LWT - Food Science and Technology 237 (2025) 118750

Contents lists available at ScienceDirect

LWT

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

Integrative chemometrics, multivariate, and artificial neural network-based modeling of fruit quality, fatty acid composition, and biochemical traits in olive (*Olea europaea* L.) cultivars

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ARTICLE INFO

Keywords:
Antioxidant capacity
Cold-pressed oil
Health-promoting lipids
Oxidative stability
Phenolic profile
Virgin olive oil

ABSTRACT

Olive (*Olea europaea* L.) oil quality is intricately influenced by the biochemical composition, fatty acid profile, and pomological traits of the cultivar. Given the increasing consumer demand for functional foods and health-promoting dietary fats, the comprehensive evaluation of these traits using multivariate and chemometric approaches is crucial. Traditional analyses often fall short of revealing the complex interactions between phenolic compounds, fatty acid composition, and oxidative stability. Therefore, integrative methods such as multivariate analyses and artificial neural network (ANN)-based modeling offer powerful tools for optimizing varietal selection aligned with nutritional value and oil quality. In this study, 22 olive cultivars were assessed under uniform agroecological conditions for fruit weight (2.15–6.55 g), oil content (16.23–30.92 %), total phenolic content (125.00–284.83 mg CAE/kg), and antioxidant activity. The dominant fatty acids included oleic acid (67.20–77.50 %), palmitic acid (9.20–15.55 %), and linoleic acid (7.20–13.50 %). Substantial genotypic variation was observed in UFAs/SFAs ratios (4.36–8.00) and oxidative stability indicators such as peroxide value and free fatty acid content. PCA revealed that the first two principal components explained 60.68 % of the total variance, primarily driven by oleic acid, total phenolics, and oil content. Hierarchical cluster analysis (HCA) grouped cultivars into four main clusters with distinct compositional traits, while correlation matrix analysis (CMA) indicated a strong positive correlation between oleic acid and total phenolic content ($r = 0.88^{**}$). Multiple regression analysis (MRA) further identified oleic acid and free fatty acids as significant predictors ($p \leq 0.01$) of overall oil quality. In parallel, an ANN model based on 10 input traits achieved a prediction accuracy of 92.3 % during validation, with an R^2 of 0.91 and a mean squared error (MSE) of 0.024. Notably, cultivars such as 'Savrani', 'Memecik', 'Memeli', 'Halhalı', and 'Ayvalık' demonstrated superior profiles with high oleic acid, phenolic content, and favorable unsaturation indices. The integration of chemometric techniques and ANN-based modeling provided a comprehensive framework for elucidating trait interrelationships and predicting olive oil quality. The study highlights key biochemical markers and compositional traits as reliable predictors of nutritional and oxidative stability. The identification of superior cultivars supports evidence-based selection strategies for high-value olive oil production with enhanced health benefits. These findings not only contribute to precision breeding and functional food development but also address growing public health concerns regarding dietary fat quality.

1. Introduction

The olive tree (*Olea europaea* L.) is one of the most ancient and symbolically significant fruit crops in the Mediterranean Basin, where its cultivation has shaped cultural, economic, and ecological landscapes for millennia (Avramidou et al., 2024). Beyond its long-standing heritage,

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https://doi.org/10.1016/j.lwt.2025.118750
Received 11 August 2025; Received in revised form 9 November 2025; Accepted 11 November 2025
Available online 11 November 2025
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the olive fruit and its derivative—virgin olive oil—are renowned for their distinctive sensory attributes and remarkable nutritional properties, making them central components of the Mediterranean diet (Estruch et al., 2018). In recent decades, a growing body of evidence has underscored the multifaceted health benefits associated with olive oil consumption, including its protective effects against cardiovascular diseases (Covas et al., 2009), metabolic syndromes, neurodegenerative conditions (Rigacci & Stefani, 2016), and certain forms of cancer (Fabiani, 2016). These effects are primarily attributed to the oil's unique biochemical profile, particularly its high content of monounsaturated fatty acids (MUFAs), polyphenols, and antioxidant compounds (Mititelu et al., 2024; Visioli & Bernardini, 2011).

Among these constituents, oleic acid (C18:1) plays a pivotal role as the dominant MUFA, offering superior oxidative stability and contributing to favorable lipid profiles in human blood by reducing LDL-cholesterol and increasing HDL-cholesterol levels (Peña-Vázquez et al., 2024; Schwingshackl & Hoffmann, 2012). Polyunsaturated fatty acids (PUFAs), such as linoleic (C18:2) and linolenic acids (C18:3), although present in lower quantities, are essential for human health but may compromise oil stability when present in excess due to their susceptibility to oxidation (Frankel, 2011). Similarly, the saturated fatty acid fraction—chiefly palmitic (C16:0) and stearic acids (C18:0)—influences the nutritional balance and metabolic implications of olive oil. While stearic acid is metabolically neutral, excessive palmitic acid intake has been associated with increased cardiovascular risk (Boskou, 2006). Concurrently, olive oil is an exceptional source of bioactive phenolic compounds, such as hydroxytyrosol, tyrosol, and oleuropein, whose antioxidant, anti-inflammatory, and antimicrobial activities have been extensively documented (Karković Marković et al., 2019). These components work synergistically to reduce oxidative stress, modulate inflammatory responses, and inhibit lipid peroxidation, thereby enhancing both health benefits and shelf-life properties.

Given this nutritional relevance, the selection and characterization of olive cultivars based on their fruit quality, fatty acid composition, and biochemical traits have gained considerable importance (Comlekcioglu et al., 2022; Guo et al., 2018). Cultivar-specific differences in these traits are often shaped by genotypic factors, environmental influences, and agricultural practices, necessitating integrative evaluation approaches to unravel the complex interactions among them. While previous studies have explored phenotypic diversity or compositional attributes in isolation, few have holistically assessed their interdependence using advanced statistical and predictive tools (Gumus et al., 2018; Sakar et al., 2022). Moreover, the application of chemometric and artificial intelligence techniques, such as principal component analysis (PCA), multiple regression analysis (MRA), and artificial neural networks (ANNs), offers powerful frameworks to model trait interrelationships and predict oil quality outcomes with greater precision (Katritzky et al., 2010).

From a public health perspective, such multidimensional evaluations are not merely of academic interest but are instrumental in guiding breeding programs and industrial practices aimed at producing functionally enriched olive oils (Laroussi-Mezghani et al., 2015). Identifying cultivars with optimal levels of beneficial fatty acids, antioxidant capacity, and favorable oil indices can significantly contribute to nutritional security, especially in light of increasing consumer awareness and global demand for health-promoting dietary fats (Matías et al., 2024).

In this context, the present study aimed to conduct an integrative multivariate analysis of fruit quality parameters, biochemical traits, and fatty acid composition across 22 olive cultivars grown under uniform agroecological conditions. By combining chemometric analyses with an ANN-based predictive model, we sought to identify cultivars with superior compositional profiles, elucidate trait interdependencies, and propose a decision-making framework for functional cultivar selection targeting both oil quality and human health benefits.

2. Materials and methods

2.1. Plant material and research region

The study was conducted during the 2021, 2022, and 2023 growing seasons in the "Olive Collection Parcel" located in the Antakya district of Hatay province (36°19′56"N, 36°11′38"E), under the jurisdiction of the Faculty of Agriculture, Hatay Mustafa Kemal University, Türkiye (Fig. 1). The experimental orchard is at an altitude of approximately 80–100 m above sea level. The region is characterized by alluvial soils, which are generally deep, well-drained, and rich in minerals, making them suitable for olive cultivation. According to the United States Department of Agriculture (USDA) soil taxonomy (Soil Survey Staff, 1999; 2022), these soils predominantly fall under the Typic Xerofluvents subgroup. The Antakya region exhibits a typical Mediterranean climate, with hot and dry summers and mild, rainy winters. The mean annual temperature, recorded between 2021 and 2023, ranged from 18.2 (2022) to 19 °C (2023) (Supplementary Table S1), while the annual rainfall averaged between 57.4 (2021) and 78 mm (2023) (Supplementary Table S2), with precipitation mainly concentrated in the winter months. This combination of climatic and edaphic conditions supports the optimal development of olive trees and contributes significantly to the expression of cultivar-specific traits. All climate data were obtained from the Turkish State Meteorological Service (TSMS, 2025). In this study, a total of 22 olive cultivars were evaluated, including 'Arbequina', 'Ascolana', 'Ayvalık', 'Domat', 'Edincik Su', 'Gemlik', 'Halhalı', 'Hojiblanca', 'İzmir Sofralık', 'Kilis Yağlık', 'Manzanilla de Sevilla', 'Memecik', 'Memeli', 'Meski', 'Nizip Yağlık', 'Samanlı', 'Sarı Haşebi', 'Sarı Ulak', 'Sarı Yaprak', 'Savrani', 'Silifke Yağlık', and 'Uslu'. All cultivars were 18 years old, grown on their roots, and maintained under uniform orchard conditions. The trees were spaced at 5 × 5 m. Standard cultural practices—such as regular irrigation, fertilization, pruning, soil management, and integrated pest and disease control—were consistently applied to preserve tree health and ensure the reliability of phenotypic and biochemical evaluations.

2.2. Fruit quality assessment

Fruit quality characteristics were evaluated using a total of three replications for each olive cultivar. In each replication, fifty randomly selected fruits were subjected to measurement and analysis in order to ensure statistical robustness and reliable representation of each cultivar. Fruit weight was determined individually using a precision digital balance (Epilons Diamond SF-400D, İstanbul, Türkiye). The length and width of each fruit were measured with a digital caliper (INSIZE 1108-150, Suzhou, China; accuracy ±0.01 mm), by recording the longitudinal and equatorial dimensions, respectively. Based on these measurements, fruit shape was assessed by calculating the ratio of fruit length to fruit width (L/W), and cultivars were categorized accordingly. To determine the fruit flesh ratio, the mesocarp (flesh) was manually separated from the endocarp (stone), and the flesh weight was expressed as a percentage of the total fruit weight. All measurements were performed under controlled laboratory conditions and at room temperature, to minimize potential variations caused by environmental factors. The average values obtained from the three replications per cultivar were used for subsequent statistical analyses aimed at identifying inter-cultivar differences in fruit quality traits.

This methodology is consistent with earlier and more recent studies that have employed precise measurement tools in olive fruit research. For example, Mirzabe et al. (2013) evaluated the physical properties of virgin olive fruits using digital calipers with a sensitivity of ±0.01 mm, emphasizing the importance of standardized and accurate measurements in olive characterization studies. Building on this approach, Ponce et al. (2018) applied image analysis techniques to estimate olive fruit mass and size, achieving relative errors as low as 0.78 %. Their findings further highlighted the critical role of precision instruments in

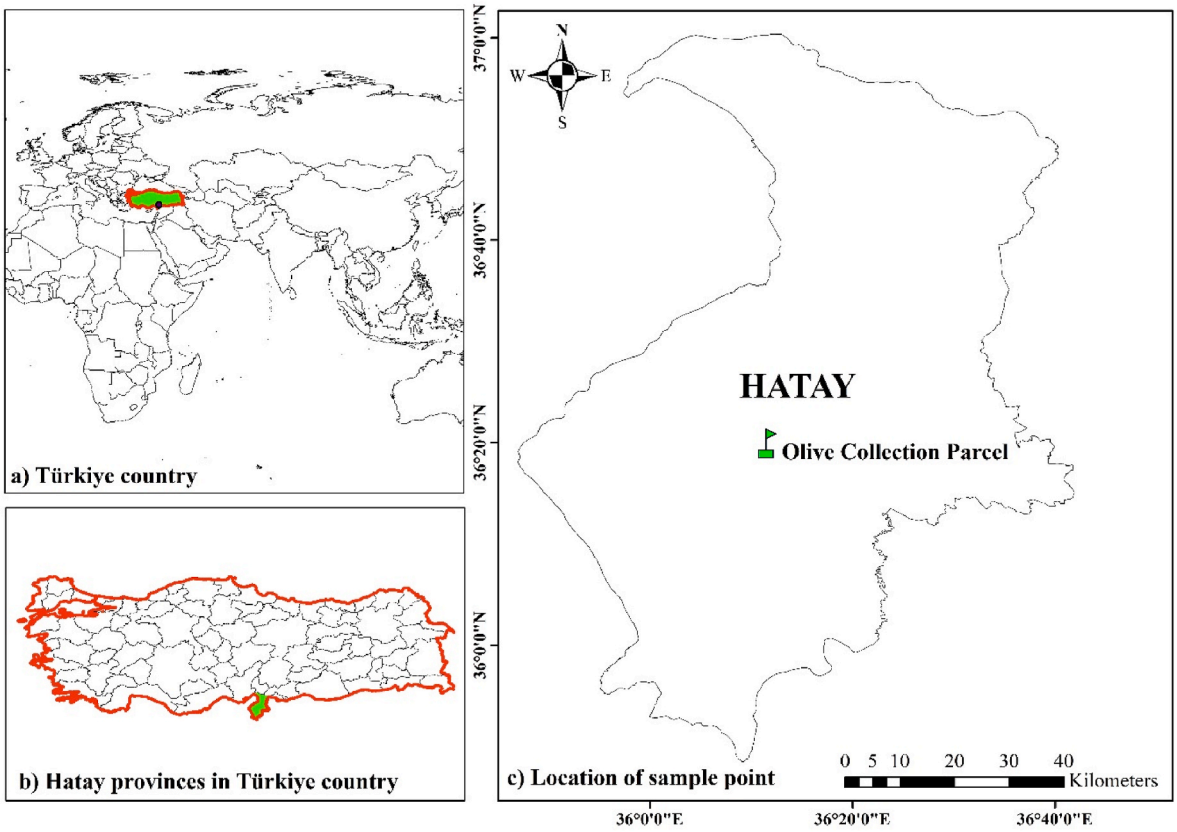


Fig. 1. Geographic location of the collection site of the studied cultivars.

enhancing the accuracy of fruit assessment, particularly in the context of developing automated systems for olive processing.

2.3. Biochemical properties and oil quality analysis

2.3.1. Sample preparation

For the biochemical analyses, olive oil was extracted from the fruit samples using a cold-press method. The fruits were manually pitted, and the mesocarp was homogenized using a stainless steel laboratory blender (Waring Commercial, Model 7011HS, Torrington, CT, USA) under cold conditions to avoid thermal degradation of bioactive compounds. The homogenate was then centrifuged at 4000 rpm for 20 min at 4 °C using a refrigerated centrifuge (Elektromag M 4808 PR, İstanbul, Türkiye) to facilitate oil separation. The extracted oil was filtered through Whatman No. 1 filter paper (GE Healthcare Life Sciences, Little Chalfont, UK) to remove any remaining solid residues. Finally, the oil samples were transferred into amber glass bottles and stored at 4 ± 1 °C in the dark to prevent oxidation and degradation of sensitive compounds until analysis.

This sample preparation procedure was also employed for the fatty acid composition analysis, ensuring consistency in oil extraction across all analytical procedures.

2.3.2. Total phenolic compounds (TPC)

The total phenolic content in olive oil was determined using the Folin-Ciocalteu colorimetric method. Before analysis, 5 g of olive oil was extracted with 10 mL of a methanol-water mixture (80:20, v/v) under vortex agitation to isolate the phenolic compounds. The resulting mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was collected for analysis. An aliquot of 0.5 mL of the extract was mixed with 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent and 2 mL of 7.5 % (w/v) sodium carbonate solution. The reaction mixture was incubated in the dark at room temperature for 60 min to allow complete color development. Absorbance was measured at 765 nm using a Shimadzu UV-1800 UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The results were expressed as milligrams of caffeic acid equivalents (CAE) per kilogram of oil (mg CAE/kg). A standard calibration curve was constructed using caffeic acid solutions in the range of 0–500 mg/L. This method is based on the color change resulting from the reducing capacity of phenolic compounds and is widely recognized for its reliability in determining the antioxidant capacity of olive oils (Grossi et al., 2023; Singleton et al., 1999).

2.3.3. Free fatty acids

The free acidity, expressed as a percentage of oleic acid (OA), was determined using the official titration method as outlined by the International Olive Council (IOC) in the COI/T.20/Doc. No 34/Rev.1/2017 (IOC, 2017a). This method involves dissolving the oil sample in a solvent mixture of ethanol and diethyl ether, followed by titration with a standardized potassium hydroxide (KOH) solution using phenolphthalein as an indicator. The endpoint is identified by a persistent pink coloration, indicating neutralization of the free fatty acids. Before titration, oil samples were brought to room temperature (22 ± 1 °C) to avoid temperature-related fluctuations in acid-base reaction kinetics. All reagents used were of analytical grade and freshly prepared to ensure consistency in endpoint detection. The titration was performed using a Mettler Toledo DL50 Titrator (Mettler Toledo DL50, Greifensee, Switzerland), equipped with an automatic burette and pH-sensitive electrode, ensuring precise delivery of titrant and real-time monitoring of pH changes.

2.3.4. Oil content

Oil content was determined using Near-Infrared (NIR) spectroscopy, a non-destructive, rapid, and reliable analytical technique widely employed for quality assessment in olive breeding and postharvest research. Measurements were performed using a FOSS NIRSystems 5000

scanning monochromator (FOSS Analytical A/S, Hillerød, Denmark), operating within the wavelength range of 1100–2500 nm and equipped with an integrating sphere and rotating quartz sample cup designed for whole-fruit analysis. All measurements were conducted under standardized ambient conditions (22 ± 1 °C and relative humidity 50–60 %) to avoid spectral interference caused by temperature or humidity fluctuations. The system was pre-calibrated using a robust prediction model developed from reference samples analyzed via the Soxhlet method (ISO 659:2009) (ISO, 2009), which is considered the gold standard for oil content determination. Calibration accuracy and model performance were regularly verified using internal validation sets and certified olive fruit standards to ensure predictive reliability throughout the analysis. This approach enables high-throughput screening of oil content by quantifying overtone and combination bands of C–H, O–H, and N–H bonds associated with lipid absorption in the near-infrared spectrum. The oil content was expressed as a percentage (%) of the fresh fruit weight. This procedure was conducted following the method described by Cayuela et al. (2009), who demonstrated the effectiveness of NIR spectroscopy for the rapid evaluation of oil content in diverse olive cultivars with high prediction accuracy ($R^2 > 0.90$). This method allows for the estimation of oil content in intact olive fruits by analyzing the absorbance spectra in the NIR region, providing accurate results without the need for extensive sample preparation.

2.3.5. Peroxide value

The peroxide value, indicating the extent of primary oxidation, was measured according to the iodometric titration method specified in COI/T.20/Doc. No 35/Rev.1/2017 by the IOC (IOC, 2017b). In this procedure, the oil sample reacts with a mixture of acetic acid and chloroform, followed by the addition of potassium iodide. The reaction is carried out under light-protected conditions to prevent photooxidation of iodine. After a defined incubation period (1 min), the liberated iodine is titrated with 0.01 N sodium thiosulfate solution using starch as an endpoint indicator. The volume of thiosulfate consumed corresponds to the amount of peroxides present in the sample, and the results are expressed in milliequivalents of active oxygen per kilogram of oil (meq O_2/kg). The titration was conducted using a Mettler Toledo DL50 Titrator (Mettler Toledo DL50, Greifensee, Switzerland), which ensures precise titrant delivery and real-time monitoring, providing reliable and reproducible results.

All analyses were performed in triplicate, with three independent oil samples per cultivar, and each replicate was analyzed separately for all parameters to ensure accuracy and reproducibility.

2.4. Fatty acid composition

2.4.1. Preparation of fatty acid methyl esters (FAMEs)

The preparation of FAMEs was conducted following the International Olive Council (IOC) official method COI/T.20/Doc. No 33/Rev.1/2017 (IOC, 2017c). Approximately 0.1 g of olive oil was placed into a test tube, to which 2 mL of a 0.5 N methanolic potassium hydroxide (KOH) solution was added. The mixture was vortexed for 30 s using a Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA) set at 3200 rpm to ensure complete mixing. Subsequently, 2 mL of n-heptane (Sigma-Aldrich, USA) was added, and the solution was vortexed again for 30 s under the same conditions. The mixture was then allowed to stand until phase separation occurred. The upper n-heptane layer containing the FAMEs was carefully collected and transferred into a GC vial for analysis.

2.4.2. Gas chromatography analysis

The FAMEs were analyzed using a gas chromatograph equipped with a flame ionization detector (GC-FID). The analysis was performed on a SCION 8500 GC system (SCION 8500, Gorinchem, Netherlands) equipped with a split/splitless injector and an 8400PRO autosampler (SCION Instruments, 2023). A SCION-FAME capillary column (100 m × 0.25 mm ID × 0.2 μm film thickness) was used for the separation of FAMEs. The carrier gas was high-purity hydrogen (99.999 %), with a constant flow rate of 1.5 mL/min. The injector temperature was set at 250 °C, and the detector temperature was maintained at 260 °C. The oven temperature program was as follows: initial temperature at 100 °C, held for 5 min; increased to 240 °C at a rate of 4 °C/min, and held for 15 min. The injection volume was 1 μL in split mode with a split ratio of 1:50.

2.4.3. Identification and quantification

Individual FAMEs were identified by comparing their retention times with those of a standard FAME mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, USA). Quantification was achieved by calculating the relative percentage of each fatty acid based on the area normalization method, as recommended by the IOC (IOC, 2017c). All analyses were performed in three independent replications per cultivar, and in each replication, one FAME extract was prepared and analyzed three times on the GC-FID, resulting in a total of nine analytical readings per cultivar to ensure accuracy, repeatability, and statistical robustness. As part of the fatty acid composition analysis, the following fatty acids were detected and quantified: palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), and gadoleic acid (C20:1).

2.5. Statistical analysis

The study was conducted over three consecutive years: 2021, 2022, and 2023. A randomized complete block design (RCBD) with three replications per cultivar was employed to minimize environmental variability. To improve the robustness of the results and reduce the impact of year-to-year variability, statistical analyses were performed based on the average values across all years. All statistical procedures were carried out using JMP® Pro 17 software (SAS Institute Inc., Cary, NC, USA) (JMP®, 2024). Group comparisons were evaluated using Tukey's Honestly Significant Difference (HSD) test at a 5 % significance level ($p < 0.05$), and all results were reported as mean ± standard deviation.

For multivariate assessments, correlation matrix analysis (CMA), principal component analysis (PCA), and heat map analysis (HMA) were performed using Origin Pro® 2025b software (OriginLab®, 2024). The Pearson correlation coefficient (r) was used to assess the strength and direction of associations between traits. PCA was conducted using the Varimax rotation method with Kaiser normalization to improve the interpretability of component loadings. A biplot based on the first two principal components (PC1 and PC2) was generated to visualize the distribution of cultivars concerning their traits. Heat map clustering, based on Ward's method and Euclidean distance metrics, was used to group both genotypes and variables, offering a detailed graphical representation of their relationships.

Furthermore, multiple regression analysis (MRA) was applied to determine the variables influencing key leaf parameters, with selected leaf traits defined as dependent variables. The MRA was executed using the "stepwise" approach under the "linear regression" module of SPSS® software (SPSS Inc., Chicago, IL, USA) (Efe et al., 2000; Norusis, 1998; SPSS, 2009). The integration of these multivariate statistical techniques provided valuable insights into the complex interactions among traits, supporting a more precise classification and characterization of the olive cultivars under investigation.

2.6. Artificial neural network (ANN) modeling

To further explore the complex relationships between key pomological, biochemical, and fatty acid traits and the resulting olive oil quality, an ANN model was constructed using a feed-forward multilayer architecture (Basheer & Hajmeer, 2000; Khaki & Wang, 2019; Kuan, 2018). The model was developed based on ten input variables selected

according to their biological relevance, statistical significance, and contribution to olive oil quality parameters observed in the current study.

The ANN architecture consisted of four sequential layers: (i) an input layer comprising 10 neurons representing the selected traits — fruit weight, fruit flesh ratio, oleic acid, linoleic acid, total phenolic compounds, free fatty acids, peroxide value, UFAs/SFAs ratio, oil content, and palmitic acid; (ii) a pattern layer with 5 hidden neurons that capture latent nonlinear interactions; (iii) a summation layer with 3 neurons to integrate the processed signals; and (iv) an output layer with a single neuron representing the predicted olive oil quality score.

Each neuron in a given layer was fully connected to all neurons in the subsequent layer using weight coefficients optimized through supervised learning (Cuomo et al., 2022). The model training was performed using the backpropagation algorithm under a sigmoid activation function, and the learning rate was empirically adjusted to minimize mean squared error (MSE) between predicted and observed quality scores. Before training, all input variables were normalized between 0 and 1 using min-max scaling to ensure comparability across different units.

This modeling approach provides a robust framework for capturing nonlinear interactions among complex biochemical and fruit quality traits and offers predictive insights that can guide cultivar selection and targeted breeding programs, achieving 92.3 % prediction accuracy during validation ($R^2 = 0.91$, MSE = 0.024).

3. Results and discussion

3.1. Fruit quality, biochemical properties, antioxidant capacity, oil quality, and fatty acid composition analyses

Detailed statistical comparisons of fruit quality traits among the cultivars are provided in Table 1. The morphological evaluation of fruit quality traits among the 22 olive cultivars revealed substantial inter-cultivar variability, particularly in fruit weight, size dimensions, and flesh ratio, highlighting the influence of genetic background on pomological characteristics. Fruit weight values ranged from 2.15 ± 0.18 g in 'Kilis Yağlık' to 6.55 ± 0.16 g in 'Ascolana', with a mean of 3.98 ± 1.19 g and a coefficient of variation (CV) of 29.80 %, indicating a high degree of heterogeneity across cultivars. 'Ascolana' (6.55 ± 0.16 g) and 'İzmir Sofralık' (6.15 ± 0.24 g) displayed significantly larger fruits, confirming their suitability for table olive production, while smaller-fruited cultivars such as 'Kilis Yağlık' (2.15 ± 0.18 g), 'Nizip Yağlık' (2.30 ± 0.13 g), 'Meski' (2.33 ± 0.08 g), and 'Arbequina' (2.75 ± 0.15 g) are more likely aligned with oil production purposes. This morphological divergence corresponds well with prior classifications of olive cultivars based on end-use, as also reported by Mirzabe et al. (2013) and Ponce et al. (2018), who emphasized size parameters as reliable discriminators in cultivar characterization. Fruit length and width exhibited relatively lower variability, with CVs of 11.78 % and 9.54 %, respectively. Nonetheless, statistically significant differences were observed, with 'Ascolana' presenting the greatest fruit length (27.90 ± 0.89 mm) and width (22.05 ± 0.81 mm), reinforcing its commercial potential in the fresh market. Conversely, 'Kilis Yağlık' (length 17.80 ± 1.76, width 15.65 ± 1.23) again ranked lowest in both dimensions. Despite these dimensional disparities, fruit shape index, calculated as the length-to-width ratio, varied within a narrower range (CV = 6.70 %), from 1.11 ± 0.06 in 'Samanlı' to 1.44 ± 0.12 in 'Sarı Ulak'. The observed values denote a general tendency towards elliptical morphology among the cultivars, with 'Sarı Ulak' (1.44 ± 0.12), 'Sarı Yaprak' (1.39 ± 0.14), and 'Memeli' (1.35 ± 0.08) exhibiting a more elongated form, which is often preferred in high-grade table olives. The low variability in fruit shape index suggests that fruit shape is a relatively stable trait among the studied genotypes, making it a potentially heritable feature with low environmental influence. The fruit flesh ratio, representing the proportion of edible mesocarp, ranged from 75.00 ± 2.00 % in 'Meski' to 86.50 ± 2.43 % in 'Halhalı', with a group mean of 82.17 ± 2.92 % and a remarkably low CV of 3.55 %. Such limited variation points to the trait's consistency across growing seasons and its potential utility in breeding programs focused on enhancing fruit quality. Notably, cultivars such as 'Halhalı' (86.50 ± 2.43 %), 'Gemlik' (85.50 ± 2.43 %), and 'Edincik Su' (85.33 ± 2.16 %) demonstrated superior fruit flesh ratio values, suggesting dual-purpose applicability for both table and oil production. The combination of high flesh ratio and medium fruit size, as observed in these cultivars, may provide an optimal balance for producers aiming to diversify marketable outputs.

These findings are consistent with previous studies conducted on diverse olive germplasms. For instance, Zeinanloo (2022) reported a fruit weight variation ranging from 1.20 g in 'Koroneiki' to 11.36 g in 'Toffahi', and a fruit flesh ratio between 79.19 % in 'Tiaki' and 93.64 %

Table 1
Detailed Tukey's HSD test results for fruit quality traits of the studied olive cultivars.

Cultivar	Fruit weight [FWe (g)]	Fruit length [FL (mm)]	Fruit width [FWi (mm)]	Fruit shape index [FSI]	Fruit flesh ratio [FFR (%)]
'Arbequina'	2.75 ± 0.15^{i}	19.60 ± 1.47^{jk}	17.05 ± 0.61^{e-h}	1.15 ± 0.10^{cd}	81.58 ± 1.80^{a-f}
'Ascolana'	6.55 ± 0.16^{a}	27.90 ± 0.89^{a}	22.05 ± 0.81^{a}	1.27 ± 0.06^{a-d}	78.75 ± 2.44^{d-g}
'Ayvalık'	3.65 ± 0.17^{e-g}	21.80 ± 0.99^{g-j}	17.85 ± 0.82^{d-h}	1.22 ± 0.06^{a-d}	83.00 ± 3.03^{a-d}
'Domat'	5.78 ± 0.17^{c}	25.98 ± 0.96^{a-c}	19.90 ± 1.16^{a-d}	1.31 ± 0.10^{a-d}	83.00 ± 3.41^{a-d}
'Edincik Su'	4.70 ± 0.14^{d}	22.10 ± 1.68^{e-j}	18.55 ± 1.11^{c-f}	1.20 ± 0.15^{b-d}	85.33 ± 2.16^{a-c}
'Gemlik'	3.95 ± 0.21^{e}	23.10 ± 1.65^{c-h}	19.55 ± 1.39^{b-d}	1.19 ± 0.13^{b-d}	85.50 ± 2.43^{ab}
'Halhalı'	3.65 ± 0.24^{e-g}	19.95 ± 1.37^{i-k}	16.95 ± 0.82^{e-h}	1.18 ± 0.05^{b-d}	86.50 ± 2.43^{a}
'Hojiblanca'	4.50 ± 0.17^{d}	23.75 ± 1.25^{c-g}	18.95 ± 0.73^{c-e}	1.25 ± 0.06^{a-d}	83.58 ± 2.20^{a-d}
'İzmir Sofralık'	6.15 ± 0.24^{b}	27.85 ± 0.83^{ab}	21.25 ± 1.18^{ab}	1.31 ± 0.04^{a-d}	84.00 ± 1.67^{a-c}
'Kilis Yağlık'	2.15 ± 0.18^{i}	17.80 ± 1.76^{k}	15.65 ± 1.23^{h}	1.14 ± 0.16^{cd}	84.17 ± 2.14^{a-c}
'Manzanilla de Sevilla'	3.88 ± 0.17^{e}	20.55 ± 1.11^{h-k}	17.75 ± 0.71^{d-h}	1.16 ± 0.08^{cd}	84.75 ± 1.72^{a-c}
'Memecik'	4.75 ± 0.12^{d}	24.95 ± 0.69^{b-e}	19.85 ± 1.20^{a-d}	1.26 ± 0.11^{a-d}	80.92 ± 2.29^{b-f}
'Memeli'	4.65 ± 0.15^{d}	25.55 ± 0.64^{a-c}	18.95 ± 0.97^{c-e}	1.35 ± 0.08^{a-c}	82.50 ± 2.88^{a-e}
'Meski'	2.33 ± 0.08^{i}	19.55 ± 1.33^{jk}	16.25 ± 0.83^{gh}	1.21 ± 0.09^{b-d}	75.00 ± 2.00^{g}
'Nizip Yağlık'	2.30 ± 0.13^{i}	21.95 ± 1.77^{f-j}	17.85 ± 1.18^{d-h}	1.23 ± 0.04^{a-d}	83.50 ± 2.59^{a-d}
'Samanlı'	4.60 ± 0.22^{d}	22.55 ± 1.30^{d-i}	20.35 ± 0.86^{a-c}	1.11 ± 0.06^{d}	83.50 ± 1.64^{a-d}
'Sarı Haşebi'	3.25 ± 0.25^{h}	22.20 ± 1.51^{d-j}	16.75 ± 0.95^{e-h}	1.33 ± 0.13^{a-d}	80.17 ± 2.79^{c-g}
'Sarı Ulak'	3.75 ± 0.15^{ef}	23.95 ± 2.00^{c-g}	16.65 ± 1.37^{f-h}	1.44 ± 0.12^{a}	76.83 ± 1.94^{fg}
'Sarı Yaprak'	4.40 ± 0.17^{d}	25.05 ± 1.03^{a-d}	18.20 ± 1.50^{c-g}	1.39 ± 0.14^{ab}	82.42 ± 2.94^{a-e}
'Savrani'	3.30 ± 0.14^{gh}	21.95 ± 1.31^{f-j}	17.10 ± 1.14^{e-h}	1.29 ± 0.14^{a-d}	82.50 ± 2.35^{a-e}
'Silifke Yağlık'	3.15 ± 0.17^{h}	20.50 ± 1.92^{h-k}	15.85 ± 0.82^{h}	1.30 ± 0.14^{a-d}	77.67 ± 2.80^{e-g}
'Uslu'	3.45 ± 0.11^{f-g}	24.80 ± 1.47^{c-f}	19.65 ± 1.34^{b-d}	1.27 ± 0.12^{a-d}	82.58 ± 3.41^{a-e}
Minimum	2.15	17.80	15.65	1.11	75.00
Maximum	6.55	27.90	22.05	1.44	86.50
Mean ± SD	3.98 ± 1.19	22.80 ± 2.70	18.32 ± 1.75	1.25 ± 0.08	82.17 ± 2.92
CV	29.80	11.78	9.54	6.70	3.55

±*SD* Standard Deviation, *CV* Coefficient of Variation. Levels not connected by the same letters are significantly different ($p < 0.05$).

in 'Jlot'. Similarly, Nikpeyma (2023) observed substantial inter-cultivar diversity, with fruit weight ranging from 1.76 g ('Kilis Yağlık') to 7.5 g ('İzmir Sofralık'), fruit length from 17.59 mm ('Kilis Yağlık') to 33.62 mm ('Çelebi'), fruit width from 14.98 mm ('Sarı Ulak') to 23.13 mm ('Karamürsel Su'), fruit shape index from 1.08 ('Büyük Topak Ulak') to 1.64 ('Sarı Ulak'), and fruit flesh ratio ranging between 71.82 % ('Sarı Ulak') and 89.44 % ('Edincik Su'). In another recent investigation, Dehghan-Seresht, Khadivi, and Tunç (2024) documented ranges of 2.03–9.73 g for fruit weight, 16.50–32.68 mm for fruit length, and 13.58–23.80 mm for fruit width. A similar study conducted in India also reported comparable findings (Sisodiya et al., 2025). These data support the assertion that considerable morphological diversity exists within and across olive cultivars, validating the significance of the findings in the present study.

Overall, the substantial diversity observed in fruit weight and size parameters, contrasted with the relatively stable nature of flesh ratio and shape index, underlines the importance of comprehensive morphological profiling in olive germplasm evaluation. The statistically significant differences across cultivars, as verified by Tukey's HSD test at $p < 0.05$, reinforce the robustness of the phenotypic data. These findings underscore the value of detailed fruit quality assessment in guiding both cultivar selection for specific agronomic targets and the broader conservation of olive genetic resources.

The statistical comparisons of oil quality parameters and phenolic content among the olive cultivars are presented in detail in Table 2. The findings reveal significant differences ($p < 0.05$) among cultivars in terms of both chemical quality and biochemical composition. The observed variation, particularly in traits such as oil content, free fatty acidity, peroxide value, and total phenolic compounds, reflects the combined influence of genetic factors and micro-environmental conditions.

Total oil content varied among cultivars, ranging from 16.23 ± 1.00 % in 'Ascolana' to 30.92 ± 2.50 % in 'Kilis Yağlık', with an overall mean of 23.28 ± 3.86 %. The highest oil contents were observed in 'Kilis Yağlık' (30.92 ± 2.50 %), 'Nizip Yağlık' (29.50 ± 1.05 %), and 'Savrani' (28.33 ± 1.21 %), highlighting their potential as high-yielding oil cultivars. These findings reflect the genetic variability in the total lipid biosynthesis capacity among olive cultivars. The recorded values are consistent with the oil content ranges previously reported by Nikpeyma (2023) (16.76–31.86 %) and Gagour et al. (2024) (12.25–31.81 %). Moreover, environmental stress factors, as emphasized by Roussos et al. (2025), may also contribute to the observed inter-cultivar differences in oil yield.

Free fatty acid content is a critical parameter in the classification of olive oil quality (Bajoub et al., 2018). In this study, free fatty acid levels ranged from 0.40 ± 0.09 % in 'Ayvalık' to 0.87 ± 0.05 % in 'Sarı Yaprak', with an overall mean of 0.64 ± 0.11 %. The lowest free fatty acid values ranged from 0.40 ± 0.09 ('Ayvalık') to 0.78 ± 0.08 % ('Edincik Su' and 'İzmir Sofralık'), and these cultivars are suitable for high-quality oil production and fall within the "extra virgin" category. According to the criteria of the International Olive Council (IOC, 2023), olive oils with a free acidity below 0.8 % are classified as extra virgin. In the study conducted by Armenta et al. (2007), free fatty acid content was reported to range from 0.07 % to 1.66 %. The results obtained in the present study fall within this previously reported range, supporting the consistency of free acidity values across different olive oil investigations. Moreover, our findings are consistent with previous studies that emphasized cultivar-dependent variation in free fatty acid content under standardized agricultural conditions (El Yamani et al., 2022; García Martín, 2022; Sakar et al., 2022). The ability of certain cultivars to maintain low acidity under identical ecological conditions suggests that genetic background plays a decisive role in oil quality stability and potential shelf life. Within this context, the cultivar 'Sarı Yaprak', which exhibited a value above 0.8 %, may fall into a lower quality category due to factors such as oxidative stress, delayed harvesting, or poor postharvest handling, which are known to increase lipase activity and thus free acidity (Frangipane et al., 2025; Manai-Djebali et al., 2023).

Table 2
Detailed Tukey's HSD test results for oil quality and biochemical traits of the studied olive cultivars.

Cultivar	Oil content	Free fatty acids	Peroxide value	Total phenolic compounds
	[OC w/w (%)]	[FFA (% OA)]	[PV (meq O_2/kg)]	[TPC (mg CAE/kg)]
'Arbequina'	21.58 ± 1.50^{g-j}	0.57 ± 0.08^{d-g}	6.35 ± 1.30^{a-e}	184.83 ± 15.01^{ef}
'Ascolana'	16.23 ± 1.00^{l}	0.72 ± 0.08^{a-d}	4.57 ± 0.73^{de}	165.00 ± 17.88^{fg}
'Ayvalık'	24.17 ± 1.60^{d-h}	0.40 ± 0.09^{g}	6.23 ± 1.12^{a-e}	280.33 ± 19.10^{ab}
'Domat'	20.83 ± 1.17^{h-k}	0.70 ± 0.09^{a-e}	6.77 ± 0.71^{ab}	179.67 ± 24.01^{ef}
'Edincik Su'	18.50 ± 1.76^{j-l}	0.78 ± 0.08^{ab}	7.42 ± 0.88^{ab}	145.17 ± 21.18^{fg}
'Gemlik'	24.83 ± 1.94^{d-g}	0.53 ± 0.05^{e-g}	6.60 ± 1.56^{a-c}	164.67 ± 13.14^{fg}
'Halhalı'	24.58 ± 1.28^{d-g}	0.58 ± 0.08^{d-f}	7.22 ± 0.53^{ab}	240.00 ± 26.83^{b-d}
'Hojiblanca'	18.00 ± 1.38^{kl}	0.77 ± 0.05^{a-c}	5.75 ± 0.82^{b-e}	155.00 ± 16.43^{fg}
'İzmir Sofralık'	18.83 ± 1.47^{j-l}	0.78 ± 0.08^{ab}	7.42 ± 0.86^{ab}	145.33 ± 14.51^{fg}
'Kilis Yağlık'	30.92 ± 2.50^{a}	0.58 ± 0.10^{d-f}	6.77 ± 0.71^{ab}	270.00 ± 10.95^{a-c}
'Manzanilla de Sevilla'	19.75 ± 1.25^{i-k}	0.66 ± 0.10^{b-f}	6.50 ± 0.37^{a-d}	160.00 ± 14.91^{fg}
'Memecik'	23.83 ± 1.94^{e-h}	0.50 ± 0.09^{fg}	4.77 ± 0.73^{c-e}	265.00 ± 28.49^{a-c}
'Memeli'	23.50 ± 1.76^{e-h}	0.58 ± 0.08^{d-f}	4.40 ± 0.74^{e}	230.00 ± 23.45^{cd}
'Meski'	27.33 ± 1.72^{b-d}	0.58 ± 0.08^{d-f}	7.17 ± 0.68^{ab}	210.17 ± 16.36^{de}
'Nizip Yağlık'	29.50 ± 1.05^{ab}	0.52 ± 0.08^{fg}	7.13 ± 0.87^{ab}	264.67 ± 23.89^{a-c}
'Samanlı'	21.58 ± 2.01^{g-j}	0.73 ± 0.12^{a-d}	5.63 ± 0.97^{b-e}	145.33 ± 21.69^{fg}
'Sarı Haşebi'	25.17 ± 1.17^{c-f}	0.72 ± 0.08^{a-d}	6.42 ± 1.01^{a-d}	260.00 ± 20.98^{a-c}
'Sarı Ulak'	24.50 ± 1.22^{d-g}	0.60 ± 0.06^{c-f}	5.47 ± 0.53^{b-e}	250.00 ± 18.71^{a-d}
'Sarı Yaprak'	22.58 ± 1.74^{f-i}	0.87 ± 0.05^{a}	8.18 ± 1.38^{a}	135.17 ± 11.91^{g}
'Savrani'	28.33 ± 1.21^{a-c}	0.58 ± 0.08^{d-f}	5.82 ± 0.94^{b-e}	284.83 ± 27.18^{a}
'Silifke Yağlık'	26.83 ± 1.72^{b-e}	0.67 ± 0.10^{b-f}	6.42 ± 0.57^{a-d}	240.17 ± 20.25^{b-d}
'Uslu'	20.83 ± 1.94^{h-k}	0.60 ± 0.13^{c-f}	5.80 ± 1.38^{b-e}	125.00 ± 11.56^{g}
Minimum	16.23	0.40	4.40	125.00
Maximum	30.92	0.87	8.18	284.83
Mean ± SD	23.28 ± 3.86	0.64 ± 0.11	6.31 ± 0.97	204.56 ± 54.50
CV	16.57	17.52	15.40	26.64

±*SD* Standard Deviation, *CV* Coefficient of Variation. Levels not connected by the same letters are significantly different ($p < 0.05$).

Peroxide value, which is directly related to the oxidative stability of olive oil, ranged from 4.40 ± 0.74 meq O_2/kg in 'Memeli' to 8.18 ± 1.38 meq O_2/kg in 'Sarı Yaprak' among the cultivars examined. The overall mean was calculated as 6.31 ± 0.97 meq O_2/kg. The cultivars with the lowest peroxide value included 'Memeli' (4.40 ± 0.74), 'Ascolana' (4.57 ± 0.73), and 'Memecik' (4.77 ± 0.73), which are notable for their longer shelf life and lower risk of oxidative degradation. The maximum peroxide value limit recommended by the International Olive Council (IOC, 2023) is 20 meq O_2/kg; therefore, the fact that all cultivars in this study remained below this threshold indicates that the oils obtained are oxidatively stable. However, the relatively high peroxide value observed in cultivars such as 'Sarı Yaprak' (8.18 ± 1.38), 'Edincik Su' (7.42 ± 0.88), and 'İzmir Sofralık' (7.42 ± 0.86) underscore the importance of

post-processing storage conditions. The peroxide value serves as a practical and sensitive indicator for detecting the initial onset of oxidative degradation in olive oil (Gharby & Charrouf, 2022). In a study conducted by Armenta et al. (2007), peroxide values were reported to range between 1.8 and 17.2 meq O_2/kg. Similarly, Veloso et al. (2020), Longobardi et al. (2021), Revelou et al. (2021), El Yamani et al. (2022), Sakar et al. (2022), and Gagour et al. (2024) also documented peroxide values below the 20 meq O_2/kg threshold established by the International Olive Council. These findings align well with the results of the present study, reinforcing the observation that olive oils produced under diverse agroecological conditions generally maintain oxidative stability within internationally accepted quality standards.

Total phenolic compounds are among the most important quality indicators in terms of antioxidant capacity and functional constituents (Shi et al., 2022). The results revealed that total phenolic compound levels ranged from 125 ± 11.56 mg CAE/kg in 'Uslu' to 284.83 ± 27.18 mg CAE/kg in 'Savrani', with an overall mean of 204.56 ± 54.50 mg CAE/kg. Cultivars such as 'Savrani' (284.83 ± 27.18), 'Ayvalık' (280.33 ± 19.10), 'Kilis Yağlık' (270.00 ± 10.95), 'Memecik' (265.00 ± 28.49), 'Nizip Yağlık' (264.67 ± 23.89), and 'Sarı Haşebi' (260.00 ± 20.98) stood out due to their high phenolic contents, suggesting their suitability for the production of functional olive oils with enhanced health-promoting properties. In the study conducted by Veloso et al. (2020), total phenolic compound levels were reported to range from 88 ± 7 to 144 ± 4 mg CAE/kg, while Reboredo-Rodríguez et al. (2021) documented a broader range between 75 ± 2.18 and 302 ± 29.45 mg CAE/kg. The findings of the present study are partially consistent with those reported by Veloso et al. (2020) and fall within the range observed by Reboredo-Rodríguez et al. (2021), indicating that the antioxidant potential of the evaluated cultivars is within the expected variability range for virgin olive oils. Variations in phenolic content may stem not only from genetic differences but also from agronomic practices, ripening stage, and processing methods. Recent studies, such as that by Granado-Rodríguez et al. (2021), emphasize that phenolic profiles are strongly genotype-dependent and are significantly influenced by environmental factors such as climate, soil type, and water availability, which together shape the metabolic response of olive trees.

The fatty acid composition of olive oils derived from the studied cultivars demonstrated a marked variation across genotypes, reflecting the influence of genetic background on oil quality attributes (Table 3). The fatty acid profile and associated nutritional quality indices of virgin olive oils from 22 cultivars are presented in Supplementary Table S3. This table includes detailed data on saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs), along with key lipid health indices such as the MUFA/PUFA and oleic/linoleic (C18:1/C18:2) ratios. The ratio of UFAs (comprising both MUFAs and PUFAs) to SFAs ranged from 4.36 ± 0.24 in 'İzmir Sofralık' to 8.00 ± 0.58 in 'Kilis Yağlık', with a mean of 5.86 ± 1.26. A higher UFAs/SFAs ratio, as observed in cultivars like 'Kilis Yağlık' (8.00 ± 0.58), 'Silifke Yağlık' (7.95 ± 0.72), and 'Memecik' (7.90 ± 0.40), is generally indicative of better nutritional value and increased oxidative stability, aligning with prior findings that underscore the importance of this ratio as a benchmark for oil healthfulness and shelf-life performance (Abd El-Moneim et al., 2022; El Moudden et al., 2020).

Palmitic acid, the predominant saturated fatty acid, exhibited considerable variation from 9.20 ± 0.66 % in 'Kilis Yağlık' to 15.55 ± 1.54 % in 'İzmir Sofralık', with an overall mean of 12.30 ± 2.11 %. Lower palmitic acid levels are typically preferred due to their association with cardiovascular risk when consumed in high amounts (Annevelink et al., 2023). Conversely, oleic acid, the principal MUFA and a cornerstone of the Mediterranean diet, ranged from 67.20 ± 2.98 % in 'İzmir Sofralık' to 77.50 ± 1.42 % in 'Ayvalık', averaging 71.56 ± 3.22 %. Cultivars such as 'Ayvalık' (77.50 ± 1.42 %), 'Memecik' (76.20 ± 1.50 %), and 'Savrani' (75.80 ± 1.43 %), which exhibited higher oleic acid concentrations, stand out for their favorable oil profiles, as oleic acid contributes to oxidative stability, palatability, and

Table 3
Detailed Tukey's HSD test results for fatty acid composition traits of the studied olive cultivars.

Cultivar	UFAs(MUFAs + PUFAs)/SFAs [Ratio]	Palmitic acid [C16:0 (%)]	Palmitoleic acid [C16:1 (%)]	Stearic acid [C18:0 (%)]
'Arbequina'	5.74 ± 0.45^{f-i}	13.12 ± 0.87^{b-d}	0.92 ± 0.10^{gh}	1.44 ± 0.10^{h}
'Ascolana'	5.05 ± 0.23^{h-l}	12.85 ± 0.71^{b-d}	1.32 ± 0.06^{c-f}	3.22 ± 0.32^{ab}
'Ayvalık'	7.32 ± 0.51^{a-c}	9.58 ± 0.74^{fg}	1.50 ± 0.09^{b-d}	2.20 ± 0.12^{d-f}
'Domat'	5.26 ± 0.77^{g-k}	12.85 ± 1.49^{b-d}	0.94 ± 0.11^{gh}	2.82 ± 0.20^{bc}
'Edincik Su'	4.47 ± 0.30^{kl}	14.25 ± 1.27^{a-c}	1.12 ± 0.11^{fg}	3.60 ± 0.37^{a}
'Gemlik'	5.47 ± 0.21^{g-j}	12.24 ± 0.97^{c-e}	1.33 ± 0.08^{c-f}	2.85 ± 0.15^{bc}
'Halhalı'	4.92 ± 0.23^{i-l}	14.44 ± 1.05^{ab}	1.55 ± 0.18^{a-c}	2.12 ± 0.28^{e-g}
'Hojiblanca'	5.05 ± 0.35^{h-l}	13.84 ± 0.98^{a-c}	1.22 ± 0.09^{ef}	2.25 ± 0.25^{d-f}
'İzmir Sofralık'	4.36 ± 0.24^{l}	15.55 ± 1.54^{a}	0.82 ± 0.09^{h}	2.62 ± 0.28^{cd}
'Kilis Yağlık'	8.00 ± 0.58^{a}	9.20 ± 0.66^{g}	1.78 ± 0.18^{a}	1.60 ± 0.21^{h}
'Manzanilla de Sevilla'	4.72 ± 0.22^{j-l}	14.22 ± 0.51^{a-c}	0.92 ± 0.08^{gh}	2.84 ± 0.19^{bc}
'Memecik'	7.90 ± 0.40^{ab}	9.30 ± 0.56^{g}	1.22 ± 0.10^{ef}	1.60 ± 0.16^{h}
'Memeli'	7.11 ± 0.43^{b-d}	10.25 ± 0.78^{e-g}	1.32 ± 0.10^{c-f}	1.80 ± 0.19^{f-h}
'Meski'	6.42 ± 0.29^{d-f}	11.62 ± 0.66^{de}	1.44 ± 0.11^{b-e}	1.55 ± 0.13^{h}
'Nizip Yağlık'	7.10 ± 0.47^{b-d}	10.25 ± 0.85^{e-g}	1.60 ± 0.15^{ab}	1.70 ± 0.20^{gh}
'Samanlı'	4.57 ± 0.24^{kl}	14.25 ± 1.06^{a-c}	1.45 ± 0.15^{b-e}	3.24 ± 0.29^{ab}
'Sarı Haşebi'	6.02 ± 0.27^{e-g}	11.55 ± 0.62^{d-f}	0.80 ± 0.09^{h}	2.40 ± 0.18^{c-e}
'Sarı Ulak'	6.82 ± 0.42^{c-e}	10.64 ± 0.81^{e-g}	1.62 ± 0.05^{ab}	1.82 ± 0.14^{f-h}
'Sarı Yaprak'	4.39 ± 0.32^{kl}	14.80 ± 1.12^{ab}	0.94 ± 0.10^{gh}	3.40 ± 0.30^{a}
'Savrani'	5.80 ± 0.35^{f-h}	11.20 ± 0.95^{d-g}	1.30 ± 0.14^{d-f}	3.20 ± 0.31^{ab}
'Silifke Yağlık'	7.95 ± 0.72^{ab}	9.32 ± 1.08^{g}	1.52 ± 0.11^{b-d}	1.60 ± 0.08^{h}
'Uslu'	4.56 ± 0.30^{kl}	15.25 ± 0.85^{a}	1.32 ± 0.08^{c-f}	2.40 ± 0.23^{c-e}
Minimum	4.36	9.20	0.80	1.44
Maximum	8.00	15.55	1.78	3.60
Mean ± SD	5.86 ± 1.26	12.30 ± 2.11	1.27 ± 0.28	2.37 ± 0.69
CV	21.49	17.14	22.24	29.01

UFAs Unsaturated Fatty Acids, *MUFAs* Monounsaturated Fatty Acids, *PUFAs* Polyunsaturated Fatty Acids, *SFAs* Saturated Fatty Acids, ±*SD* Standard Deviation, *CV* Coefficient of Variation. Levels not connected by the same letters are significantly different ($p < 0.05$).

Cultivar	Oleic acid [C18:1 (%)]	Linoleic acid [C18:2 (%)]	Linolenic acid [C18:3 (%)]	Arachidic acid [C20:0 (%)]	Gadoleic acid [C20:1 (%)]
'Arbequina'	72.82 ± 2.01^{b-f}	10.75 ± 1.30^{c-e}	0.55 ± 0.08^{d-h}	0.33 ± 0.06^{cd}	0.06 ± 0.01^{c-f}
'Ascolana'	68.90 ± 1.53^{fg}	12.72 ± 0.46^{a-c}	0.45 ± 0.06^{h}	0.48 ± 0.07^{ab}	0.05 ± 0.01^{ef}
'Ayvalık'	77.50 ± 1.42^{a}	8.10 ± 0.69^{fg}	0.78 ± 0.08^{a-c}	0.28 ± 0.03^{d}	0.04 ± 0.01^{f}
'Domat'	70.62 ± 2.13^{d-g}	11.60 ± 0.97^{a-d}	0.62 ± 0.07^{c-h}	0.48 ± 0.10^{ab}	0.06 ± 0.01^{d-f}
'Edincik Su'	67.50 ± 1.98^{g}	12.30 ± 1.18^{a-d}	0.64 ± 0.08^{c-g}	0.48 ± 0.06^{ab}	0.09 ± 0.02^{a}

(*continued on next page*)

Table 3 (*continued*)

Cultivar	Oleic acid [C18:1 (%)]	Linoleic acid [C18:2 (%)]	Linolenic acid [C18:3 (%)]	Arachidic acid [C20:0 (%)]	Gadoleic acid [C20:1 (%)]
'Gemlik'	71.20 ± 2.40^{c-g}	11.20 ± 0.96^{b-e}	0.72 ± 0.10^{a-d}	0.38 ± 0.05^{b-d}	0.07 ± 0.01^{b-f}
'Halhalı'	70.20 ± 2.67^{e-g}	10.42 ± 1.07^{de}	0.82 ± 0.06^{ab}	0.36 ± 0.05^{b-d}	0.07 ± 0.01^{b-e}
'Hojiblanca'	69.50 ± 2.43^{e-g}	12.12 ± 0.77^{a-d}	0.52 ± 0.06^{e-h}	0.48 ± 0.06^{ab}	0.06 ± 0.01^{b-f}
'İzmir Sofralık'	67.20 ± 2.98^{g}	12.72 ± 0.99^{a-c}	0.48 ± 0.06^{f-h}	0.52 ± 0.06^{a}	0.08 ± 0.01^{a-d}
'Kilis Yağlık'	75.20 ± 2.68^{a-c}	11.20 ± 1.19^{b-e}	0.62 ± 0.11^{c-h}	0.34 ± 0.06^{cd}	0.05 ± 0.01^{ef}
'Manzanilla de Sevilla'	68.62 ± 3.58^{fg}	12.42 ± 0.66^{a-d}	0.46 ± 0.07^{h}	0.42 ± 0.06^{a-c}	0.08 ± 0.02^{ab}
'Memecik'	76.20 ± 1.50^{ab}	10.40 ± 0.65^{de}	0.82 ± 0.09^{ab}	0.36 ± 0.06^{b-d}	0.08 ± 0.01^{a-d}
'Memeli'	74.80 ± 1.59^{a-d}	10.60 ± 1.71^{de}	0.85 ± 0.13^{a}	0.32 ± 0.04^{cd}	0.05 ± 0.01^{ef}
'Meski'	70.80 ± 1.38^{d-g}	13.50 ± 0.93^{a}	0.68 ± 0.07^{a-e}	0.32 ± 0.05^{cd}	0.07 ± 0.01^{a-e}
'Nizip Yağlık'	75.80 ± 2.09^{ab}	9.50 ± 0.84^{ef}	0.65 ± 0.05^{b-g}	0.43 ± 0.05^{a-c}	0.06 ± 0.01^{b-f}
'Samanlı'	68.20 ± 1.57^{g}	11.60 ± 0.74^{a-d}	0.65 ± 0.07^{b-f}	0.52 ± 0.06^{a}	0.08 ± 0.01^{a-e}
'Sarı Haşebi'	71.20 ± 2.17^{c-g}	12.90 ± 0.74^{ab}	0.76 ± 0.07^{a-c}	0.32 ± 0.03^{cd}	0.06 ± 0.01^{b-f}
'Sarı Ulak'	72.60 ± 1.00^{b-f}	12.40 ± 1.11^{a-d}	0.48 ± 0.08^{gh}	0.36 ± 0.07^{b-d}	0.06 ± 0.01^{b-f}
'Sarı Yaprak'	67.40 ± 1.30^{g}	12.40 ± 1.03^{a-d}	0.56 ± 0.07^{d-h}	0.42 ± 0.08^{a-c}	0.06 ± 0.01^{b-f}
'Savrani'	75.80 ± 1.43^{ab}	7.20 ± 0.64^{g}	0.85 ± 0.08^{a}	0.35 ± 0.07^{b-d}	0.08 ± 0.01^{a-c}
'Silifke Yağlık'	73.50 ± 1.97^{a-e}	13.10 ± 0.81^{ab}	0.55 ± 0.11^{d-h}	0.32 ± 0.05^{cd}	0.07 ± 0.01^{a-e}
'Uslu'	68.80 ± 0.84^{fg}	11.22 ± 1.06^{b-e}	0.55 ± 0.10^{d-h}	0.38 ± 0.08^{b-d}	0.06 ± 0.01^{b-f}
Minimum	67.20	7.20	0.45	0.28	0.04
Maximum	77.50	13.50	0.85	0.52	0.09
Mean ± SD	71.56 ± 3.22	11.38 ± 1.58	0.64 ± 0.13	0.39 ± 0.07	0.07 ± 0.01
CV	4.50	13.92	20.72	18.34	18.70

UFAs Unsaturated Fatty Acids, *MUFAs* Monounsaturated Fatty Acids, *PUFAs* Polyunsaturated Fatty Acids, *SFAs* Saturated Fatty Acids, ±*SD* Standard Deviation, *CV* Coefficient of Variation. Levels not connected by the same letters are significantly different ($p < 0.05$).

health-promoting properties (Clodoveo, 2021).

Polyunsaturated fatty acids, represented by linoleic and linolenic acids, also varied notably among cultivars. Linoleic acid ranged between 7.20 ± 0.64 % in 'Savrani' and 13.50 ± 0.93 % in 'Meski', with a mean of 11.38 ± 1.58 %, while linolenic acid varied from 0.45 ± 0.06 % in 'Ascolana' to 0.85 ± 0.08 % in 'Savrani', averaging 0.64 ± 0.13 %. Although PUFAs play essential roles in human nutrition, elevated concentrations can predispose oils to faster oxidation (Oliver et al., 2021). Therefore, cultivars with moderate PUFA levels—such as 'Samanlı' (12.25 ± 0.77), 'Domat' (12.22 ± 0.95), 'Gemlik' (11.92 ± 0.96), and 'Kilis Yağlık' (11.82 ± 1.22)—strike a favorable balance between nutritional value and oxidative resistance (Supplementary Table S3) (Frankel, 2005).

Among the less abundant fatty acids, palmitoleic acid levels ranged from 0.80 ± 0.09 % in 'Sarı Haşebi' to 1.78 ± 0.18 % in 'Kilis Yağlık', with an average of 1.27 ± 0.28 %. Although present in relatively low concentrations, palmitoleic acid has attracted increasing scientific interest due to its potential metabolic benefits, including anti-inflammatory properties and the enhancement of insulin sensitivity, which may contribute positively to cardiovascular and metabolic health (Brown et al., 2015). Stearic acid, a saturated fatty acid, showed a range of 1.44 ± 0.10 % in 'Arbequina' to 3.60 ± 0.37 % in 'Edincik Su', with a mean value of 2.37 ± 0.69 %. Despite being a saturated fatty acid, stearic acid is generally regarded as metabolically neutral, as it has minimal impact on plasma LDL-cholesterol levels when compared to other SFAs, particularly palmitic acid (Gonçalinho et al., 2023). Arachidic acid, a long-chain saturated fatty acid, ranged from 0.28 ± 0.03 % in 'Ayvalık' to 0.52 ± 0.06 % in 'İzmir Sofralık' and 'Samanlı', with a mean value of 0.39 ± 0.07 %. Although present in small amounts, high levels of long-chain SFAs such as arachidic acid may influence lipid metabolism and are generally considered less desirable from a cardiovascular health perspective (Gunstone, 2001; Mensink, 2016). Gadoleic acid, a minor monounsaturated fatty acid, exhibited a narrow range between 0.04 ± 0.01 % in 'Ayvalık' and 0.09 ± 0.02 % in 'Edincik Su'. Although nutritionally insignificant due to its trace concentration, it may serve as a chemotaxonomic helpful marker in distinguishing olive cultivars (Li et al., 2023). Previous studies have reported comparable values for these fatty acids. For instance, El Moudden et al. (2020) reported palmitoleic acid levels ranging from 0.43 to 0.95 %, stearic acid from 2.19 to 2.98 %, arachidic acid from 0.24 to 0.34 %, and gadoleic acid between 0.28 and 0.38 %. Revelou et al. (2021) found palmitoleic acid ranging from 0.56 ± 0.13 to 1.53 ± 0.19 %, stearic acid from 2.23 ± 0.14 to 2.78 ± 0.28 %, and gadoleic acid between 0.23 ± 0.03 and 0.34 ± 0.00 %. Similarly, Rodrigues et al. (2021) reported palmitoleic acid values ranging from 0.48 ± 0.01 to 1.41 ± 0.02 %, stearic acid from 1.84 ± 0.04 to 3.35 ± 0.45 %, and arachidic acid from 0.34 ± 0.01 to 0.53 ± 0.05 %. Abd El-Moneim et al. (2022) detected palmitoleic acid values between 1.46 ± 0.08 and 1.48 ± 0.08 %, while Nikpeyma (2023) identified palmitoleic acid in the range of 0.71–1.45 % and stearic acid between 2.27 and 4.97 %. Lu et al. (2024) and Roussos et al. (2025) also confirmed similar levels in their recent analysis. According to the International Olive Council (IOC, 2019), palmitoleic acid in olive oil typically ranges from 0.30 to 3.50 %, arachidic acid is generally present at levels below 0.60 %, and gadoleic acid does not usually exceed 0.50 %. As the levels found in our study fall well within these established ranges, the results are in agreement with those reported in earlier literature.

Our findings indicate that most of the examined cultivars possess compositional profiles suitable for the production of high-quality virgin olive oils. The observed variation in oleic and linoleic acid levels suggests that cultivar selection can be optimized according to specific nutritional or industrial application goals. Moreover, the high oleic/linoleic ratios observed in cultivars such as 'Savrani' (10.58 ± 0.85), 'Ayvalık' (9.63 ± 0.79), and 'Nizip Yağlık' (8.02 ± 0.65) confer advantages for markets that demand extended shelf life and enhanced thermal stability (Supplementary Table S3).

3.2. Correlation matrix analysis (CMA)

The CMA revealed several significant associations between total phenolic compounds, oil quality traits, and individual fatty acids in the studied olive cultivars (Fig. 2 and Supplementary Table S4). Total phenolic content was positively correlated with oil content ($r = 0.76$, $p < 0.01$), the UFAs/SFAs ratio ($r = 0.82$, $p < 0.01$), oleic acid ($r = 0.88$, $p < 0.01$), and palmitoleic acid ($r = 0.48$, $p < 0.05$), indicating that phenolic-rich cultivars tend to accumulate higher amounts of monounsaturated fatty acids and exhibit more favorable unsaturation profiles. In contrast, total phenolics were negatively correlated with free fatty acids ($r = -0.65$, $p < 0.01$), palmitic acid ($r = -0.85$, $p < 0.01$), stearic acid ($r = -0.55$, $p < 0.01$), linoleic acid ($r = -0.52$, $p < 0.05$), and arachidic acid ($r = -0.69$, $p < 0.01$), suggesting an inverse relationship between antioxidant content and the abundance of saturated or highly polyunsaturated fractions.

The UFAs/SFAs ratio was positively associated with oil content ($r = 0.70$, $p < 0.01$), palmitoleic acid ($r = 0.55$, $p < 0.01$), and oleic acid ($r = 0.88$, $p < 0.01$), but negatively correlated with free fatty acids ($r = -0.64$, $p < 0.01$), palmitic acid ($r = -0.97$, $p < 0.01$), stearic acid ($r = -0.75$, $p < 0.01$), and arachidic acid ($r = -0.68$, $p < 0.01$). These results highlight the importance of the UFAs/SFAs ratio as a composite

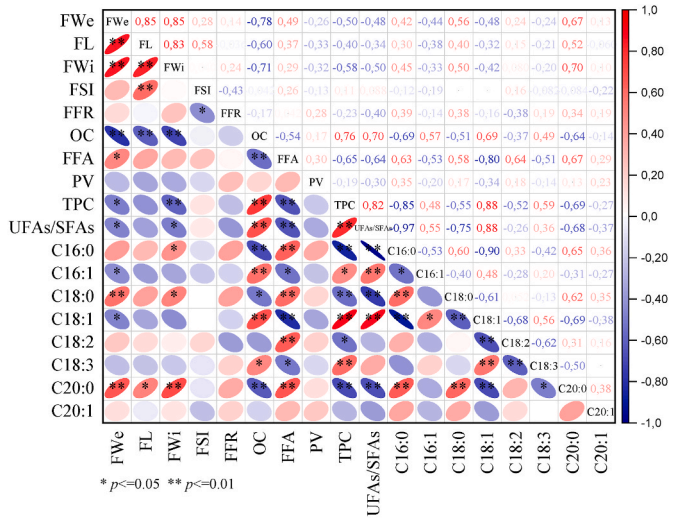


Fig. 2. Simple correlations between the variables utilized in studied olive cultivars. For abbreviations, please see Tables 1–3.

indicator of lipid quality and metabolic health potential.

Oleic acid showed strong positive correlations with total phenolics ($r = 0.88, p < 0.01$), UFAs/SFAs ($r = 0.88, p < 0.01$), and palmitoleic acid ($r = 0.48$, $p < 0.05$), indicating its central role in defining both oil composition and functional quality. It was negatively associated with palmitic acid ($r = -0.90, p < 0.01$), stearic acid ($r = -0.61, p < 0.01$), linoleic acid ($r = -0.68, p < 0.01$), linolenic acid ($r = -0.56, p < 0.01$), and arachidic acid ($r = -0.69$, $p < 0.01$), supporting the known antagonism between oleic acid and less desirable saturated or polyunsaturated fatty acids.

Linolenic acid exhibited negative correlations with linoleic acid ($r = -0.62, p < 0.01$) and arachidic acid ($r = -0.50, p < 0.05$) while showing a significant positive correlation with oleic acid ($r = 0.56$, $p < 0.01$), which may reflect a cultivar-specific trend in polyunsaturated fatty acid biosynthesis or a tightly regulated balance in fatty acid desaturation pathways.

Taken together, these correlation patterns indicate that olive cultivars rich in phenolics and oleic acid, with a high UFAs/SFAs ratio and low levels of saturated and free fatty acids, represent superior candidates for developing health-promoting and stable olive oils. The strong link between antioxidant and fatty acid profiles further reinforces the potential of these traits as integrated selection criteria in olive breeding programs aimed at enhancing nutritional quality. From a human health perspective, these results are highly relevant, as high oleic acid content and elevated phenolic compounds are both associated with reduced risk of cardiovascular disease, improved lipid metabolism, anti-inflammatory action, and greater oxidative stability (de Souza et al., 2017; Farràs et al., 2020; Santa-María et al., 2023). Conversely, the negative correlations with palmitic, stearic, and arachidic acids—known contributors to elevated LDL cholesterol and systemic inflammation—highlight the importance of minimizing saturated fatty acid fractions in health-oriented cultivar development (Cofrades & Álvarez, 2021).

Our correlation findings are fully consistent with those reported by Revelou et al. (2021), who demonstrated a similarly strong positive association between oleic acid and phenolic content in Mediterranean olive genotypes. Moreover, partial similarities were observed with the studies of El Yamani et al. (2022) and Sakar et al. (2022), particularly concerning the inverse relationships between saturated fatty acids and antioxidant parameters. This alignment across different research settings suggests that the observed relationships may be genetically conserved and environmentally stable, thus reinforcing the reliability of these traits as functional selection markers in olive breeding programs focused on enhancing both nutritional value and consumer health outcomes.

3.3. Multiple regression analysis (MRA)

The multiple regression analysis (MRA) results provided in Table 4 revealed significant associations between key fruit and oil quality traits in the studied olive cultivars. Each dependent character showed distinct predictor variables with statistically significant standardized beta (β) coefficients ($p \leq 0.05$), indicating their influence on the trait of interest.

Oleic acid, a major monounsaturated fatty acid and a central quality determinant of olive oil, was negatively influenced by several fatty acids. Palmitic acid ($\boldsymbol{\beta = -0.65, p \leq 0.00}$) and linoleic acid ($\boldsymbol{\beta = -0.49, p \leq 0.00}$) exerted the strongest negative effects, highlighting a competitive metabolic interaction where increased synthesis of these fatty acids leads to a corresponding reduction in oleic acid levels. Similarly, stearic acid also had a moderate but significant negative effect ($\boldsymbol{\beta = -0.21, p \leq 0.00}$). In contrast, palmitoleic acid ($\boldsymbol{\beta = 0.09, p \leq 0.00}$) exhibited a positive, albeit minor, association, while other minor fatty acids such as linolenic, arachidic, and gadoleic showed negligible or non-significant effects.

The ratio of unsaturated to saturated fatty acids, an essential nutritional indicator, was significantly and negatively influenced by both palmitic acid ($\boldsymbol{\beta = -0.81, p \leq 0.00}$) and stearic acid ($\boldsymbol{\beta = -0.27, p \leq 0.00}$). These findings confirm that saturated fatty acids substantially compromise the overall degree of unsaturation, thus reducing the health value of the oil.

For total phenolic compounds, which are crucial for antioxidant capacity, two predictors were identified. Oleic acid had a strong and positive effect ($\boldsymbol{\beta = 0.77, p \leq 0.00}$), suggesting a potential co-regulation between oil quality and phenolic biosynthesis. Conversely, fruit width showed a moderate negative association ($\boldsymbol{\beta = -0.25, p \leq 0.03}$), implying that larger fruits may exhibit lower phenolic content, possibly due to dilution effects or lower tissue density.

In terms of free fatty acids, which indicate oil degradation and lower quality, a significant negative relationship with oleic acid was observed ($\boldsymbol{\beta = -0.81, p \leq 0.00}$), consistent with the known oxidative stability of oleic-rich oils. However, fruit shape index showed a moderate positive association ($\beta = 0.27, p \leq 0.04$), suggesting that morphological factors may influence oil stability or mechanical damage during processing.

Regarding linoleic acid, a key polyunsaturated fatty acid, its levels were strongly and negatively associated with oleic acid ($\beta = -1.81, p \leq 0.00$). This result highlights the inverse metabolic relationship between mono- and polyunsaturated fatty acids in olive oil biosynthesis. Additionally, linoleic acid was positively influenced by the ratio of unsaturated to saturated fatty acids ($\beta = 1.36, p \leq 0.00$), and negatively by palmitoleic acid ($\beta = -0.18, p \leq 0.00$) and fruit flesh ratio ($\beta = -0.14, p \leq 0.01$), although these latter effects were less pronounced. The bold values are supported by the CMA.

Collectively, the findings illustrate complex interdependencies between morphological and biochemical traits in olive cultivars. Two dominant patterns emerge: one characterized by high oleic acid, high phenolics, and low free fatty acids, and another by elevated palmitic/linoleic acids and higher free fatty acids, indicating differential cultivar potential for oil quality optimization.

To the best of our knowledge, no previous studies have applied multiple regression analysis to investigate these specific trait interrelationships in olive cultivars. Consequently, the findings of the present study were evaluated within their own statistical and biological framework, thereby offering original insights that may enrich the existing body of knowledge in olive research.

From a human health perspective, these results emphasize the importance of selecting olive cultivars with high oleic acid content and favorable unsaturation profiles (UFAs/SFAs), which are associated with improved lipid metabolism, reduced cardiovascular risk, and enhanced oxidative stability (Pérez-Saucedo et al., 2021). Additionally, the strong

Table 4
The characteristics associated with related traits in studied olive cultivars as revealed using MRA and coefficients.

Dependent character	Independent character	*r*	r^2	*β*	*t*-value	*p*-value
Oleic acid (C18:1)	Palmitic acid (C16:0)	0.896[a]	0.80	**−0.65**	−1464.07	**0.00**[a]
	Linoleic acid (C18:2)	0.983[b]	0.97	**−0.49**	−1289.35	**0.00**[a]
	Stearic acid (C18:0)	0.997[c]	0.99	**−0.21**	−517.40	**0.00**[a]
	Palmitoleic acid (C16:1)	1.000[d]	1.00	**0.09**	252.98	**0.00**[a]
	Linolenic acid (C18:3)	1.000[e]	1.00	**−0.04**	−92.80	**0.00**[a]
	Arachidic acid (C20:0)	1.000[f]	1.00	**−0.02**	−45.25	**0.00**[a]
	Gadoleic acid (C20:1)	1.000[g]	1.00	0.00	−14.41	0.00
UFAs/SFAs	Palmitic acid (C16:0)	0.968[a]	0.94	**−0.81**	−21.67	**0.00**[a]
	Stearic acid (C18:0)	0.991[b]	0.98	**−0.27**	−7.12	**0.00**[a]
Total phenolic compounds	Oleic acid (C18:1)	0.881[a]	0.78	**0.77**	7.38	**0.00**[a]
	Fruit width	0.910[b]	0.83	**−0.25**	−2.43	**0.03**[a]
Free fatty acids	Oleic acid (C18:1)	0.804[a]	0.65	**−0.81**	−6.65	**0.00**[a]
	Fruit shape index	0.848[b]	0.72	0.27	2.22	0.04
Linoleic acid (C18:2)	Oleic acid (C18:1)	0.676[a]	0.46	−1.81	−18.10	0.00
	UFAs/SFAs	0.964[b]	0.93	1.36	12.07	0.00
	Palmitoleic acid (C16:1)	0.977[c]	0.95	−0.18	−3.41	0.00
	Fruit flesh ratio	0.984[d]	0.97	−0.14	−2.74	0.01

UFAs/SFAs ratio of unsaturated to saturated fatty acids, *r* Correlation coefficient, r^2 Coefficient of determination, *β* Standardized beta coefficients.
[a] Multiple regression analysis correlations supported by the correlation matrix analysis.

link between oleic acid and phenolic content reinforces the dual benefit of such cultivars in providing both heart-healthy fats and potent antioxidants (Montaño et al., 2016). Conversely, the negative impact of elevated palmitic and linoleic acid levels—particularly concerning free fatty acid accumulation—highlights the necessity of minimizing saturated and excessively polyunsaturated fractions to preserve both nutritional integrity and shelf life (Frydrych et al., 2025; Smolińska et al., 2024). Therefore, cultivar selection strategies guided by these biochemical markers may serve as a pivotal approach in developing functional olive oils aligned with public health priorities.

3.4. Principal component analysis (PCA)

PCA was performed to identify the major sources of variation among the studied olive cultivars based on fruit quality traits, fatty acid composition, antioxidant parameters, and biochemical attributes (Table 5). Four principal components with eigenvalues greater than 1.0 were extracted, cumulatively explaining 79.43 % of the total variance, indicating a high level of data representation and suitability for dimensionality reduction.

The first principal component (PC1) accounted for 45.81 % of the total variance and was primarily associated with saturated fatty acids and fruit weight. Specifically, palmitic acid (0.30), arachidic acid (0.29), free fatty acids (0.28), fruit weight (0.26), and stearic acid (0.25) exhibited strong positive loadings. In contrast, negative loadings were observed for oil content (−0.30), the ratio of unsaturated to saturated fatty acids (−0.31), total phenolic compounds (−0.31), and oleic acid (−0.32). This component reflects a trade-off between saturated fatty acid accumulation and traits indicative of oil quality and antioxidant potential. Cultivars characterized by higher levels of saturated fatty acids and heavier fruits tended to exhibit reduced oleic acid content and lower phenolic concentrations, suggesting inferior oil quality despite increased fruit size.

The second principal component (PC2), explaining 14.87 % of the variance, was mainly influenced by morphometric characteristics. Positive loadings were found for fruit length (0.45), fruit shape index (0.37), fruit weight (0.33), and fruit width (0.30), indicating that this axis represents general fruit size and shape variation. Negative loadings for gadoleic acid (−0.25) and peroxide value (−0.45) suggest that larger fruits may be associated with lower oxidative susceptibility and decreased concentrations of certain long-chain unsaturated fatty acids. This component emphasizes the contribution of morphological traits to fruit quality and storage potential.

Table 5
Eigenvalues of the principal component axes from the PCA of traits in the studied olive cultivars.

Eigenvector	Component			
	1	2	3	4
Fruit weight	**0.26**[a]	**0.33**[a]	0.14	0.03
Fruit length	0.21	**0.45**[a]	0.07	0.14
Fruit width	0.24	**0.30**[a]	**0.26**[a]	−0.18
Fruit shape index	0.02	**0.37**[a]	**−0.27**[a]	**0.54**[a]
Fruit flesh ratio	0.09	−0.24	**0.49**[a]	0.01
Oil Content	**−0.30**[a]	−0.15	−0.04	0.19
Free fatty acids	**0.28**[a]	−0.06	−0.24	0.21
Peroxide value	0.06	**−0.45**[a]	−0.12	**0.32**[a]
Total phenolic compounds	**−0.31**[a]	0.09	0.05	0.21
Ratio of unsaturated to saturated fatty acids	**−0.31**[a]	0.17	−0.10	−0.06
Palmitic acid (C16:0)	**0.30**[a]	−0.20	0.05	−0.01
Palmitoleic acid (C16:1)	−0.21	0.00	0.03	**−0.40**[a]
Stearic acid (C18:0)	**0.25**[a]	−0.05	0.22	0.24
Oleic acid (C18:1)	**−0.32**[a]	0.16	0.16	0.04
Linoleic acid (C18:2)	0.17	−0.03	**−0.51**[a]	−0.13
Linolenic acid (C18:3)	−0.19	0.01	**0.38**[a]	**0.37**[a]
Arachidic acid (C20:0)	**0.29**[a]	0.00	0.10	−0.15
Gadoleic acid (C20:1)	0.12	**−0.25**[a]	0.10	0.19
Eigenvalue	*8.25*	*2.68*	*2.18*	*1.20*
Component degree of significance	**	**	**	**
Variance (%)	*45.81*	*14.87*	*12.10*	*6.65*
∑ variance (%)	*45.81*	*60.68*	*72.78*	*79.43*

[a]Bold values indicate the characteristics that most influence each PC (Eigenvalues are significant ≥0.25). Component degree of significance: *$p < 0.05$, **$p < 0.01$.

The third principal component (PC3), which explained 12.10 % of the total variance, was characterized by a combination of biochemical and morphological traits. High positive loadings were recorded for flesh ratio (0.49), linolenic acid (0.38), and fruit width (0.26), whereas negative associations were observed for linoleic acid (−0.51) and fruit shape index (−0.27). This component highlights a divergence in polyunsaturated fatty acid profiles and suggests that wider fruits with higher flesh content may also possess elevated linolenic acid levels, potentially enhancing nutritional value. Meanwhile, cultivars with elongated fruit shapes and higher linoleic acid content appear to cluster at the opposite end of this axis, reflecting differences in both visual appearance and fatty acid composition.

The fourth principal component (PC4) accounted for an additional

6.65 % of the variance and was primarily associated with linolenic acid (0.37), peroxide value (0.32), and fruit shape index (0.54), with palmitoleic acid displaying a negative loading (−0.40). This axis may reflect a subtle interplay between shape-related morphological traits and lipid oxidation characteristics. The positive relationship between linolenic acid and peroxide value suggests that cultivars with elevated omega-3 fatty acid content may exhibit reduced oxidative stability, while the inverse relationship with palmitoleic acid may indicate alterations in monounsaturated fatty acid composition.

Overall, the PCA revealed distinct and interpretable patterns of covariation among fruit quality traits, fatty acid profiles, and antioxidant properties. These findings underscore the complexity of olive cultivar characterization and offer a multivariate framework for identifying superior genotypes with favorable combinations of nutritional quality and marketable fruit characteristics.

In addition, the biplot analysis revealed that both traits and cultivars were meaningfully distributed across the four principal component axes (Fig. 3). Notably, the cultivars 'Ascolana', 'İzmir Sofralık', 'Kilis Yağlık', and 'Edincik Su' were positioned outside the 95 % confidence ellipse defined within the two-dimensional PCA plane. This finding indicates that these cultivars differ significantly from the general sample in terms of the evaluated parameters and possess distinct characteristics.

This result is of particular important for identifying genotypes located at the extremes of variation. Cultivars positioned outside the confidence ellipse may exhibit unique genetic backgrounds, distinctive biochemical profiles, or extreme morphological traits. Therefore, these genotypes could be considered potential selection materials in terms of both oil quality and fruit attributes. Moreover, their distinctive positions suggest that they could serve as valuable resources in future breeding programs, particularly when supported by advanced molecular and metabolomic evaluations.

It is also worth noting that although previous studies focusing on similar trait groups have applied PCA and biplot analysis (El Moudden et al., 2020; Nikpeyma, 2023; Revelou et al., 2021; Sakar et al., 2022), the distribution patterns and angular relationships of both traits and cultivars in those studies differed from the present findings. The biplot structure observed in this study shows a unique configuration, especially in terms of variable orientations and cultivar clustering, reflecting the distinctive biochemical and morphological variation of the studied olive genotypes.

In conclusion, the identification of cultivars located outside the confidence ellipse in biplot analysis provides a robust basis for better understanding population structure and strategically utilizing genetic resources (Yan & Kang, 2003).

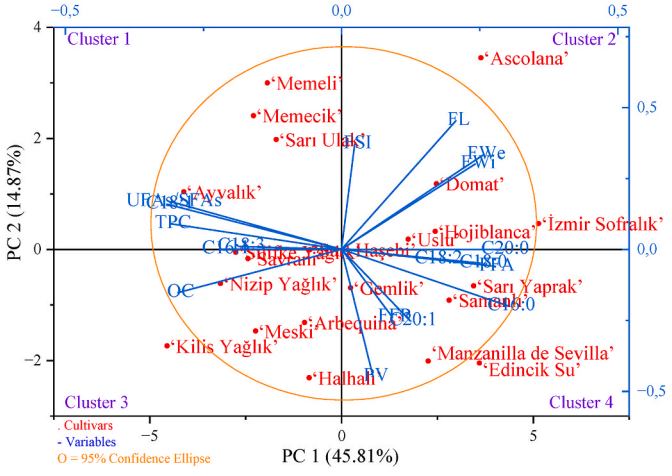


Fig. 3. Biplot for the studied olive cultivars and variables based on PC1/PC2 of traits. For abbreviations, please see Tables 1–3.

3.5. Heat map analysis (HMA)

According to the hierarchical clustering analysis presented in the heat map (Fig. 4), both the olive cultivars and evaluated traits were classified into distinct sub-clusters, providing complementary insights into the phenotypic and biochemical diversity observed across the cultivars. The clustering of traits resulted in four main sub-groups, namely A1, A2, B1, and B2, each reflecting specific dimensions of variability.

Trait cluster A1 comprised fruit weight, fruit length, fruit width, oil content, peroxide value, palmitic acid, stearic acid, linoleic acid, and the ratio of unsaturated to saturated fatty acids. This group primarily reflects morphometric characteristics combined with key fatty acid components and general oil quality parameters, suggesting that these variables co-vary and jointly influence fruit yield potential and basic compositional quality. Cluster A2 included fruit flesh ratio, total phenolic compounds, and oleic acid, indicating a strong emphasis on nutritional and antioxidant traits, which are crucial for both consumer health and oxidative stability of the oil. Cluster B1 consisted of fruit shape index, free fatty acids, palmitoleic acid, linolenic acid, and arachidic acid. This grouping represents traits associated with lipid oxidation sensitivity and specific minor fatty acids, in conjunction with fruit morphology. These variables may be particularly relevant in post-harvest quality assessments. Lastly, cluster B2, composed solely of gadoleic acid, appears to behave independently from other traits, underscoring its unique variability and potential as a discriminative biochemical marker among cultivars.

Olive cultivars were similarly grouped into four main clusters: C1, C2, D1, and D2. Cluster C1 included 'Domat', 'Edincik Su', 'İzmir Sofralık', 'Manzanilla de Sevilla', 'Samanlı', and 'Sarı Yaprak'. These cultivars showed a clear association with A1 traits, suggesting a shared pattern of large fruit size, elevated oil content, and prominent saturated and unsaturated fatty acid levels. Notably, 'Edincik Su' was associated with both fruit width and length, while 'İzmir Sofralık' was linked to oil content and peroxide value, indicating an overall emphasis on yield and compositional bulk. Cluster C2, containing 'Ascolana', 'Hojiblanca', and 'Uslu', was closely aligned with A2 traits. These cultivars displayed

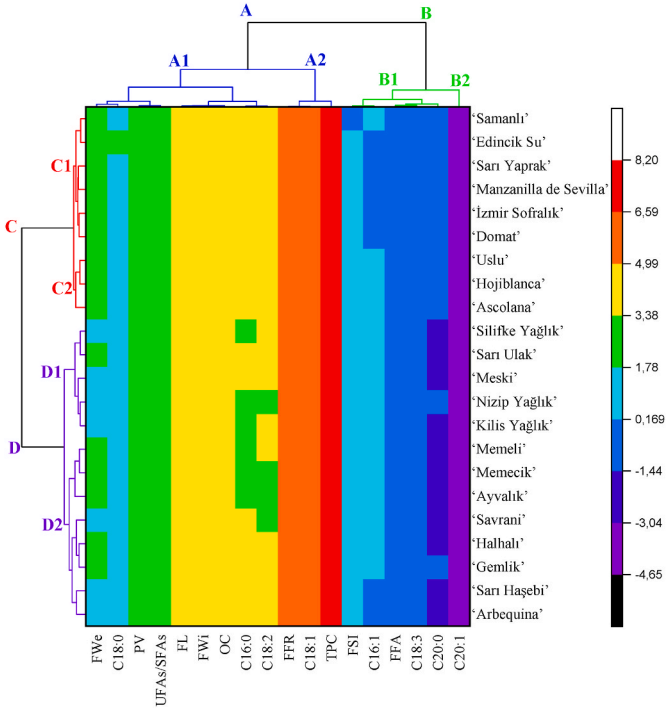


Fig. 4. Visualization of clustering patterns of olive cultivars and traits using heat map. For abbreviations, please see Tables 1–3.

elevated oleic acid and total phenolic contents, as well as a higher fruit flesh ratio, marking them as promising candidates for high-quality olive oil production with enhanced antioxidant profiles. Cluster D1 comprised 'Kilis Yağlık', 'Meski', 'Nizip Yağlık', 'Sar Ulak', and 'Silifke Yağlık'. This group showed strong associations with B1 traits, which include high free fatty acid content, polyunsaturated fatty acids prone to oxidation, and distinctive fruit shape. Such a profile may reflect greater susceptibility to oil degradation or shorter shelf life, although environmental adaptation should also be considered. Cluster D2 included 'Arbequina', 'Ayvalık', 'Gemlik', 'Halhalı', 'Memecik', 'Memeli', 'Sarı Haşebi', and 'Savrani', which were uniquely linked to B2. The exclusive association with gadoleic acid suggests that these cultivars possess a distinct long-chain fatty acid profile, potentially relevant for cultivar differentiation or specialized oil profiles.

Overall, the clustering analysis revealed coherent associations between specific trait clusters and cultivar groups, highlighting biologically meaningful relationships. The co-occurrence of A1 and C1 underscores the dominance of size- and yield-related traits in those cultivars, whereas the alignment of A2 with C2 points toward cultivars of superior oil quality. Meanwhile, the D1–B1 interaction signals the presence of cultivars with more challenging compositional features, and the unique behavior of D2 concerning B2 suggests unexplored diversity in gadoleic acid metabolism. These results reinforce the value of hierarchical clustering in identifying functional groupings among cultivars and traits, contributing to the strategic selection of cultivars for breeding, conservation, and quality improvement purposes. Similar analytical approaches were employed in a recent study by Roussos et al. (2025), where heat map-based clustering yielded different grouping patterns. This divergence may reflect variations in cultivar origin, analytical parameters, or trait emphasis, underscoring the influence of dataset-specific factors on clustering outcomes.

3.6. Integration of ANN modeling into trait analysis

To complement the multivariate statistical approaches, an ANN model was developed to assess the predictive relationships between key pomological, biochemical, and fatty acid traits and the overall olive oil quality. The ANN model, composed of 10 input variables, effectively captured nonlinear interactions that traditional linear models may overlook. Notably, traits such as oleic acid, UFAs/SFAs ratio, and total phenolic compounds emerged as strong positive predictors of oil quality, whereas elevated levels of palmitic acid and free fatty acids showed a negative influence. These findings were consistent with the results obtained from correlation analysis and regression models, confirming the robustness of the selected traits.

The ANN model's layered architecture, comprising a pattern and summation layer, enabled the integration of complex multidimensional inputs into a single output reflecting predicted oil quality scores (Fig. 5). This approach not only strengthens the interpretability of the biochemical dataset but also provides a practical framework for cultivar selection in olive breeding programs aiming to enhance nutritional and functional oil traits.

ANNs offer a powerful, data-driven approach to analyzing complex biological interactions that cannot be easily modeled using conventional statistical methods. Multivariate statistical techniques have long been employed to classify olive oils based on cultivar origin, extraction method, and geographical source (Chen et al., 2022; Gumus et al., 2018; Lechhab et al., 2021; Sakar et al., 2017, 2021). Notably, Gumus et al. (2018) effectively combined ANN and principal component analysis to classify Turkish olive oils based on analytical parameters. Similarly, Sakar et al. (2022) integrated ANN with CMA and principal component analysis in a study conducted in Morocco, while Cervera-Gascó et al. (2023) successfully applied ANN modeling to discriminate Spanish

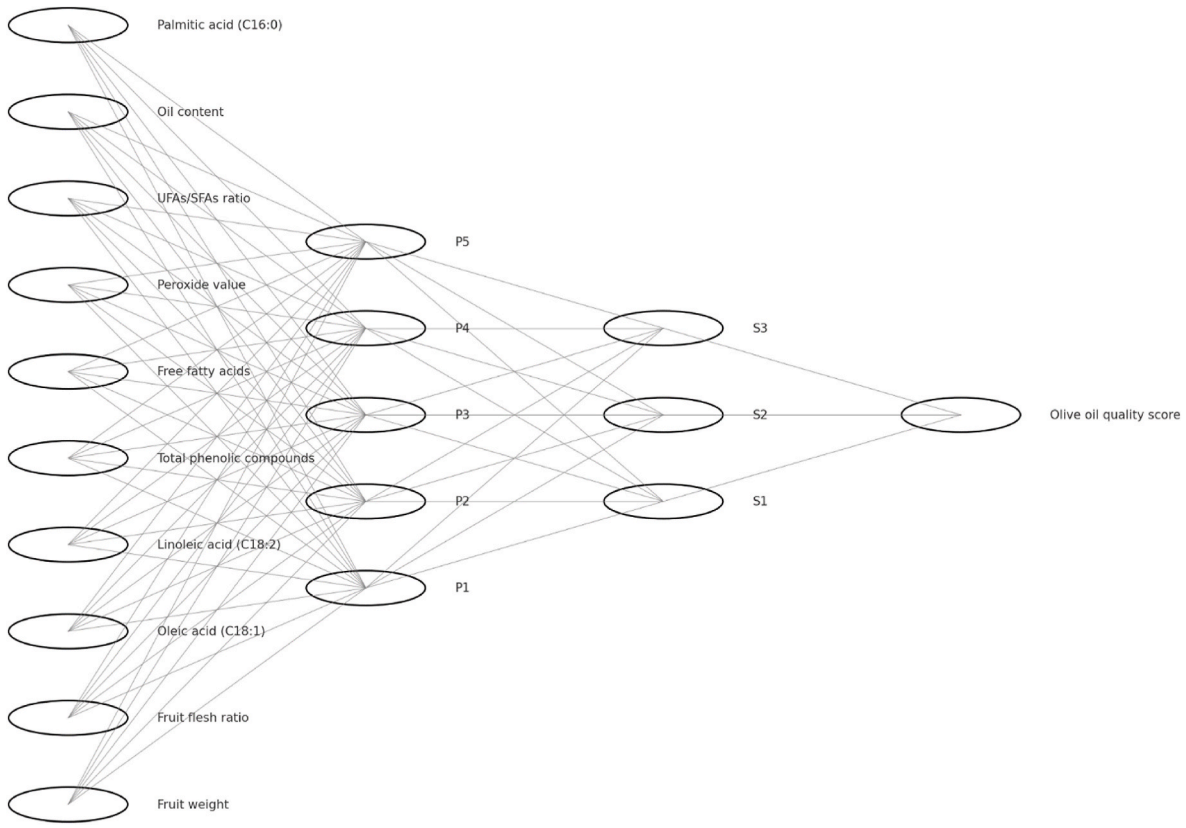


Fig. 5. Schematic architecture of the ANN model used to predict olive oil quality based on 10 selected input traits. The model comprises four layers: input layer (10 variables), pattern layer (5 neurons), summation layer (3 neurons), and output layer (olive oil quality score). Arrows represent the flow of information through weighted connections.

monovarietal olive oils. Inspired by recent advances, the present study employs ANN modeling in combination with a comprehensive dataset encompassing biochemical, pomological, and fatty acid characteristics, alongside various multivariate analytical techniques. Notably, this work represents one of the first integrated efforts to simultaneously apply CMA, MRA, PCA, HMA, and ANN modeling for an in-depth evaluation of olive oil quality and the identification of key discriminative traits across diverse olive cultivars.

In general, the present findings provide valuable insights into the biochemical diversity and oil quality traits of the studied olive cultivars. These results highlight the significance of genotype-dependent variation in determining the functional value of olive products.

The findings of this study also hold substantial implications for functional cultivar selection within ongoing olive breeding programs. Cultivars such as 'Savrani', 'Memecik', 'Ayvalık', and 'Halhalı', which exhibited superior profiles in terms of oleic acid content, total phenolic concentration, and unsaturation indices, can serve as promising parental lines for the development of high-quality and health-promoting genotypes. These traits are directly associated with oxidative stability, nutritional value, and consumer acceptance—critical parameters in modern olive breeding efforts. Furthermore, the integration of multivariate statistical and ANN-based predictive modeling offers a powerful framework to identify key functional traits with high heritability and predictive potential. This integrated approach facilitates informed decision-making in parent selection, accelerates breeding progress, and strengthens the development of functionally enhanced cultivars adapted to both nutritional and industrial requirements.

Overall, these outcomes contribute to the long-term objectives of olive improvement programs focused on enhancing oil quality, antioxidant potential, and overall functional value.

4. Conclusions

This study provides an integrative chemometric assessment of 22 olive (*Olea europaea* L.) cultivars, evaluating their fruit quality attributes, fatty acid composition, and biochemical traits under uniform agroecological conditions. By applying a multivariate statistical framework, combined with an ANN-based predictive model, we successfully identified complex trait interrelationships that contribute to olive oil quality and nutritional value. The findings highlight considerable genotypic variability among cultivars, with notable differences in key parameters such as oleic acid content, unsaturation/saturation ratios (UFAs/SFAs), total phenolic content, free fatty acids, and peroxide values.

Oleic acid, as the predominant monounsaturated fatty acid, showed strong positive associations with phenolic content and oxidative stability indices, reinforcing its role as a cornerstone of both oil quality and human health. Conversely, elevated levels of palmitic and linoleic acids were linked with increased free fatty acid content and reduced oil stability. The strong correlations observed between antioxidant capacity and key fatty acid traits further underscore the functional synergy between biochemical and nutritional parameters in olive oils.

PCA and biplot visualizations enabled the identification of cultivar clusters with distinct compositional profiles, facilitating the selection of superior cultivars with desirable trait combinations. Cluster groups A1 and B2, for example, demonstrated elevated oleic acid, high total phenolics, and favorable UFAs/SFAs ratios, suggesting their suitability for premium oil production with enhanced health-promoting characteristics. Among the studied genotypes, 'Savrani', 'Memeli', 'Halhalı', 'Memecik', and 'Ayvalık' emerged as the top-performing cultivars, displaying optimal combinations of fruit weight, oil content, oleic acid proportion, and total phenolic concentration.

Moreover, the ANN model provided robust predictive accuracy in estimating overall oil quality based on ten selected input variables. The successful deployment of ANN modeling underscores the potential of machine learning approaches to support precision agriculture, varietal selection, and quality forecasting in olive breeding programs. These models can be valuable tools for integrating multidimensional datasets and offering real-time recommendations for cultivar deployment based on specific biochemical profiles and end-use objectives.

From a broader perspective, the study's outcomes contribute to the growing demand for scientifically informed cultivar selection strategies aligned with public health goals and functional food production. The integration of chemometric and ANN-based approaches not only improves our understanding of trait interdependencies but also enhances decision-making capacity in selecting cultivars with optimized fatty acid profiles, strong antioxidant potential, and low oxidative degradation.

In conclusion, the combination of multivariate analysis and ANN modeling offers a comprehensive platform for characterizing olive cultivars and identifying those with superior nutritional and oil quality attributes. The findings have direct implications for cultivar improvement, sustainable olive production, and the development of high-value olive oils tailored to health-conscious markets. Future studies may further refine these models by incorporating additional environmental variables and exploring genotype-by-environment interactions to enhance predictive robustness across diverse growing conditions.

CRediT authorship contribution statement

Celil Toplu: Investigation, Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Yazgan Tunç:** Investigation, Conceptualization, Formal analysis, Software, Supervision, Writing – original draft. **Ali Khadivi:** Investigation, Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. **Daya Shankar Mishra:** Investigation, Methodology, Visualization, Writing – review & editing.

Informed consent

Not applicable.

Ethics approval and consent to participate

Not Applicable.

Research involving human participants and, or animals

Not applicable.

Clinical trial study

Not applicable.

Clinical trial number

Not applicable.

Statement specifying permissions

For this study, we obtained permission to collect and study olive issued by the Agricultural and Forestry Ministry of Türkiye.

Statement on experimental research and field studies on plants

The either cultivated or wild-growing plants sampled comply with relevant institutional, national, and international guidelines and domestic legislation of Türkiye.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the co-corresponding authors upon reasonable request.

Funding

Not applicable.

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.

Acknowledgment

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118750.

Data availability

Data will be made available on request.

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