on duplicate analysis was ≤0.4‰.

2.3. Meteorological data

All meteorological data (Table S1) comprising average annual temperature at 2 m above the ground (Avg T, °C), annual maximum temperature (Max T, °C), annual minimum temperature (Min T, °C), average relative humidity (RH, %), average annual rainfall (mm), average solar exposure (h) and number of hot days throughout a year ($T > 30$ °C) were recorded at ARSO Meteo, Portorož station. Meteorological variables were considered on an annual basis and were calculated according to the methodology of the Slovenian Environment Agency (ARSO). Specifically, annual average air temperature was obtained as the arithmetic mean of daily mean temperatures. Annual precipitation was determined as the sum of daily precipitation totals, whereas annual mean maximum and minimum temperatures were calculated as the arithmetic means of daily maximum and daily minimum temperatures, respectively (ARSO

Meteo, 2024).

2.4. Statistical analysis

Basic statistical analyses, including a Kruskal–Wallis test and Pearson correlation coefficients, were conducted using XLSTAT 2024 (Addinsoft, Long Island, NY, USA). Differences between means were considered statistically significant at $p < 0.05$. Linear regression analysis was performed using Microsoft Excel Professional Plus 2021 (Microsoft Corporation, Redmond, WA, USA). Multivariate analyses, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were performed in RStudio (version 2023.12.1) and SIMCA (version 15; Sartorius Stedim Biotech, Umeå, Sweden), respectively.

3. Results and discussion

3.1. Impact of meteorological conditions on fatty acid and isotopic composition

3.1.1. Fatty acid composition with respect to meteorological conditions

The results (Table 1) show that oleic acid is the predominant FA regardless of harvest year, ranging from 71.88% in 2018 to 78.87% in 1996, and remains within the limits established by the current EU Regulation 2022/2104 for olive oil. All other FAs are also compliant, except for eicosenoic acid (C20:1) in 1995, which exceeded the 0.5% limit by 74%. That year was characterized by one of the lowest annual temperatures and the highest relative humidity recorded during the study period (Table S1). Such environmental conditions may have altered the physiological and biochemical processes involved in olive maturation and fatty acid biosynthesis, thereby contributing to the elevated level of C20:1.

Compared to the FA composition of EVOOs from 2019, the 1993 samples showed a decrease of 26.3% and 20.5% in the amounts of palmitoleic and linoleic acids, respectively, while the content of linolenic acid increased by 15.9%. No significant differences were observed for oleic, stearic, and palmitic acids between 1993 and 2019. These differences are likely attributable to variations in climatic conditions: in 2019, both the annual average and maximum temperatures were approximately 2 °C higher than in 1993, and the total annual rainfall was also significantly greater (1145.1 mm compared to 783.2 mm) (Table S1).

Oils with the lowest oleic acid content were observed in 2018 and 2019, coinciding with some of the highest average, maximum, and minimum temperatures (Table S1) recorded during the study period. Compared to 1996, which showed the highest average oleic acid content, levels in 2018 and 2019 were 8.9% and 7.4% lower, respectively. For palmitic and palmitoleic acids, an increase in their contents was observed with increasing temperature; however, the relative influence of temperature was not confirmed by statistical modelling. Notably, in 2018, a year characterized by some of the highest temperatures, the contents of palmitic and palmitoleic acids were 23.2% and 57.9% higher, respectively, than in 1996, which experienced among the lowest annual temperatures (average: 12.7 °C, maximum: 17.8 °C) and fewest hot days (8 days). For linoleic acid, the lowest level was observed in 2005, when annual temperatures were the lowest (average: 12.6 °C). The average temperature that year was 2.2 °C lower than in 2018, which had the highest linoleic acid content (+35.3%).

The variability among EVOO samples from different harvest years can be explained by Beltrán et al. (2004), who found that temperature during oil biosynthesis affects the variability of palmitic and linoleic acid levels. In contrast, summer rainfall primarily influences oleic acid content. The monounsaturated fatty acid (MUFA)/PUFA ratio showed a strong negative correlation with both annual average ($r = -0.643$) and maximum ($r = -0.725$) temperatures, suggesting that higher temperatures reduce the biosynthesis of MUFAs while promoting that of PUFAs.

Table 1
Mean values and standard deviations of fatty acids in Slovenian virgin olive oil samples from 1993 to 2019.

	Fatty Acids (%, *w/w*)							
YEAR	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1
Limits*	7.00–20.0	0.3–3.5	0.5–5.0	55.0–85.0	2.5–21.0	≤1.0	≤ 0.60	≤0.50
1993	13.99 ± 0.76^{a}	1.01 ± 0.09^{cd}	3.03 ± 0.38^{a}	74.71 ± 1.04^{b}	5.58 ± 0.63^{bc}	0.73 ± 0.11^{a}	0.44 ± 0.07^{ab}	0.33 ± 0.06^{a}
1994	13.77 ± 1.28^{a}	1.05 ± 0.28^{cd}	2.60 ± 0.60^{ab}	75.90 ± 1.20^{a}	5.18 ± 0.77^{c}	0.71 ± 0.10^{a}	0.41 ± 0.08^{b}	0.30 ± 0.05^{b}
1995	11.66 ± 1.05^{de}	0.77 ± 0.16^{d}	2.40 ± 0.39^{bc}	78.49 ± 1.55^{a}	5.17 ± 1.13^{c}	0.87 ± 0.07^{a}	0.42 ± 0.06^{b}	0.87 ± 0.07^{a}
1996	11.01 ± 0.61^{e}	0.70 ± 0.07^{d}	2.33 ± 0.26^{bc}	78.87 ± 0.83^{a}	5.61 ± 0.78^{b}	0.63 ± 0.07^{b}	0.43 ± 0.11^{b}	0.28 ± 0.02^{bc}
1997	13.13 ± 1.16^{b}	0.97 ± 0.22^{cd}	2.65 ± 0.38^{ab}	74.94 ± 1.51^{b}	6.86 ± 0.39^{a}	0.68 ± 0.08^{a}	0.43 ± 0.06^{b}	0.25 ± 0.04^{cd}
1998	12.93 ± 0.69^{abc}	1.07 ± 0.12^{cd}	2.38 ± 0.27^{bc}	76.67 ± 0.81^{a}	5.55 ± 0.31^{c}	0.60 ± 0.06^{b}	0.41 ± 0.04^{b}	0.25 ± 0.02^{cd}
1999	13.89 ± 1.12^{a}	1.04 ± 0.14^{cd}	2.73 ± 0.26^{ab}	75.48 ± 1.32^{b}	5.60 ± 0.48^{bc}	0.59 ± 0.05^{bc}	0.38 ± 0.05^{c}	0.22 ± 0.02^{d}
2000	13.96 ± 0.58^{a}	1.14 ± 0.16^{bc}	2.60 ± 0.34^{b}	74.81 ± 0.88^{b}	5.96 ± 0.52^{b}	0.72 ± 0.06^{a}	0.43 ± 0.05^{b}	0.26 ± 0.02^{d}
2001	10.83 ± 0.59^{e}	1.13 ± 0.14^{c}	2.66 ± 0.29^{b}	77.83 ± 0.78^{a}	5.58 ± 0.52^{c}	0.63 ± 0.06^{b}	0.52 ± 0.06^{a}	0.37 ± 0.02^{a}
2002	11.87 ± 0.46^{d}	1.10 ± 0.11^{c}	2.55 ± 0.29^{b}	77.31 ± 0.45^{a}	5.59 ± 0.29^{c}	0.54 ± 0.05^{c}	0.42 ± 0.06^{b}	0.31 ± 0.03^{a}
2003	12.82 ± 0.74^{c}	1.26 ± 0.28^{b}	2.84 ± 0.34^{a}	74.80 ± 1.47^{b}	6.47 ± 0.84^{a}	0.68 ± 0.06^{a}	0.45 ± 0.05^{b}	0.30 ± 0.03^{b}
2004	11.86 ± 0.55^{e}	1.11 ± 0.13^{c}	2.84 ± 0.31^{a}	76.86 ± 0.87^{a}	5.61 ± 0.57^{c}	0.56 ± 0.04^{c}	0.47 ± 0.06^{a}	0.32 ± 0.02^{a}
2005	11.52 ± 0.48^{de}	1.02 ± 0.10^{cd}	2.84 ± 0.24^{a}	77.75 ± 0.50^{a}	5.11 ± 0.34^{c}	0.57 ± 0.04^{bc}	0.49 ± 0.04^{a}	0.32 ± 0.01^{a}
2006	12.15 ± 0.56^{d}	1.28 ± 0.11^{ab}	2.73 ± 0.33^{ab}	76.32 ± 0.49^{a}	5.84 ± 0.36^{b}	0.56 ± 0.04^{c}	0.45 ± 0.06^{ab}	0.30 ± 0.02^{b}
2007	13.13 ± 0.40^{ab}	1.33 ± 0.13^{a}	2.53 ± 0.19^{ab}	75.34 ± 0.56^{b}	5.98 ± 0.30^{ab}	0.61 ± 0.05^{b}	0.43 ± 0.04^{b}	0.30 ± 0.02^{b}
2008	12.59 ± 0.45^{bc}	1.15 ± 0.11^{abc}	2.92 ± 0.44^{a}	75.11 ± 0.69^{b}	6.61 ± 0.68^{a}	0.59 ± 0.07^{bc}	0.44 ± 0.07^{ab}	0.28 ± 0.02^{bc}
2009	13.37 ± 0.68^{b}	1.27 ± 0.16^{b}	3.05 ± 0.37^{a}	73.62 ± 1.05^{c}	7.00 ± 0.66^{a}	0.61 ± 0.05^{b}	0.46 ± 0.06^{b}	0.28 ± 0.02^{c}
2010	12.24 ± 0.98^{d}	1.14 ± 0.14^{c}	2.33 ± 0.45^{c}	77.34 ± 1.21^{a}	5.37 ± 0.82^{c}	0.56 ± 0.08^{c}	0.39 ± 0.07^{c}	0.30 ± 0.03^{b}
2011	13.43 ± 0.67^{b}	1.18 ± 0.10^{c}	3.05 ± 0.38^{a}	73.73 ± 0.90^{c}	6.88 ± 0.64^{a}	0.62 ± 0.06^{b}	0.48 ± 0.06^{a}	0.28 ± 0.01^{c}
2012	13.68 ± 0.74^{a}	1.42 ± 0.20^{a}	2.97 ± 0.44^{a}	73.83 ± 1.57^{c}	6.37 ± 1.08^{b}	0.62 ± 0.05^{b}	0.47 ± 0.06^{a}	0.28 ± 0.02^{c}
2013	12.43 ± 0.79^{d}	1.09 ± 0.16^{d}	2.77 ± 0.36^{b}	76.61 ± 1.19^{a}	5.40 ± 0.66^{c}	0.60 ± 0.06^{c}	0.45 ± 0.06^{b}	0.29 ± 0.02^{b}
2014	12.56 ± 0.71^{d}	1.36 ± 0.20^{a}	2.38 ± 0.37^{c}	75.53 ± 1.38^{b}	6.34 ± 1.07^{b}	0.70 ± 0.09^{a}	0.43 ± 0.06^{b}	0.33 ± 0.02^{a}
2015	12.94 ± 0.66^{c}	1.15 ± 0.13^{c}	2.74 ± 0.46^{b}	75.11 ± 1.16^{b}	6.39 ± 0.75^{b}	0.64 ± 0.07^{b}	0.43 ± 0.07^{b}	0.28 ± 0.02^{c}
2016	13.18 ± 0.68^{b}	1.18 ± 0.15^{c}	2.90 ± 0.35^{a}	74.37 ± 1.40^{c}	6.70 ± 1.10^{a}	0.61 ± 0.07^{b}	0.44 ± 0.05^{b}	0.27 ± 0.02^{d}
2017	13.07 ± 0.58^{b}	1.28 ± 0.17^{b}	2.71 ± 0.44^{b}	75.15 ± 0.92^{b}	6.10 ± 0.61^{b}	0.63 ± 0.08^{b}	0.44 ± 0.06^{b}	0.27 ± 0.02^{c}
2018	14.34 ± 1.55^{a}	1.66 ± 0.67^{a}	2.44 ± 0.64^{c}	71.88 ± 3.04^{d}	7.89 ± 2.07^{a}	0.77 ± 0.15^{a}	0.40 ± 0.09^{c}	0.29 ± 0.04^{b}
2019	13.69 ± 0.50^{a}	1.37 ± 0.11^{a}	3.11 ± 0.41^{a}	73.06 ± 1.04^{c}	7.02 ± 0.69^{a}	0.63 ± 0.08^{b}	0.48 ± 0.06^{a}	0.29 ± 0.01^{b}

* Limits established by the current EU Regulation 2022/2104 for olive oil. C16:0: palmitic acid; C16:1: palmitoleic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: linolenic acid; C20:0: arachidic acid; C20:1: eicosenoic acid. Within each FA column, means with different superscript letters differ significantly, while means sharing at least one letter do not differ significantly.

The impact of meteorological parameters over the years was also reflected in the FA ratios (Table S2). The oleic to linoleic acid ratio was highest in 2005 and 1995, showing a noticeable decrease in 2018, which had the highest annual temperatures. The oleic to palmitic acid ratio remained relatively stable, with the highest values recorded in 1996 and 2001, suggesting that this ratio is less sensitive to climatic fluctuations. The linoleic to linolenic acid ratio exhibited the highest variability among the ratios, with the lowest values observed in 1995, a year characterized by the lowest annual temperatures and higher rainfall. These climatic conditions likely contributed to a shift in the balance of PUFAs, with cooler and wetter years promoting linolenic acid production. Additionally, when averaging the linoleic to linolenic acid ratio over three periods, we observe an increasing trend: from 8.37 in the first period (1993–2000) to 9.99 in the second period (2001−2010), and 10.16 in the third period (2011–2019). This trend suggests that higher temperatures generally increase the content of linoleic acid and decrease the content of linolenic acid. The palmitic to stearic acid ratio exhibited minimal variability, indicating that the synthesis of these saturated fatty acids (SFAs) is less influenced by meteorological factors. Overall, meteorological parameters had the most significant impact on the production of linoleic and linolenic acids, highlighting the greater susceptibility of PUFAs to climatic conditions.

Pearson correlations, presented as a heatmap (Fig. S3), were used to examine the relationship between FA composition and different meteorological conditions. No strong correlations were observed between FAs and either annual rainfall or number of hot days ($T > 30$ °C). However, a moderate negative correlation was observed between annual average, minimum and maximum temperatures and oleic acid content, indicating that oleic acid tends to decrease as temperatures rise. Conversely, palmitoleic and linoleic acids showed moderate positive correlations with annual average, maximum, and minimum temperatures. Oleic and linoleic acids also showed an opposite trend, with a strong negative correlation ($r = -0.850$), likely reflecting the activity of the oleate desaturase enzyme, which converts oleic acid to linoleic acid (El Qarnifa et al., 2019). To better understand the influence of temperature on FA composition in olive oil, it is important to consider the molecular mechanisms involved. Temperature regulates key enzymes involved in FA biosynthesis, such as acetyl-CoA carboxylase and FA desaturases, which are essential for the production of both MUFAs and PUFAs. Higher temperatures typically enhance the activity of oleate desaturase and linoleate desaturase, resulting in an increased conversion of oleic acid to linoleic acid. Temperature stress may also affect the expression of genes involved in lipid metabolism, which can further influence the FA profile. This highlights the significant role of enzymatic activity in determining the balance of oleic and linoleic acids in response to temperature variations (El Qarnifa et al., 2019).

The results are consistent with García-Inza et al. (2014), who also reported decreases in oleic acid and increases in linoleic and palmitoleic acids as temperature increased. Interestingly, it contrasts with that observed in oilseed crops (e.g., sunflower, maize, soybean), where high temperatures during fruit growth are associated with increased oleic acid content (Zuil et al., 2012). Several authors suggest that different responses to temperature depend on the cultivar and the tissues in which oil accumulates. In olives, the oil accumulates in the mesocarp, whereas in oilseeds it accumulates in the embryo/endosperm (García-Inza et al., 2024). Previous studies have also concluded that temperature is the main factor affecting FA metabolism in olives (El Qarnifa et al., 2019), findings consistent with our results.

3.1.2. Isotope composition of bulk olive oil with respect to meteorological conditions

The δ^{13}C values of bulk olive oil (Fig. 2) revealed that EVOOs harvested in 2007 and 2008 had less negative δ^{13}C values (−29.5 ± 0.5‰ and − 29.1 ± 0.4‰, respectively), which were significantly different from those of 2006 and 2019 (−30.3 ± 0.4‰ and − 29.7 ± 0.4‰, respectively). These results are consistent with the fact that olive trees are C3 plants, whose δ^{13}C values typically range between approximately −22 to −33‰. Previous studies have shown that δ^{13}C in bulk olive oil varies with temperature, precipitation, and relative humidity, which affect plant oil biosynthesis pathways and lead to carbon isotope fractionation (Bontempo et al., 2019). The lowest δ^{13}C values among observed years, recorded in 2006, coincided with the lowest annual average (13.7 °C) and minimum temperatures (8.6 °C) of the study period. Interestingly, despite temperatures being 1 °C higher and rainfall 258.6 mm higher in 2019 than in 2006, no statistically significant difference in δ^{13}C values was observed. Furthermore, no strong correlations were found between δ^{13}C values and meteorological parameters (Table S4).

In contrast, we observed a strong positive correlation between the δ^{18}O values of Slovenian EVOO and rainfall ($r = 0.839$), relative humidity ($r = 0.871$) and number of hot days (>30 °C) ($r = 0.922$) (Table S4). Iacumin et al. (2009) similarly reported a strong correlation between the δ^{18}O values of Italian EVOO and average annual temperature (positive), and relative humidity (negative) for the years 2004 and 2005. However, they found no correlation with average annual rainfall. Furthermore, δ^{18}O values in our EVOO samples were 22.9‰ in both 2006 and 2007, whereas in 2008 the δ^{18}O value was 1.4‰ higher. This increase coincided with a significantly greater rainfall (993.8 mm) and higher relative humidity (71%) in 2008.

Although the average annual temperature in 2008 was not higher, the number of hot days (>30 °C) was greater. In contrast, Portarena et al. (2014) reported that EVOOs from northern Italian regions had significantly lower δ^{18}O values than those from southern regions, due to higher precipitation and lower temperatures. Furthermore, Song et al.

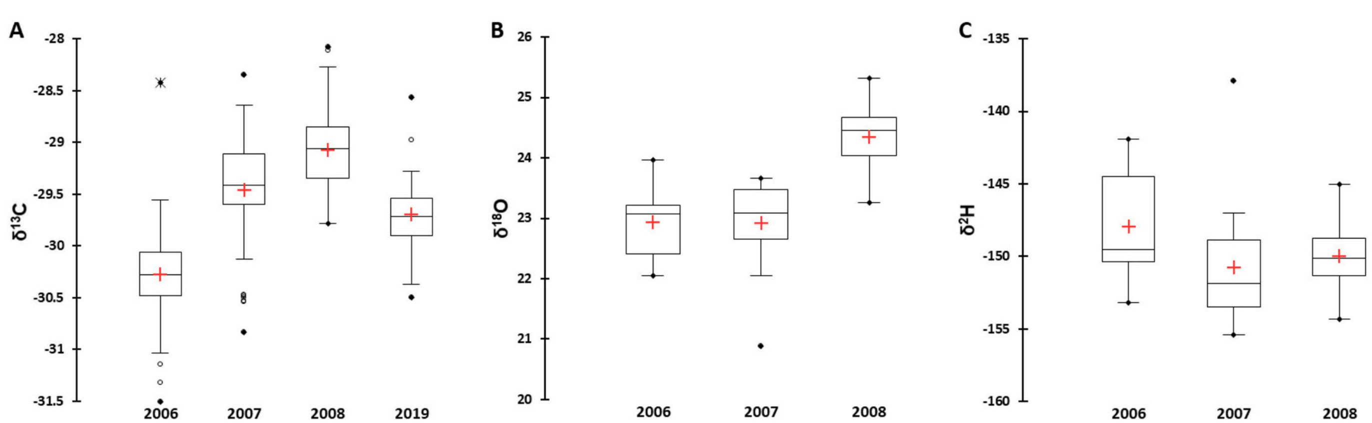

Fig. 2. Box plots of (**A**) δ^{13}C, (**B**) δ^{18}O, (**C**) δ^{2}H values (‰) of bulk extra virgin olive (EVOO samples from Slovenia according to the year of production (2006–2008, 2019). δ^{18}O and δ^{2}H values were not detected in the 2019 samples.

(2022) found that the ^{18}O content of precipitation decreases with lower condensation temperatures and higher rainfall amounts. Consequently, years with high temperatures and low rainfall are generally characterized by heavier, i.e., ^{18}O-enriched precipitation.

The δ^2H values in plants are known to be influenced by the isotopic composition of the primary hydrogen source (source water via leaf water) as well as geography and climate (Zhao et al., 2024). The δ^2H values were on average − 148 ± 4‰ in 2006, −151 ± 4‰ in 2007, and -150 ± 3‰ in 2008, and were not statistically different across production years. These results suggest that δ^2H is relatively stable across years and less sensitive to interannual meteorological variation. Moreover, δ^{18}O and δ^2H values in bulk olive oil showed a weak negative correlation (r = −0.237) (Table S4), which is not consistent with the findings of Bontempo et al. (2009), who reported a strong correlation between δ^{18}O and δ^2H values in Italian olive oils. In contrast, δ^{13}C values were strongly positively correlated with δ^{18}O (r = 0.773) and negatively correlated with δ^2H (r = −0.800).

From the linear regression analysis, the relationships between δ^{13}C and meteorological parameters are uniformly weak ($r^2 \leq 0.11$), indicating that carbon isotopic composition is insensitive to the examined meteorological variables in this dataset (Table S5). In contrast, δ^{18}O shows a strong to moderate correlation with several parameters. The most pronounced being the number of hot days (>30 °C) ($r^2 = 0.85$, slope = 0.30), followed by relative humidity ($r^2 = 0.76$, slope = 0.72) and rainfall ($r^2 = 0.70$, slope = 0.01).

The equation "Rainfall–δ^{18}O" shows that a change of 100 mm in annual rainfall corresponds to a higher values of approximately +1.0‰ in δ^{18}O. For δ^2H, the strongest relationship was observed with average temperature ($r^2 = 0.94$, slope = −4.71), showing a depletion of deuterium with increasing temperature. Substantial negative slopes were also observed for minimum and maximum temperatures, whereas rainfall, relative humidity, and the number of hot days showed negligible associations. Overall, these results suggest that δ^{13}C remains relatively stable with respect to meteorological variability. At the same time, δ^{18}O responds primarily to hot-day frequency and relative humidity, and δ^2H shows a strong relationship with temperature, making it potentially the most climate-sensitive isotope of the three.

3.1.3. *Isotopic composition of individual fatty acids with respect to meteorological conditions*

The δ^{13}C values were determined in the five most abundant FAs in Slovenian EVOOs, harvested in 2006–2008 and 2019, namely palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids (Fig. 3). The δ^{13}C values for the palmitoleic acid exhibited the broadest range (from −45.4‰ to −21.9‰) when compared to the δ^{13}C values for other FAs. Specifically, the δ^{13}C values of other FAs vary from −31.8‰ to −27.9‰, −35.4‰ to −29.0‰, −32.2‰ to −26.7‰ and − 31.5‰ to −27.9‰ for palmitic, stearic, oleic and linoleic acids, respectively. The δ^{13}C trends in FAs are consistent with those reported for vegetable oils in previous studies (Bontempo et al., 2019; Faberi et al., 2014; Spangenberg & Ogrinc, 2001).

The variability in the δ^{13}C values of FAs is expected due to known fractionation effects that occur during different FA biosynthesis. A multienzyme complex catalyzes both chain elongation, through the addition of acetyl groups, and desaturation of FAs. Our results indicate an initial isotopic fractionation from palmitic to stearic acid during the elongation step, with stearic acid generally showing lower δ^{13}C values. The following desaturation step leads to higher δ^{13}C values for oleic acid than for stearic acid, whereas a second desaturation reaction results in a slight decrease in the δ^{13}C values of linoleic acid. Observed fractionations can also be related to the different cell compartments (chloroplasts vs cytosol) in which these reactions occur (Portarena et al., 2019).

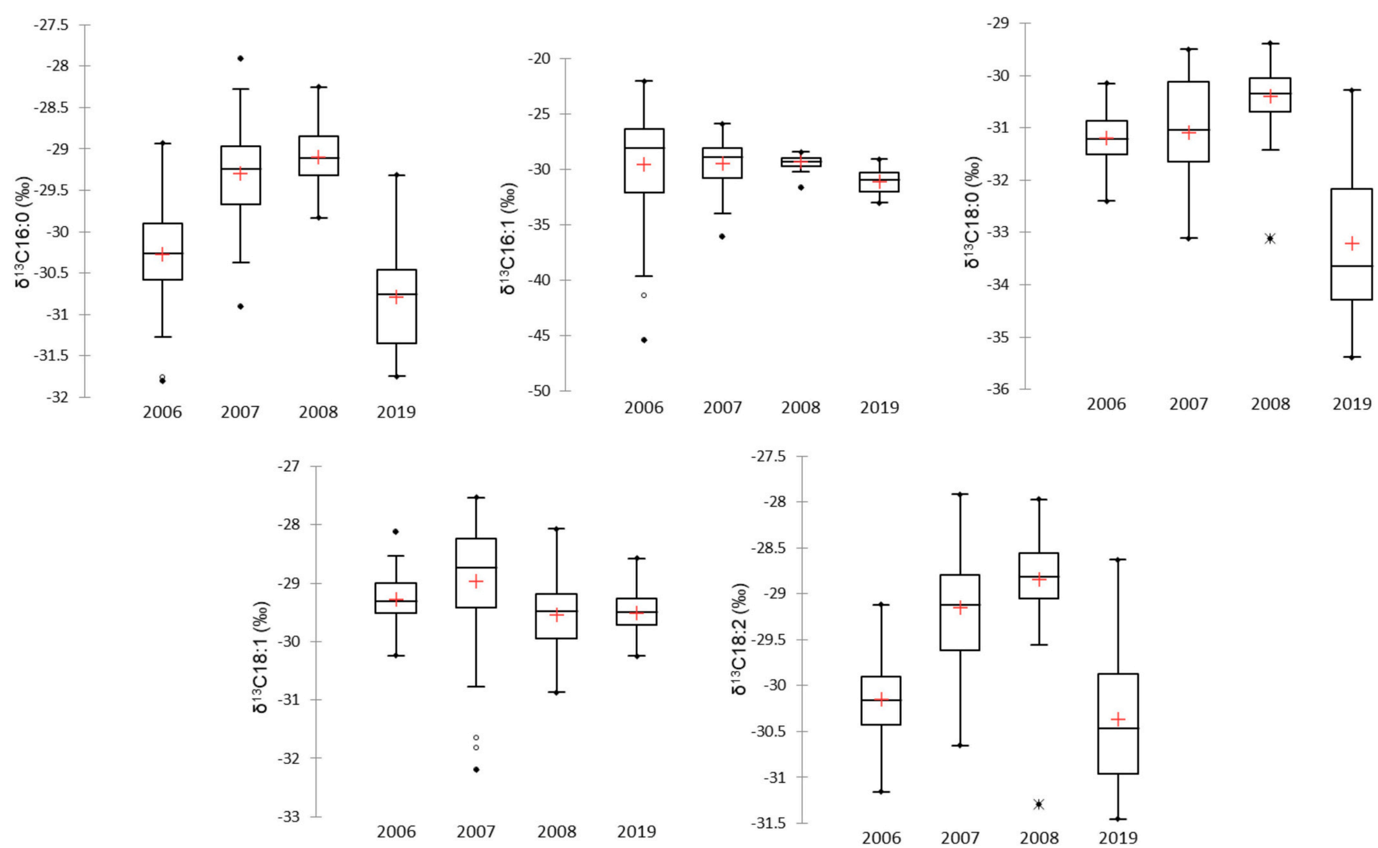

Fig. 3. Box plots of carbon isotope ratios (δ^{13}C) of individual fatty acids of Slovenian extra virgin olive oil (EVOO) according to different production years (2006–2008, 2019). δ^{13}C16:0: carbon isotope ratios of palmitic acid; δ^{13}C16:1: carbon isotope ratios of palmitoleic acid; δ^{13}C18:0: carbon isotope ratios of stearic acid; δ^{13}C18:1: carbon isotope ratios of oleic acid; δ^{13}C18:2: carbon isotope ratios of linoleic acid.

The mean δ^{13}C values of C16:0, C16:1 and C18:0 in 2019 were significantly lower than those observed in other production years. Similarly, the mean δ^{13}C18:2 value in 2019 was significantly lower than in 2007 and 2008, while the mean δ^{13}C18:1 value in 2019 was significantly lower than in 2006 and 2007. The δ^{13}C value of stearic acid exhibited a moderate negative correlation with annual maximum temperature ($r = -0.556$), number of hot days ($r = -0.512$) and average temperature ($r = -0.392$), while showing a weak correlation with other meteorological parameters across 2006–2008 and 2019. The δ^{13}C values of individual FAs appear less influenced by meteorological conditions, likely because the samples originated from Istria. This finding is in agreement with the study by Iacumin et al. (2009), which also reported that meteorological parameters do not correlate well with δ^{13}C values of Italian olive oils, especially within a limited geographic area.

The δ^{13}C values of palmitic and linoleic acids showed the strongest correlation with the δ^{13}C values of bulk oil (r = 0.786 and 0.798, respectively) and with each other ($r = 0.909$). The δ^{13}C values of stearic, oleic, and linoleic acids moderately correlated with the δ^{18}O values of bulk oil (0.560, 0.549, 0.416, respectively) and with δ^{2}H values (0.473, 0.408, 0.373, respectively).

3.1.4. Chemometric analysis

Partial Least Squares Discriminant Analysis (PLS-DA) was used to explore differences between years of production (2006–2008, 2019) of Slovenian EVOOs according to eleven variables ($\delta^{13}C_{bulk}$, δ^{13}C16:0, δ^{13}C16:1, δ^{13}C18:0, δ^{13}C18:1, δ^{13}C18:2; palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid). The original two-component model (Fig. 4A) achieved a cross-validated correlation coefficient (Q^2 = 0.44), indicating moderate predictive ability, with an accuracy of 0.83 and a correlation coefficient (R^2) of 0.45. Permutation testing ($n = 200$) confirmed that the model was not overfitted, with all permuted R^2 and Q^2 intercepts near zero or negative (2006: $R^2 = -0.0007$, $Q^2 = -0.0725$; 2007: $R^2 = -0.0064$, $Q^2 = -0.0657$; 2008: $R^2 = -0.0013$, $Q^2 = -0.0694$; 2019: $R^2 = 0.0055$, $Q^2 = -0.0502$), indicating predictive performance significantly better than chance. The model's classification ability was further supported by high AUC values for each year (2006: 0.945; 2007: 0.981; 2008: 0.948; 2019: 0.881), demonstrating consistently good to excellent discrimination between olive oils from different production years.

The model explained 56.5% of the variance, with Component 1 accounting for 33.7% of the total variance, primarily separating the 2006 and 2019 samples from those produced in 2007 and 2008, while Component 2, accounting for 22.8% of the total variance, particularly distinguished the 2019 samples from the 2006 samples and the 2007 samples from the 2008 samples. The most relevant variables for the model (VIP > 1, variable importance in projection) were the levels of palmitic acid, δ^{13}C18:2, δ^{13}C16:0, δ^{13}C18:0, $\delta^{13}C_{bulk}$ and oleic acid (Fig. 4B).

Although the score plot showed partial overlap across years, several 2019 samples clustered distinctly from earlier years, likely reflecting the exceptional meteorological conditions of 2019, characterized by the highest annual average, maximum, and minimum temperatures, the hottest days, and the highest annual rainfall in the dataset. In contrast, 2007 recorded the lowest annual rainfall, with more than 133 mm less precipitation, and the lowest relative humidity among the analyzed years. These interannual meteorological differences may have

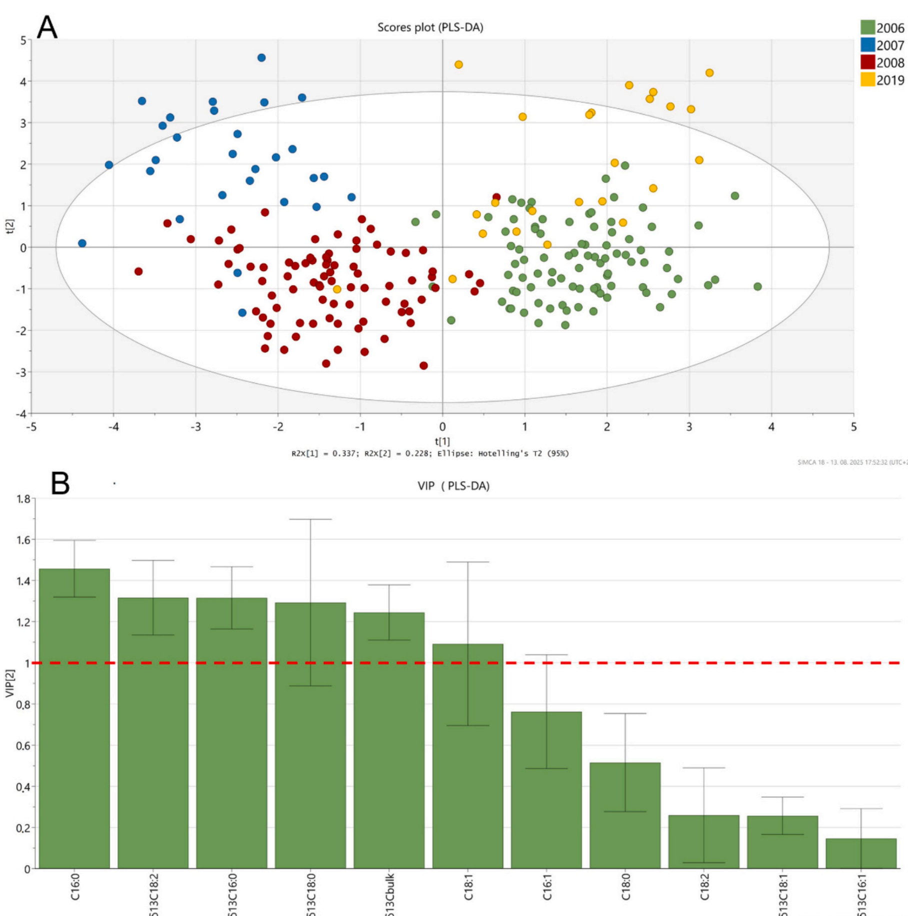

Fig. 4. PLS-DA score plot (A) and VIP values (B) in the pairwise comparisons between different groups of Slovenian extra virgin olive oil (EVOO) from different production years (2006–2008, 2019) derived from fatty acids data and carbon isotopic composition data ($\delta^{13}C_{bulk}$, δ^{13}C16:0: δ^{13}C value of palmitic acid; δ^{13}C16:1: δ^{13}C value of palmitoleic acid; δ^{13}C18:0: δ^{13}C value of stearic acid; δ^{13}C18:1: δ^{13}C value of oleic acid; δ^{13}C18:2: δ^{13}C value of linoleic acid).

contributed to moderate yet measurable shifts in FA composition and carbon-isotopic signatures.

3.2. *Influence of meteorological conditions on the isotopic composition of olive oils of different origins*

3.2.1. *Isotopic composition of bulk olive oil*

Fig. 5 shows the distribution of the δ^{13}C, δ^{2}H and δ^{18}O values determined for bulk EVOOs, grouped by country of production. According to meteorological averages for the study period (1990–2009), the average annual temperature in Cordoba, Spain, was 19 °C, and the average annual precipitation was 674 mm (Orlandi et al., 2014). In the Marche Region of Italy, the average annual temperature was 13.9 °C, and annual precipitation was 930 mm during 1991–2020 (Gentilucci et al., 2019, 2020). For the Istrian region of Croatia (meteorological station Rovinj), the average annual temperature for the period 1991–2010 was 14.1 °C and annual precipitation was 798 mm (Ugarković & Ugarković, 2013). The Montenegrin location of EVOOs was the Adriatic coastal region, characterized by a typical Mediterranean climate, with an average annual temperature of 15–16 °C and total annual precipitation of 1200 mm (Ćulafić et al., 2020).

When comparing data from Slovenia, Croatia, Italy, Spain and Montenegro for 2007, significantly higher δ^{13}C, δ^{2}H, and δ^{18}O values were observed in bulk oils from Spain than from other countries. In addition, statistically significant differences in δ^{13}C values were found between Slovenian samples and those from Italy and Croatia. Interestingly, samples from Croatia, Italy and Montenegro did not show significant differences in δ^{13}C values.

The δ^{18}O values of the Montenegrin samples were significantly different from those of the Italian and Slovenian samples. The δ^{18}O and δ^{2}H values in bulk olive oil were significantly positively correlated ($r = 0.645$), while moderate positive correlations were found between δ^{13}C and both δ^{18}O ($r = 0.481$) and δ^{2}H ($r = 0.508$). Bontempo et al. (2019) suggested that each production area may have site-specific fractionation processes affecting C, H and O isotopes, likely reflecting the combined effects of regional climatic factors such as average temperature, relative humidity and drought stress.

The higher δ^{13}C, δ^{18}O and δ^{2}H values in Spanish oils can be explained by higher temperatures and lower rainfall, which cause evaporative enrichment of leaf water. Conversely, Italy's cooler and wetter conditions resulted in more depleted δ^{18}O values. Average annual temperature showed significant positive correlations with δ^{18}O and δ^{2}H values of bulk oil ($r = 0.814$ and $r = 0.607$, respectively) and moderate correlation with δ^{13}C of bulk ($r = 0.393$) across all five countries.

These results are consistent with previous findings that δ^{18}O and δ^{2}H values in EVOOs reflect both meteorological conditions and geographical gradients in precipitation isotopes (Bontempo et al., 2009; Camin et al., 2010). Furthermore, Lehmann et al. (2018) reported that δ^{18}O in plant organic matter is influenced by the isotopic composition of source water and evapotranspiration intensity.

In Mediterranean climates, rainfall δ^{2}H values are generally more enriched in drier, warmer regions, following global trends in the meteoric water line (Dansgaard, 1964). Accordingly, the enriched δ^{2}H values observed in Spanish EVOOs reflect higher temperatures and reduced rainfall, whereas the more depleted δ^{2}H values in Italian, Croatian and Montenegrin oils correspond to cooler, wetter conditions. The enrichment in ^{13}C, ^{18}O and ^{2}H isotopes in EVOOs produced in drought-prone regions (such as Spain) can be attributed to both meteorological factors (temperature and precipitation) and plant-related parameters (such as stomatal conductance), highlighting a strong genotype-environment interaction. Moreover, during dry Mediterranean summers, trees often reduce stomatal conductance to minimize water loss, thereby lowering photosynthetic rates. These physiological adjustments influence carbon isotope fractionation during CO_2 uptake and fixation, thereby modifying the δ^{13}C composition of organic matter (Portarena et al., 2025). It is noteworthy that, in the present study, δ^{18}O and δ^{2}H exhibited stronger correlations with temperature than δ^{13}C, indicating that these isotopes were more sensitive to climatic conditions in the analyzed samples.

3.2.2. *Isotopic composition of individual fatty acids in olive oil*

As shown in Fig. 6, EVOO samples from Italy exhibited the lowest δ^{13}C values among all five FAs. They were significantly different from the δ^{13}C values of FAs in the Spanish samples, which showed the highest δ^{13}C values for all FAs, except palmitoleic acid. Conversely, lower δ^{13}C values observed in Italian oils suggest more favorable moisture conditions, however, this interpretation should be made with caution, as carbon isotope composition can also be influenced by multiple interacting environmental and physiological factors. The statistical differences observed among some countries highlight the potential of δ^{13}C values to act as markers for determining the geographical origin of EVOOs. In addition, average annual temperature showed a significant moderate correlation with the δ^{13}C values of stearic and oleic acids ($r = 0.468$ and $r = 0.437$, respectively), indicating a possible relationship between meteorological conditions and carbon isotope composition, although the strength of these associations remains limited.

The average annual rainfall showed negative, weak, or negligible correlations with isotopic fingerprints. Spanish samples, characterized by the highest average annual temperature and the lowest rainfall among the analyzed countries, showed the highest δ^{13}C values for FAs. Camin et al. (2010) reported that under warmer, drier conditions, stomatal conductance decreases, leading to higher water-use efficiency and, consequently, to enrichment in ^{13}C. The Mediterranean region is already characterized by plant heat and water stress due to harsh summer weather conditions, including low precipitation, high temperatures, and intense solar radiation.

According to the Intergovernmental Panel on Climate Change

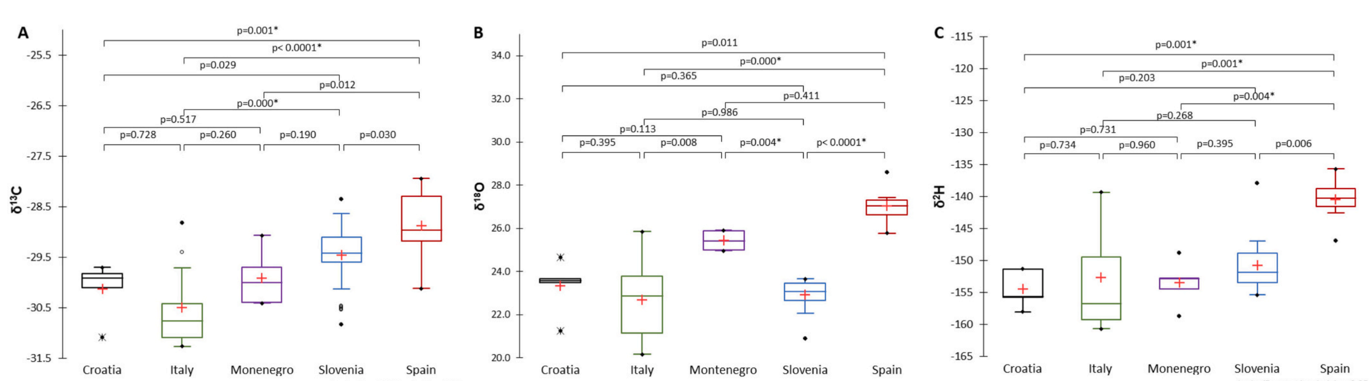

Fig. 5. Box plots of (**A**) δ^{13}C, (**B**) δ^{18}O, (**C**) δ^{2}H values of bulk extra virgin olive oils (EVOOs) produced in 2007, according to country of production (Croatia, Italy, Montenegro, Slovenia, Spain).

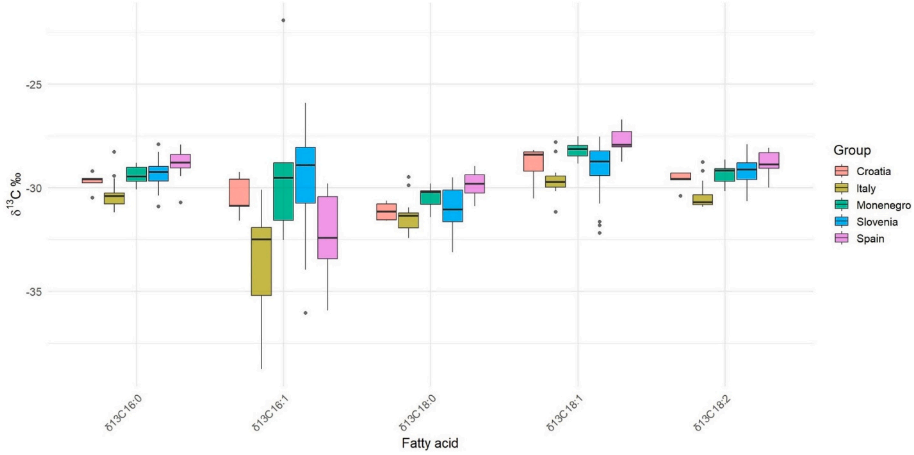

Fig. 6. Box plots of δ^{13}C values determined in fatty acids (δ^{13}C16:0: δ^{13}C value of palmitic acid; δ^{13}C16:1: δ^{13}C value of palmitoleic acid; δ^{13}C18:0: δ^{13}C value of stearic acid; δ^{13}C18:1: δ^{13}C value of oleic acid; δ^{13}C18:2: δ^{13}C value of linoleic acid) from extra virgin olive oil (EVOOs) samples produced in 2007, according to country of production (Croatia, Italy, Montenegro, Slovenia, Spain).

(IPCC), future climate projections indicate a noticeable decrease in annual precipitation and a continued rise in temperatures across the Mediterranean area (IPCC, 2013). For perennial crops such as olive trees, these climatic changes are expected to cause severe adverse effects, particularly on water relations, oxidative pathways, final yield and quality attributes, including oil composition, phenolic content, and sensory characteristics (Fraga et al., 2021).

3.2.3. Principal component analysis (PCA)

Principal Component Analysis (PCA) was performed to visualize the geographical differentiation of EVOO samples from Slovenia, Croatia, Italy, Spain, and Montenegro collected in 2007 (Fig. 7). The variables included the δ^{13}C, δ^{18}O, and δ^{2}H values of bulk oil, and the δ^{13}C values of individual FAs.

The two Principal components together explain 78.5% of the variance. PC1 explained 60.8% of the total variance and is positively influenced by the δ^{13}C values of bulk oil and of stearic, linoleic, palmitic, and oleic acids. In comparison, PC2 explained 17.7% of the total variance and was mainly influenced by the δ^{13}C of palmitoleic acid and the δ^{2}H and δ^{18}O values of bulk oil. A clear separation is visible between Spanish and Italian EVOOs and those from the Balkan macro-region. However, there was poor separation among Slovenian, Croatian, and Montenegrin oils. Notably, Slovenian EVOOs appear to be characterized primarily by the δ^{13}C values of palmitoleic acid. The δ^{2}H and δ^{18}O values of bulk oil, together with compound-specific δ^{13}C, seem to be determinant for Italian and Spanish EVOOs, with Spanish samples showing higher isotopic values of all three light elements.

4. Conclusion

This long-term study demonstrated that temperature significantly affects the FA composition of Slovenian EVOOs, with higher temperatures linked to lower oleic acid and higher linoleic and palmitoleic acid contents. Among the isotopic parameters, δ^{18}O and δ^{2}H in bulk Slovenian oils proved sensitive to climatic variability. Linear regression analysis indicated that δ^{18}O was primarily influenced by the frequency of hot days and relative humidity, while δ^{2}H showed a strong relationship with temperature. EVOOs from Mediterranean regions with lower temperatures and higher precipitation (Italy, Croatia and Montenegro) exhibited lower isotopic ratios for all three light elements, whereas Spanish samples, produced under warmer temperatures with lower rainfall, displayed significantly enriched δ^{13}C, δ^{2}H, and δ^{18}O values. Moreover, the average annual temperature positively correlates withs δ^{18}O and δ^{2}H values across all five countries.

However, several limitations of this study should be acknowledged. Cultivar variability was not fully controlled, which may have contributed to the observed differences in FA and isotopic composition. In addition, isotopic measurements were available for a limited number of

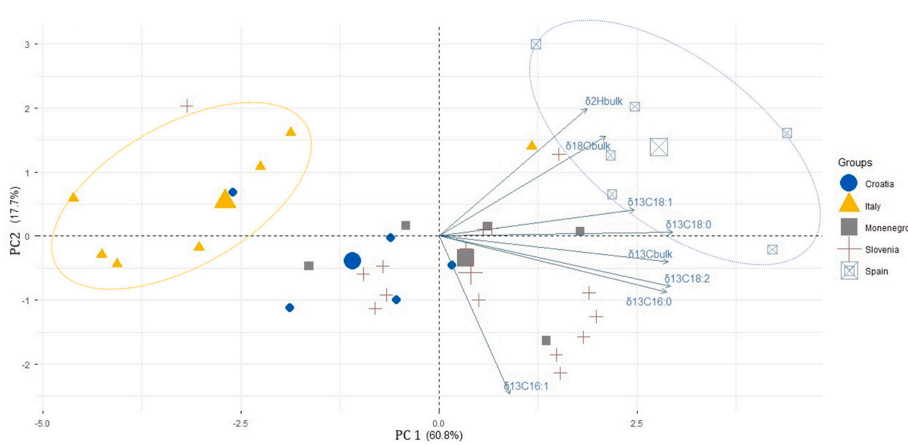

Fig. 7. PCA biplot of geographic separation of EVOOs from five European countries (Italy, Spain, Slovenia, Croatia, Montenegro) harvested the same year (2007). δ^{13}C16:0: δ^{13}C value of palmitic acid; δ^{13}C16:1: δ^{13}C value of palmitoleic acid; δ^{13}C18:0: δ^{13}C value of stearic acid; δ^{13}C18:1: δ^{13}C value of oleic acid; δ^{13}C18:2: δ^{13}C value of linoleic acid.

years, which may restrict the assessment of long-term climatic trends. Finally, the geographic sampling was uneven across countries, which may affect the comparability of regional results. Despite these limitations, the results highlight the strong influence of climate on the isotopic and FA composition of EVOOs and emphasize the need for further studies with broader cultivar representation, and extended time series to improve the robustness of future interpretations.

CRediT authorship contribution statement

Neri Bonciani: Writing – original draft, Investigation, Formal analysis, Data curation. **Marjeta Mencin:** Writing – original draft, Formal analysis, Conceptualization. **Doris Potočnik:** Methodology, Investigation, Data curation. **Claudio Natali:** Writing – review & editing, Supervision, Resources, Conceptualization. **José Manuel Moreno-Rojas:** Writing – review & editing, Investigation, Formal analysis. **Milena Bučar-Miklavčič:** Writing – review & editing, Validation, Methodology, Data curation. **Nives Ogrinc:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Data curation, 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.

Acknowledgments

We acknowledge the financial assistance of the Slovenian Research and Innovation Agency by program P1-0143, P2-0424, infrastructure I0-E006 METROFOOD-SI and HEu project SPECTRA under GA no. 101158453.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.150369.

Data availability

Data will be made available on request.

References

Aramendía, M. A., Marinas, A., Marinas, J. M., Moreno, J. M., Moalem, M., Rallo, L., & Urbano, F. J. (2007). Oxygen-18 measurement of Andalusian olive oils by continuous flow pyrolysis/isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry, 21*(4), 487–496. https://doi.org/10.1002/rcm.2862

ARSO Meteo. (2024). Official weather forecast for Slovenia - National Meteorological Service of the Republic of Slovenia. Retrieved April 10, 2024, from https://meteo.arso.gov.si/met/sl/app/webmet/#webmet==8Sdwx2bhR2cv0WZ0V2bvEGcw9ydlJWblR3LwVnaz9SYtVmYh9iclFGbt9SaulGdugXbsx3cs9mdl5WahxXYyNGapZXZ8tHZv1WYp5mOnMHbvZXZulWYnwCchJXYtVGdlJnOn0UQQdSf.

Beltrán, G., del Rio, C., Sánchez, S., & Martínez, L. (2004). Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. *Journal of Agricultural and Food Chemistry, 52*(11), 3434–3440. https://doi.org/10.1021/jf049894n

Ben Hmida, R., Gargouri, B., Chtourou, F., Abichou, M., Sevim, D., & Bouaziz, M. (2022a). Study on the effect of climate changes on the composition and quality parameters of virgin olive oil "Zalmati" harvested at three consecutive crop seasons: Chemometric discrimination. *ACS Omega, 7*(44), 40078–40090. https://doi.org/10.1021/acsomega.2c04813

Ben Hmida, R., Gargouri, B., Chtourou, F., Sevim, D., & Bouaziz, M. (2022b). Fatty acid and triacyglycerides as markers of virgin olive oil from mediterranean region: Traceability and chemometric authentication. *European Food Research and Technology, 248*(7), 1749–1764. https://doi.org/10.1007/s00217-022-04002-1

Bontempo, L., Camin, F., Larcher, R., Nicolini, G., Perini, M., & Rossmann, A. (2009). Coast and year effect on H, O and C stable isotope ratios of Tyrrhenian and Adriatic Italian olive oils. *Rapid Communications in Mass Spectrometry, 23*(7), 1043–1048. https://doi.org/10.1002/rcm.3968

Bontempo, L., Paolini, M., Franceschi, P., Ziller, L., García-González, D. L., & Camin, F. (2019). Characterization and attempted differentiation of European and extra-European olive oils using stable isotope ratio analysis. *Food Chemistry, 276*, 782–789. https://doi.org/10.1016/j.foodchem.2018.10.077

Bortoluzzi, L., Casal, S., Cruz, R., Peres, A. M., Baptista, P., & Rodrigues, N. (2026). Impact of olive grove altitude on the chemical and sensory characteristics of olive oils from centenarian trees. *European Food Research and Technology, 252*(2), Article 59. https://doi.org/10.1007/s00217-025-04940-6

Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M., & Prohaska, T. (2014). Assessment of international reference materials for isotope-ratio analysis (IUPAC technical report). *Pure and Applied Chemistry, 86*(3), 425–467. https://doi.org/10.1515/pac-2013-1023

Camin, F., Larcher, R., Nicolini, G., Bontempo, L., Bertoldi, D., Perini, M., Schlicht, C., Schellenberg, A., Thomas, F., Heinrich, K., Voerkelius, S., Horacek, M., Ueckermann, H., Froeschl, H., Wimmer, B., Heiss, G., Baxter, M., Rossmann, A., & Hoogewerff, J. (2010). Isotopic and elemental data for tracing the origin of European olive oils. *Journal of Agricultural and Food Chemistry, 58*(1), 570–577. https://doi.org/10.1021/jf902814s

Commission Regulation. (2013). Regulation (EU) no 1308/2013 of the European Parliament and of the council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing council regulations (EEC) no 922/72, (EEC) no 234/79, (EC) no 1037/2001 and (EC) no 1234/2007. Official journal of the European Union. Retrieved October 31, 2025, from https://eur-lex.europa.eu/eli/reg/2013/1308/oj/eng.

Commission Regulation. (2022a). Commission delegated regulation (EU) 2022/2104 of 29 July 2022 supplementing regulation (EU) no 1308/2013 of the European Parliament and of the council as regards marketing standards for olive oil, and repealing commission regulation (EEC) no 2568/91 and commission implementing regulation (EU) no 29/2012. Official Journal of the European Union, L284, 1–22. Retrieved June 24, 2025, from https://data.europa.eu/eli/reg_del/2022/2104/oj.

Commission Regulation. (2022b). Commission implementing regulation (EU) 2022/2105 of 29 July 2022 laying down rules on conformity checks of marketing standards for olive oil and methods of analysis of the characteristics of olive oil. *Official Journal of the European Union, L284*, 23–48. Retrieved June 24, 2025, from https://data.europa.eu/eli/reg_impl/2022/2105/oj.

Contreras, C., Pierantozzi, P., Maestri, D., Tivani, M., Searles, P., Brizuela, M., … Torres, M. (2023). How temperatures may affect the synthesis of fatty acids during olive fruit ripening: Genes at work in the field. *Plants, 12*(1), 54. https://doi.org/10.3390/plants12010054

Ćulafić, G., Popov, T., Gnjato, S., Bajić, D., Trbić, G., & Mitrović, L. (2020). Spatial and temporal patterns of precipitation in Montenegro. *Időjárás, 124*(4), 499–519. https://doi.org/10.28974/idojaras.2020.4.5

Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus, 16*(4), 436–468. https://doi.org/10.1111/j.2153-3490.1964.tb00181.x

El Qarnifa, S., El Antari, A., & Hafidi, A. (2019). Effect of maturity and environmental conditions on chemical composition of olive oils of introduced cultivars in Morocco. *Journal of Food Quality, 2019*(1), 1854539. https://doi.org/10.1155/2019/1854539

Everstine, K. D., Chin, H. B., Lopes, F. A., & Moore, J. C. (2024). Database of food fraud records: Summary of data from 1980 to 2022. *Journal of Food Protection, 87*(3), Article 100227. https://doi.org/10.1016/j.jfp.2024.100227

Faberi, A., Marianella, R. M., Fuselli, F., La Mantia, A., Ciardiello, F., Montesano, C., … Compagnone, D. (2014). Fatty acid composition and $\delta^{13}C$ of bulk and individual fatty acids as marker for authenticating Italian PDO/PGI extra virgin olive oils by means of isotopic ratio mass spectrometry. *Journal of Mass Spectrometry, 49*(9), 840–849. https://doi.org/10.1002/jms.3399

Fraga, H., Moriondo, M., Leolini, L., & Santos, J. A. (2021). Mediterranean olive orchards under climate change: A review of future impacts and adaptation strategies. *Agronomy, 11*(1). https://doi.org/10.3390/agronomy11010056

García-Inza, G. P., Castro, D. N., Hall, A. J., & Rousseaux, M. C. (2014). Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid composition in olive (*Olea europaea* L. var. '*Arauco*'). *European Journal of Agronomy, 54*, 107–115. https://doi.org/10.1016/j.eja.2013.12.005

García-Inza, G. P., Hernández, M. L., Miserere, A., Sicardo, M. D., Martínez-Rivas, J. M., & Rousseaux, M. C. (2024). Transcriptional regulation of oil synthesis and fatty acid desaturation in olive fruit under elevated growth temperature. *Physiologia Plantarum, 176*(1), Article e14149. https://doi.org/10.1111/ppl.14149

Gentilucci, M., Barbieri, M., Lee, H. S., & Zardi, D. (2019). Analysis of rainfall trends and extreme precipitation in the middle Adriatic side, Marche region (Central Italy). *Water, 11*(9), 1948. https://doi.org/10.3390/w11091948

Gentilucci, M., Materazzi, M., Pambianchi, G., Burt, P., & Guerriero, G. (2020). Temperature variations in Central Italy (Marche region) and effects on wine grape production. *Theoretical and Applied Climatology, 140*(1), 303–312. https://doi.org/10.1007/s00704-020-03089-4

Iacumin, P., Bernini, L., & Boschetti, T. (2009). Climatic factors influencing the isotope composition of Italian olive oils and geographic characterization. *Rapid Communications in Mass Spectrometry, 23*(3), 448–454. https://doi.org/10.1002/rcm.3896

IOC. (2017). *Determination of fatty acid methyl esters by gas chromatography (Vol. COI/T.20/Doc. No. 33/Rev. 1)*. International Olive Council. Retrieved June 24, 2025, from https://www.internationaloliveoil.org/wp-content/uploads/2023/11/COI-T20-33-rev-1-ENG.pdf.

IOC. (2022). *IOC standards, methods and guides*. International Olive Council. Retrieved September 3, 2025, from https://www.internationaloliveoil.org/what-we-do/chemistry-standardisation-unit/standards-and-methods/.

IOC. (2025). *Trade standard applying to olive oils and olive pomace oils (COI/T.15/NC No 3/Rev.21)*. International Olive Oil Council, 16. Retrieved February 2, 2026, from http s://www.internationaloliveoil.org/wp-content/uploads/2025/07/TRADE-STANDARD-REV-21_EN.pdf.

IPCC. (2013). Climate change 2013: The physical science basis. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, … P. M. Midgley (Eds.), *Contribution of working group i to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

IPCC. (2021). Climate Change 2021: The physical science basis. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, … B. Zhou (Eds.), *Contribution of working group i to the sixth assessment report of the intergovernmental panel on climate change* (p. 2391). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press. https://doi.org/10.1017/9781009157896.

Lechhab, T., Lechhab, W., Cacciola, F., & Salmoun, F. (2022). Sets of internal and external factors influencing olive oil (*Olea europaea* L.) composition: A review. *European Food Research and Technology, 248*(4), 1069–1088. https://doi.org/10.1007/s00217-021-03947-z

Lehmann, M. M., Goldsmith, G. R., Schmid, L., Gessler, A., Saurer, M., & Siegwolf, R. T. W. (2018). The effect of ^{18}O-labelled water vapour on the oxygen isotope ratio of water and assimilates in plants at high humidity. *New Phytologist, 217*(1), 105–116. https://doi.org/10.1111/nph.14788

Massaccesi, L., Cucina, M., Marinotti, A., Domesi, A., Orrico, E., Bozza, T., Fiorucci, F., Rossi, M., Cossignani, L., & Bufacchi, M. (2026). Climatic implication in bioactive compounds and fatty acids profile of olive oil derived from new olive (*Olea europaea* L.) genotypes in Central Italy. *Discover. Environment, 4*(1), 184. https://doi.org/10.1007/s44274-026-00709-0

Mikrou, T., Litsa, M., Papantoni, A., Kapsokefalou, M., Gardeli, C., & Mallouchos, A. (2023). Effect of cultivar and geographical origin on the volatile composition of Greek monovarietal extra virgin olive oils. *Chemosensors, 11*(2), 80. https://doi.org/10.3390/chemosensors11020080

Orlandi, F., Garcia-Mozo, H., Dhiab, A. B., Galán, C., Msallem, M., & Fornaciari, M. (2014). Olive tree phenology and climate variations in the Mediterranean area over the last two decades. *Theoretical and Applied Climatology, 115*(1), 207–218. https://doi.org/10.1007/s00704-013-0892-2

Paolini, M., Bontempo, L., & Camin, F. (2017). Compound-specific δ^{13}C and δ^{2}H analysis of olive oil fatty acids. *Talanta, 174*, 38–43. https://doi.org/10.1016/j.talanta.2017.05.080

Portarena, S., Gavrichkova, O., Lauteri, M., & Brugnoli, E. (2014). Authentication and traceability of Italian extra-virgin olive oils by means of stable isotopes techniques. *Food Chemistry, 164*, 12–16. https://doi.org/10.1016/j.foodchem.2014.04.115

Portarena, S., Leonardi, L., Scartazza, A., Lauteri, M., Baldacchini, C., Farinelli, D., Famiani, F., Ciolfi, M., & Brugnoli, E. (2019). Combining analysis of fatty acid composition and δ^{13}C in extra-virgin olive oils as affected by harvest period and cultivar: Possible use in traceability studies. *Food Control, 105*, 151–158. https://doi.org/10.1016/j.foodcont.2019.05.029

Portarena, S., Saurer, M., Brugnoli, E., Farinelli, D., & Cherubini, P. (2025). Drought response strategies of arbequina, Arbosana and Koroneiki olive cultivars revealed by ring growth, wood anatomy and δ^{13}C dynamics. *Frontiers in Plant Science, 16*, Article 1623127. https://doi.org/10.3389/fpls.2025.1623127

Song, X., Lorrey, A., & Barbour, M. M. (2022). Environmental, physiological and biochemical processes determining the oxygen isotope ratio of tree-ring cellulose. In R. T. W. Siegwolf, J. R. Brooks, J. Roden, & M. Saurer (Eds.), *Stable isotopes in tree rings: Inferring physiological, climatic and environmental responses* (pp. 311–329). Springer International Publishing. https://doi.org/10.1007/978-3-030-92698-4_10.

Spangenberg, J. E., & Ogrinc, N. (2001). Authentication of vegetable oils by bulk and molecular carbon isotope analyses with emphasis on olive oil and pumpkin seed oil. *Journal of Agricultural and Food Chemistry, 49*(3), 1534–1540. https://doi.org/10.1021/jf001291y

Ugarković, D., & Ugarković, N. K. (2013). Changes and trends of climate elements and indices in the region of Mediterranean Croatia. *Journal of Central European Agriculture, 14*(1), 236–249. https://doi.org/10.5513/JCEA01/14.1.1189

Yamani, M. E., Sakar, E. H., Boussakouran, A., & Rharrabti, Y. (2020). Influence of ripening index and water regime on the yield and quality of "Moroccan picholine" virgin olive oil. *OCL, 27*, Article 19. https://doi.org/10.1051/ocl/2020015

Zhang, Y., Zhang, C., Xu, C., Deng, Y., Wen, B., Xie, P., & Huang, L. (2022). Effect of geographical location and soil fertility on main phenolic compounds and fatty acids compositions of virgin olive oil from *Leccino* cultivar in China. *Food Research International, 157*, Article 111207. https://doi.org/10.1016/j.foodres.2022.111207

Zhao, L., Liu, X., Wang, N., Barbeta, A., Zhang, Y., Cernusak, L. A., & Wang, L. (2024). The determining factors of hydrogen isotope offsets between plants and their source waters. *New Phytologist, 241*(5), 2009–2024. https://doi.org/10.1111/nph.19492

Zuil, S. G., Izquierdo, N. G., Luján, J., Cantarero, M., & Aguirrezábal, L. A. N. (2012). Oil quality of maize and soybean genotypes with increased oleic acid percentage as affected by intercepted solar radiation and temperature. *Field Crops Research, 127*, 203–214. https://doi.org/10.1016/j.fcr.2011.11.019