Original scientific paper
DOI: /10.5513/JCEA01/27.2.5063
Journal of Central European Agriculture, 2026, 27(2), p.520-531

Bioactive and nutritional potential of extracts from differently processed olive leaves (*Olea europaea* L.) as by-products

Bioaktívny a nutričný potenciál extraktov z rôzne spracovaných olivových listov (*Olea europaea* L.) ako vedľajších produktov

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Received: October 17, 2025; accepted: June 5, 2026

ABSTRACT

Olive leaves (*Olea europaea* L.; OL), a by-product of olive processing and olive oil production, constitute a significant fraction of residual biomass. However, increasing scientific attention has been directed toward their therapeutic potential, biological activity, and nutraceutical properties, with promising applications in the prevention and supportive treatment of chronic diseases. In this study, the bioactive and nutritional profile of OL ('Frantoio' cultivar) was evaluated in differently processed forms. The antioxidant activity (AA; DPPH method) and total phenolic content (TPC) were compared among fresh, air-dried, and lyophilized samples prepared using common post-harvest processing methods. In addition, the mineral composition (ICP-OES) and fatty acid profile (GC-FID) were analyzed directly in lyophilized OL. The extract obtained from lyophilized OL exhibited strong AA (70.12%), followed by air-dried OL (69.84%) and fresh samples of OL (50.72%). The results obtained for TPC varied according to the sample preparation method, with lyophilized OL exhibiting the highest TPC level (45.75 mg GAE/g). The mineral composition determined directly in lyophilized OL showed the following order of predominance: Ca > K > Al > Na > Mg > Fe. Notably, a high proportion of α-linolenic acid was detected in lyophilized OL (22.20%), suggesting that this by-product may represent an interesting source of n-3 polyunsaturated fatty acids (PUFA). These findings indicate the bioactive potential of lyophilized OL and support their possible use as a functional supplement for enhancing the nutritional value of food products. Further studies are required to evaluate the bioavailability and therapeutic effects of OL-derived bioactive compounds using *in vitro*/preclinical models.

Keywords: olive leaves, post-harvest processing, antioxidant activity, total phenolic content, mineral composition, fatty acid profile

ABSTRAKT

Olivové listy (*Olea europaea* L.; OL), vedľajší produkt spracovania olív a výroby olivového oleja, predstavujú významnú frakciu reziduálnej biomasy. Rastúci vedecký záujem sa však zameriava na ich terapeutický potenciál, biologickú aktivitu a nutraceutické vlastnosti so sľubným využitím v prevencii a podpornej liečbe chronických ochorení. V tejto štúdii bol hodnotený bioaktívny a nutričný profil OL (kultivaru 'Frantoio') v rôzne spracovaných formách. Antioxidačná aktivita (AA; DPPH metóda) a celkový obsah polyfenolov (TPC) boli porovnávané medzi čerstvými, na vzduchu sušenými a lyofilizovovanými vzorkami pripravovanými pomocou bežných pozberových metód spracovania. Okrem toho bolo analyzované priamo v lyofilizovaných OL minerálne zloženie (ICP-OES) a profil mastných kyselín (GC-FID). Extrakt získaný z lyofilizovaných OL vykazoval silnú AA (70,12%), nasledovali sušené OL (69,84%) a čerstvé vzorky OL (50,72%). Výsledky získané z TPC sa líšili v závislosti od spôsobu prípravy vzorky, pričom lyofilizované OL dosahovali najvyššiu úroveň TPC (45,75 mg GAE/g). Minerálne zloženie stanovené priamo v lyofilizovaných OL vykazovalo nasledovnú prevahu týchto prvkov: Ca > K > Al > Na > Mg > Fe. Pozoruhodné je, že v lyofilizovaných OL bol detegovaný vysoký podiel kyseliny α-linolénovej (22,20%), čo naznačuje, že tento vedľajší produkt môže predstavovať zaujímavý zdroj n-3 polynenasýtených mastných kyselín (PUFA). Tieto zistenia poukazujú na bioaktívny potenciál lyofilizovaných OL a podporujú ich možné využitie ako funkčného doplnku stravy na zvýšenie nutričnej hodnoty potravinárskych výrobkov. Na zhodnotenie biologickej dostupnosti a terapeutických účinkov bioaktívnych zlúčenín derivovaných z OL sú potrebné ďalšie štúdie pomocou *in vitro*/predklinických modelov.

Kľúčové slová: olivové listy, pozberové spracovanie, antioxidačná aktivita, celkový obsah polyfenolov, minerálne zloženie, profil mastných kyselín

INTRODUCTION

The olive tree (*Olea europaea* L.) is one of the oldest and most economically important cultivated species worldwide, originating from the Mediterranean region. The cultivation of olive trees dates back more than 6,000 years (Besnard et al., 2013). This plant has profoundly influenced the agriculture, economy, and cultural heritage of Mediterranean societies (Diez et al., 2015). It serves not only as the primary source of olive oil, a key component of the Mediterranean diet, but also as a source of valuable by-products, including olive leaves (OL) (Casilla García et al., 2025). Although olive oil has been extensively studied for its sensory qualities and health-promoting properties, the potential of OL has only recently received increased scientific attention. Nevertheless, OL remain underappreciated in the food and pharmaceutical industries (Markhali et al., 2020).

Olive leaves are generated as a by-product of olive processing and olive oil production, accounting for about 10% of the total biomass (Palmeri et al., 2022). However, a large proportion of this material is still underutilized and is often discarded through unsustainable practices, such as incineration (Talhaoui et al., 2015). From an agro-environmental perspective, the valorization of OL may support sustainable olive cultivation by reducing waste and enhancing resource efficiency, thereby contributing to the development of circular bioeconomy principles within the olive-growing sector (Donner et al., 2022). Therefore, OL biomass represents a valuable resource with potential relevance for human health, environmental protection, and sustainable economic development (Markhali et al., 2020).

Extracts obtained from OL have attracted considerable attention due to their high content of bioactive compounds associated with various health-promoting properties. Several phytochemical studies have identified a wide range of compounds in OL, primarily secoiridoids, phenolic acids, alcohols, phenols, and flavonoids (luteolin-7-O-glucoside, rutin, apigenin-7-O-glucoside, luteolin-4-O-glucoside) (Bouaziz and Sayadi, 2005; Japón-Luján and Luque de Castro, 2007; Rahmanian et al., 2015), as well as triterpenoids and lignan derivatives (Hashmi et al., 2015). The concentration of the phenolic fraction in OL has been reported to be several times higher than that found in olive oil or olives themselves

and may vary depending on the olive cultivar, climate (Abaza et al., 2005), processing methods, and growing conditions (Özcan et al., 2019; Pasković et al., 2025). Due to their high levels of polyphenols, antioxidants, and other phytochemicals, OL extracts may exert synergistic biological effects, making them promising nutritional and therapeutic supplements (Mansour et al., 2023). Oleuropein, the main phenolic compound in OL, is particularly abundant and contributes to various health-promoting effects (Soldo et al., 2024). Hydroxytyrosol, another key phenolic compound derived from the hydrolysis of oleuropein, is known for its antioxidant and anti-inflammatory properties, which have been documented in food, medical, and pharmaceutical research (Jeon and Choi, 2018). Although OL are primarily recognized for their rich content of phenolic compounds, only a few studies have investigated other biologically active compounds present in this material, including fatty acids (FA). Oleic acid (C18:1cis n-9) is the main unsaturated FA in olive oil, whereas in OL a predominance of α-linolenic acid (C18:3 n-3) has been observed (Cavalheiro et al., 2015). This finding is of particular importance because this FA can be metabolized through sequential desaturation and elongation by enzyme systems, leading to the production of long-chain polyunsaturated fatty acids (PUFA) of the n-3 series (Silva et al., 2014). Therefore, the high α-linolenic acid content found in OL makes this by-product a promising additional source of n-3 PUFA.

The mineral composition of OL varies according to soil and climate conditions. Generally, the most commonly determined elements found in OL include sodium (Na), magnesium (Mg), phosphorus (P), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and lead (Pb), with calcium (Ca) and potassium (K) usually being among the most abundant minerals (Ibrahim et al., 2016; de Oliveira et al., 2023).

Several biological effects of OL have been reported in experimental studies. Poudyal et al. (2010) demonstrated that rats fed a diet rich in carbohydrates and lipids and supplemented with OL extract showed attenuated cardiac, hepatic, and metabolic alterations. In addition, Botsoglou et al. (2010) and Martins et al. (2009) reported that the inclusion of OL in animal feed improved meat quality by reducing lipid oxidation. Therefore, OL can be considered an important raw material with the potential for use as a natural antioxidant. Moreover, OL have been associated with a wide range of biological activities, including anticancer, anti-inflammatory, antidiabetic, and antihypertensive effects (Mansour et al., 2023; Al-Dabbas et al., 2025; Demirer and Samur, 2025). Due to these properties, OL may be beneficial in relation to metabolic disorders, including *diabetes mellitus* and hypercholesterolemia (Stevens et al., 2021).

Considering the importance of this crop, the present study aimed to evaluate the bioactive and nutritional profile of OL ('Frantoio' cultivar) in differently processed forms (fresh, air-dried, and lyophilized). The antioxidant activity (AA) and total phenolic content (TPC) were compared among the prepared samples. In addition, the FA profile and mineral composition were analyzed directly in lyophilized OL. The study also aimed to highlight the potential of OL as a valuable by-product for further applications, with particular emphasis on their relevance for future research related to human health and possible therapeutic applications.

MATERIAL AND METHODS

Plant material and sample preparation

Fresh OL of *Olea europaea* L. ('Frantoio' cultivar) were obtained from the Padova region (Italy). The plant material was collected from March to May in 2023, 2024, and 2025, corresponding approximately to the early spring phenological stages of olive trees, as summarized according to the BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) scale adapted for *Olea europaea* L. Branches bearing both young and fully developed leaves were randomly harvested from accessible heights around the entire tree canopy to minimize the effects of sun exposure. After harvesting, the samples were placed in plastic bags and transported to the laboratory. Olive leaves were divided into three groups and prepared using common post-harvest processing

methods. Fresh OL were stored at -20 °C. Drying was performed by spreading OL at room temperature in the laboratory (22 – 25 °C). Samples of OL were lyophilized using a LyoQuest Plus-55 ECO (Telstar, Terrassa, Spain) under vacuum for 48 h, with the condenser temperature maintained at -48 ± 2 °C. Subsequently, all air-dried and lyophilized OL samples were finely ground using a Philips ProBlend 4 blender (model HR2100/40; Amsterdam, the Netherlands), and the resulting powdered biomass (particle size < 1.0 mm) was vacuum-stored at room temperature in the laboratory and protected from light and moisture until extraction.

Extraction process

Extracts from fresh, air-dried, and lyophilized OL were prepared separately as follows: 20 mg of the ground sample (in duplicate) was mixed with 1.0 mL of extraction solvent (MetOH:H_2O: acetic acid = 80:18:2; Centralchem, Ltd., Bratislava, Slovakia). The mixture was homogenized and sonicated at 50 °C for 5 min. After centrifugation (15,616 × g, 10 min, 20 °C; Hettich Rotina 380, Tuttlingen, Germany), the supernatant was collected into clean 2.0 mL microtubes (Eppendorf, Hamburg, Germany). An additional 1.0 mL of extraction solvent was added to the pellet, and the extraction procedure was repeated.

Determination of antioxidant activity

The AA of the OL extracts was evaluated spectrophotometrically using the DPPH radical scavenging assay, as described by Brand-Williams et al. (1995), with slight modifications for a 96-well microplate protocol (Kováčiková et al., 2025). Absorbance readings were recorded at 515 nm using a DYNARead microplate reader (Dynex Technologies s.r.o., Prague, Czech Republic). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Schnelldorf, Germany; concentration range 0 - 100 mg/L; R^2 = 0.9946) was used as the standard reference substance. The power of AA was categorized as weak (0 - 29%), medium-strong (30 - 59%), or strong (≥ 60%).

Determination of total polyphenol content

The TPC was determined using the Folin–Ciocalteu method with slight modifications according to Kováčiková et al. (2025). Absorbance was measured at 765 nm using a Glomax Multi+ UV-Vis microplate reader (Promega Corp., Madison, WI, USA). Measurements were calibrated using a standard curve prepared with gallic acid (Merck KGaA, Darmstadt, Germany; concentration range 0 - 500 mg/L; R^2 = 0.9849). The TPC values were calculated based on the calibration curve and expressed as gallic acid equivalents (GAE).

Determination of fatty acid composition

The FA profile was determined directly in lyophilized OL, following the procedure described by Knazicka et al. (2025). Fatty acid methyl esters (FAME) were prepared from the lyophilized OL matrix using petroleum ether extraction and subsequently analyzed by gas chromatography (GC) with a flame ionization detector (FID), performed on an Agilent 6890A system (Agilent Technologies, Santa Clara, CA, USA) equipped with a multimode injector. The analysis was carried out on a DB-23 capillary column (Agilent Technologies 122-2361; 60 m × 250 µm × 0.15 µm). The system was calibrated using a 37-component Supelco standard mixture (Supelco 47885-U; Sigma-Aldrich, Laramie, WY, USA). Helium was used as the carrier gas at a constant flow rate of 2.225 mL/min. A 1.0 µL sample was injected for each analysis. Both the inlet and detector temperatures were set to 280 °C. Fatty acid peaks were identified by comparison with the retention times of standard methyl esters, and the results were expressed as a percentage of total FAs using Agilent OpenLab ChemStation software (OpenLab CDS ChemStation Edition B.04.01). All samples were analyzed in duplicate.

Determination of mineral composition

Prior to elemental analysis, the lyophilized OL samples were subjected to a pre-analytical procedure using a high-performance microwave digestion system, Ethos-One (Milestone Srl, Sorisole, BG, Italy). A closed-vessel microwave acid digestion method was applied, in which

5.0 mL of nitric acid (HNO_3; ≥ 69%; from Lambda Life s r.o., Bratislava, Slovakia; producer: Sigma-Aldrich Chemie GmbH, Steiheim, Germany), and 1.0 mL of hydrogen peroxide (H_2O_2; ≥ 30%; from Lambda Life s r.o., Bratislava, Slovakia; producer: Sigma-Aldrich Chemie GmbH, Steiheim, Germany) were added directly to PTFE vessels. The obtained samples, including the blank, were digested according to the manufacturer's recommended plant method to ensure the most reliable results. To minimize contamination, all chemicals used in this procedure were of high purity grade for trace analysis.

The determination of macro- and microelement contents directly in lyophilized OL followed the method described by Árvay et al. (2019). Each sample was measured in triplicate using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Agilent ICP-OES 720, Agilent Technologies Inc., Santa Clara, CA, USA), equipped with an axial plasma configuration and an SPS-3 autosampler (Agilent Technologies GmbH, Basel, Switzerland). Calibration standards for each analyzed element were prepared to match the expected concentration range in the samples. The selected elements were determined under standard operating conditions: power (0.90 kW), replicate read time (3 s), instrument stabilization (20 s), sample uptake delay (25 s), pump rate (15 rpm), rinse time (20 s), and CCD detector temperature (-35 °C). The gas flow parameters of the ICP-OES included plasma of 15 L/min, auxiliary of 1.50 L/min and nebulizer of 1.0 L/min. The following elements were quantified in the analyzed solutions: Ag (328.068 nm), Al (167.019 nm), As (188.980 nm), Ba (455.403 nm), Ca (315.887 nm), Cd (226.502 nm), Co (228.615 nm), Cr (267.716 nm), Cu (324.754 nm), Fe (234.350 nm), K (766.491 nm), Li (670.783 nm), Mg (383.829 nm), Mn (257.610 nm), Na (589.592 nm), Ni (231.604 nm), Pb (220.353 nm), Sb (206.834 nm), Se (196.026 nm), Sr (407.771 nm), and Zn (206.200 nm). In this study, the Multielement Standard Solution V for ICP (Sigma-Aldrich GmbH, Buchs, Switzerland) was used for calibration, while argon and carbon served as internal standards. The accuracy of the analytical procedure was verified using certified reference material ERM-CE278k (muscle tissue; IRMM, Geel, Belgium).

Statistical analysis

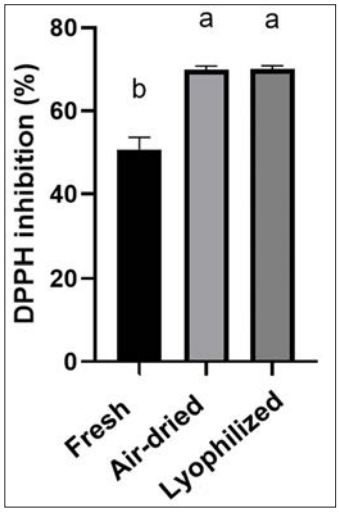
All obtained data were statistically evaluated using GraphPad Prism 8.0.1 (GraphPad Software Incorporated, San Diego, CA, USA). One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was applied to determine significant differences, with the significance level set at $P<0.05$.

RESULTS AND DISCUSSION

Evaluation of AA in differently processed OL samples

Post-harvest sample processing is considered a key factor influencing the bioactive potential of OL. Previous studies have shown that drying methods and extraction techniques can significantly affect the stability, extractability, and recovery of antioxidant compounds from olive-derived materials (Kamran et al., 2015; Cör Andrejč et al., 2022; Filgueira-Garro et al., 2022; Castillo-Luna et al., 2023). In the present study, extracts obtained from air-dried OL (69.84 ± 0.84%) and lyophilized OL (70.12 ± 0.64%) exhibited strong AA (Fig. 1), whereas fresh OL showed the lowest value (50.72 ± 2.74%; $P<0.05$). These results emphasize the importance of sample pre-treatment in the preparation of OL extracts. Lyophilization appeared to be the most effective sample preparation method for preserving AA in OL, although the difference between air-dried and lyophilized samples was minimal. Our findings are consistent with those of Filgueira-Garro et al. (2022), who reported that freeze-drying and air-drying were the most effective methods for preserving total antioxidant capacity in OL. Feng et al. (2021) showed that air-drying at room temperature (25 °C) was the most suitable drying method for OL, as it preserved leaf greenness, enhanced luminosity, and contributed to a high content of phenolic compounds. The authors also recommended avoiding high temperatures during the OL drying process, as elevated temperatures may negatively affect the quality and bioactive composition of the leaves. The importance of sample pre-treatment was also reported by Kamran et al. (2015), who observed that drying markedly influenced the recovery of antioxidant biophenols from OL. In their study, total antioxidant capacity measured by ABTS (radical cation decolorization

assay) and DPPH assays followed the order: oven-dried (105 °C) > freeze-dried > air-dried > fresh > oven-dried OL (60 °C). Yancheva et al. (2016) demonstrated the AA of methanol extracts from OL using a range of *in vitro* assays, including DPPH, ABTS, FRAP (ferric reducing antioxidant power), and CUPRAC (cupric reducing antioxidant capacity assay), in order to capture different radical-scavenging and reducing mechanisms. The highest AA values were observed in leaves of the cultivar 'Chondrolia Halkidiki'. Moreover, a strong correlation between TPC and AA was established, supporting the important contribution of phenolic compounds to the antioxidant potential of OL extracts.



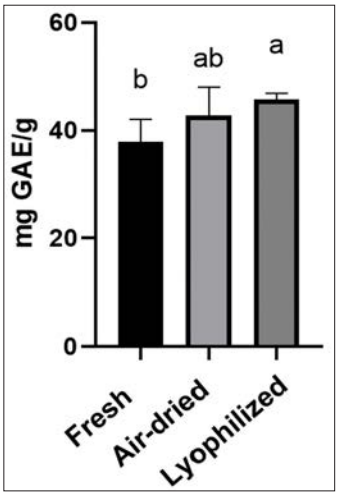
Legend: Data are presented as mean ± standard deviation; different small letters indicate statistically significant differences at the level $P<0.05$ between samples (One-way ANOVA followed by Tukey's multiple comparison test).

Figure 1. Comparison of the antioxidant activity of extracts from differently processed OL samples

Health properties of polyphenolic antioxidant plant components and their potential use as natural food additives have been the subject of considerable scientific and commercial interest (Lafka et al., 2013). The antioxidant potential of OL has also been confirmed in several *in vitro* studies. Lins et al. (2018) demonstrated that OL extracts exhibited strong AA against a wide range of reactive oxygen species and protected human erythrocytes from free radical-induced damage. Lfitat et al. (2020) confirmed the antioxidant efficacy of OL and highlighted the role of secondary metabolites in metal chelation and free radical scavenging. Similarly, Monteleone et al. (2021) reported the significant antioxidant capacity of OL extracts. Pennisi et al. (2023) further demonstrated that extracts obtained from *Olea europaea* vars *sativa* and *sylvestris* prevented lipid peroxidation in human HeLa cells and increased the activity of antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase.

Evaluation of TPC in differently processed OL samples

The highest phenolic yields were obtained using high-percentage alcohol solvents (Castillo-Correa et al., 2025). Methanol appears to be highly efficient for the extraction of phenolic compounds from OL (Orak et al., 2019), likely due to its optimal polarity and penetration capacity. The extraction solvent used in the present study consisted of methanol, water, and acetic acid. The extract from lyophilized OL samples exhibited significantly ($P<0.05$) higher TPC levels (45.75 ± 1.14 mg GAE/g) than extracts from air-dried (42.74 ± 5.27 mg GAE/g) or fresh OL samples (37.94 ± 4.14 mg GAE/g). The results are presented in Figure 2. The higher TPC observed in lyophilized OL may be associated with enhanced extractability and recovery of phenolics, possibly due to lyophilization-induced structural changes in plant tissues. These changes may have promoted the release of larger amounts of bound phenolics from disrupted cellular constituents; however, this method is limited by its long processing time and relatively high cost (Karam et al., 2016). Similar TPC ranges have been reported for 'Frantoio' OL (21.6 –106.9 mg GAE/g DW) in the study by Kamran et al. (2015). Zhang et al. (2022) compared the effects of freeze-drying and hot air-drying on the phytochemical profiles and biological activities of OL. Their study suggested that freeze-drying was a better technique than hot air-drying for enhancing the flavonoid content and biological activity of dried OL. In addition, hot air-drying was shown to be a viable alternative drying method for ensuring the maximal recovery of iridoids. These findings suggest that the effect of drying on OL bioactivity depends not only on TPC but also on the specific groups of compounds preserved or released during processing.



Legend: Data are presented as mean ± standard deviation; different small letters indicate statistically significant differences at the level $P<0.05$ between samples (One-way ANOVA followed by Tukey's multiple comparison test).

Figure 2. Comparison of the total phenol content of extracts from differently processed OL samples

Although the present results indicate an association between TPC and AA in OL extracts, further studies are needed to better understand the mechanisms involved. Future research should focus on the quantification of individual bioactive compounds (e.g., oleuropein and hydroxytyrosol) and on the evaluation of their individual and potential synergistic effects. This would help clarify whether the enhanced AA of dried and lyophilized OL extracts is mainly driven by the concentration of specific phenolic compounds or by matrix-dependent interactions affecting their extractability, stability, and antioxidant effectiveness.

Evaluation of fatty acid profile in lyophilized OL

Studies on the FA profile of lyophilized OL ('Frantoio' cultivar) are limited; therefore, the present study provides an overview of the major FA components identified directly in lyophilized OL, highlighting compounds of potential nutritional relevance (Table 1). The FA profile showed a predominance of PUFA > MUFA > SFA. A high content of α-linolenic acid (C18:3 n-3) was found in lyophilized OL (22.20%), indicating that this by-product may represent an interesting source of n-3 PUFA (29.53%), which is consistent with the findings of Cavalheiro et al. (2015). Similar observations were reported by Ferreira et al. (2023), who confirmed a predominance of this FA in leaves and leaf sprouts of three olive cultivars: 'Verdeal' (40.42 and 37.35%) > 'Madural' (37.87 and 26.48%) > 'Cobrançosa' (26.48 and 24.18%). The α-linolenic acid is a nutritionally important essential FA and a precursor of eicosanoids with anti-inflammatory and antithrombotic properties (Ruiz et al., 2002). Numerous studies have associated its intake with beneficial effects on cardiovascular health (Connor, 2000; Simopoulos, 2002; Schacky and Harris, 2007). Essential FA may influence lipoprotein concentrations, the fluidity of biological membranes, the activity of membrane enzymes and receptors, blood pressure regulation, and mineral metabolism (Orsavova et al., 2015). According to Dabbou et al. (2010), OL exhibited higher levels of PUFA than olive oil (18.11%), which contains about 17.48% linoleic acid (C18:2cis n-6) and 0.63% α-linolenic acid. The high PUFA content may increase susceptibility to oxidation; however, this effect could be counterbalanced by the presence of antioxidants that protect the OL from oxidative damage.

The 'Frantoio' cultivar also showed a considerable proportion of MUFA (24.82%), with erucic acid (C22:1 n-9) being the predominant component (13.21%). Recent evidence suggests that, at physiological concentrations, erucic acid may exert beneficial metabolic and anti-inflammatory effects. According to Galanty et al. (2023), erucic acid contributes to the modulation of lipid metabolism, reduction of hepatic fat accumulation, and support of mitochondrial function. Moreover, it has been reported to exhibit antioxidant (Goyal et al., 2024) and neuroprotective activities (Sharma et al., 2023). The second major MUFA was oleic acid (11.60%), which is widely recognized for its protective effects against coronary, autoimmune, and inflammatory disorders, as well as for its antithrombotic activity and role in blood pressure regulation (Jimenez-Lopez et al., 2020). In addition, oleic acid has been investigated for its potential protective role against tumor cell proliferation in epidemiological and animal studies (Moon et al., 2014; Lamy et al., 2016; Jiang et al., 2017; Giulitti et al., 2021).

Table 1. Fatty acid composition (%) determined directly in lyophilized OL by GC-FID

Notation	Individual of the Fatty Acids	Molecular Mass	Lyophilized of OL (x ± SD)
C14:0	Myristic acid	228.37	1.13 ± 0.008
C16:0	Palmitic acid	256.43	12.40 ± 0.037
C18:0	Stearic acid	284.48	1.68 ± 0.027
C18:1cis n-9	Oleic acid	282.47	11.60 ± 0.061
C18:2cis n-6	Linoleic acid	280.45	7.33 ± 0.030
C18:3 n-3	α-linolenic acid	278.43	22.20 ± 0.069
C20:0	Arachidic acid	312.53	1.21 ± 0.001
C22:1 n-9	Erucic acid	338.57	13.21 ± 0.087
	∑ PUFA		29.53
	∑ MUFA		24.82
	∑ SFA		16.43

Legend: PUFA – polyunsaturated fatty acid; MUFA – monounsaturated fatty acid; SFA – saturated fatty acid; OL – olive leaves.

Combined diet-drug therapies incorporating this nutraceutical have shown potential for synergistic protection against cancer progression (Carrillo et al., 2012). Our results indicate that lyophilized OL are characterized by a favorable unsaturated FA profile of potential nutritional interest.

The present study also showed that the 'Frantoio' cultivar contained lower levels of SFA (16.43%; Table 1), which followed the order: palmitic acid (12.40%) > stearic acid (1.68%) > arachidic acid (1.21%) > myristic acid (1.13%). Palmitic acid, although classified as an SFA, plays essential physiological roles. It serves as a major component of membrane phospholipids, influences the biophysical properties of cell membranes, and contributes to energy metabolism as a key substrate for β-oxidation (Carta et al., 2017). Moreover, palmitic acid participates in the process of protein palmitoylation, which regulates the localization and function of several signaling proteins (Linder and Deschenes, 2007). When consumed within balanced dietary limits, it is also necessary for maintaining normal lipid homeostasis and cellular signaling (Carta et al., 2017).

Evaluation of mineral composition in lyophilized OL

Olive leaves are considered plant material rich in both macro- and microelements, as well as bioactive compounds, and represent an alternative source with considerable nutritional and biological potential. Our results showed that the overall tendency of the analyzed elements was as follows (mg/kg DW): Ca > K > Al > Na > Mg > Fe > Sr > Mn > Zn > Ba > Cu > Sb > Pb > Se > Ni > Cr > Li > Ag (Table 2). The contents of As, Cd, and Co in the 'Frantoio' cultivar were below the limit of quantification (< LOQ) of the ICP-OES method used. The study by Antunes et al. (2021) observed that the mineral profile of OL showed higher concentrations of K and Ca, and lower concentrations of Fe, Mg, Na, Mn, P, and Zn. Cavalheiro et al. (2015) analyzed various OL varieties grown in southern Brazil using ICP-OES and concluded that OL may represent a source not only of Fe and Cu, but also of Ca, Mg, K, P, Zn, and Mn, all of which are present in moderate amounts when 50 g of dried OL are consumed.

In our study, Ca exhibited the highest concentration among the analyzed elements (7979.203 mg/kg DW).

Table 2. Mineral composition determined directly in lyophilized OL by ICP-OES

Elements	Lyophilized OL (mg/kg DW) (x ± SD)
Ca	7979.203 ± 52.920
K	6022.867 ± 11.981
Al	163.109 ± 1.794
Na	150.199 ± 0.224
Mg	77.951 ± 0.159
Fe	34.589 ± 0.126
Sr	30.993 ± 0.099
Mn	9.433 ± 0.035
Zn	5.856 ± 0.376
Ba	4.365 ± 0.007
Cu	1.731 ± 0.026
Sb	1.356 ± 0.397
Pb	0.485 ± 0.208
Se	0.437 ± 0.127
Ni	0.130 ± 0.017
Cr	0.114 ± 0.028
Li	0.054 ± 0.002
Ag	0.014 ± 0.000
As, Cd, Co	< LOQ[1]

Legend: OL – olive leaves; [1] < LOQ - below the limit of quantification.

Similar results were reported by Lee et al. (2005) and Bahloul et al. (2014), who also found high Ca contents in OL. Antunes et al. (2021) observed that Ca and K were the dominant macroelements across all analyzed varieties ('Frantoio', 'Koroneike', 'Manzanilha', 'Arbosana', and 'Arbequina'), with 'Frantoio' showing particularly high Ca accumulation, which is consistent with our findings. According to de Oliveira et al. (2023), OL represent a promising source of minerals such as Na, K, Mg, Ca, Mn, Fe, and Cu, supporting their potential role in nutritional supplementation and functional food applications. Calcium is a key macroelement involved in the development and maintenance of bones and teeth, while also contributing to the bioavailability of other nutrients (Bahloul et al., 2014). Therefore, the dietary inclusion of Ca-rich OL extracts or powders may contribute to the prevention and management of Ca deficiency. The K content in OL was 6022.867 mg/kg DW. Potassium, another important macronutrient, plays a key role in the regulation of heartbeat, maintenance of electrolyte balance, and support of muscle contraction (Terker et al., 2015; Terwoord et al., 2018; de Oliveira et al., 2023). Antunes et al. (2021) also confirmed that K is one of the most abundant mineral elements in OL. Adequate intake of both Ca and K has been associated with the prevention of cardiovascular diseases (McCarron and Reusser, 2001). Thus, the presence of these elements in lyophilized OL may contribute to the overall nutritional value of this plant material. However, despite their considerable levels, further studies are needed to verify their bioavailability, as some substances (e.g., phytates) may interfere with mineral absorption (Akwaowo et al., 2000; Cavalheiro et al., 2015).

CONCLUSION

Post-harvest sample processing had a significant effect on the bioactive and nutritional profile of OL. The present study demonstrated that lyophilized OL exhibited the highest AA and TPC, whereas fresh samples showed lower values. Lyophilization was the most effective pre-treatment method for preserving the bioactive potential of OL from the 'Frantoio' cultivar. In addition to their bioactive properties, lyophilized OL also presented a valuable FA profile, characterized by a predominance of PUFA, particularly α-linolenic acid, suggesting that OL may represent a potential source of n-3 FA. The presence of MUFA, especially erucic and oleic acids, together with considerable levels of macroelements such as Ca and K, further supports the nutritional potential of this agro-industrial by-product.

Overall, the obtained results highlight OL as a sustainable plant material with promising applications in functional foods, dietary supplements, and nutraceutical formulations. Since many commercial OL extracts are commonly prepared from fresh or air-dried leaves, further systematic research is needed to optimize ex-

traction and processing conditions, with particular attention to bioavailability, stability, and possible interactions of the identified compounds. These findings may be relevant for the food, nutraceutical, pharmaceutical, and cosmetic industries seeking to utilize OL extracts for their functional properties.

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of the Faculty of Agrobiology and Food Resources (FAFR), Slovak University of Agriculture in Nitra (Slovakia), No. 2/2024 "New perspectives on the utilization of olive tree leaves and determination of the effects of oleuropein on human health", co-financed by the Slovak Research and Development Agency APVV-21-0168, and VEGA 1/0357/26.

REFERENCES

Abaza, L., Taamalli, W., Temine, S.B., Daound, D., Gutierrez, F., Zarrouk, M. (2005) Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. Rivista Italiano Delle Sostanze Grasse, 132 (1), 12-18.

Akwaowo, E.U., Ndon, B.A., Etuk, E.U. (2000) Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook f.). Food Chemistry, 70 (2), 235-240. DOI: https://doi.org/10.1016/S0308-8146(99)00207-1

Al-Dabbas, M.M., Al-Jaloudi, R., Hamad, H.J., Al-Nawasrah, B.A., Al-Refaie, D., Abughoush, M., Aldabbas, A., Iqbal, S. (2025) Antioxidant, Antibacterial, and Antidiabetic Activities of Different Extracts from Wild Olive Leaves Grown in United Arab Emirates. Processes, 13 (4), 998. DOI: https://doi.org/10.3390/pr13040998

Antunes, B. da F., Otero, D.M., Bonemann, D.H., Ribeiro, A.S., Jacques, A.C., Zambiazi, R.C. (2021) Evaluation of physicochemical, bioactive composition and profile of fatty acids in leaves of different olive cultivars. Revista Ceres, 68 (6), 511-520. DOI: https://doi.org/10.1590/0034-737X202168060002

Árvay, J., Šnirc, M., Hauptvogl, M., Bilčíková, J., Bobková, A., Demková, L., Hudáček, M., Hrstková, M., Lošák, T., Král, M., Kováčik, A., Štefániková, J. (2019) Concentration of micro- and macro-elements in green and roasted coffee: Influence of roasting degree and risk assessment for the consumers. Biological Trace Element Research, 190, 226-233. DOI: https://doi.org/10.1007/s12011-018-1519-3

Bahloul, N., Kechaou, N., Mihoubi, N.B. (2014) Comparative investigation of minerals, chlorophylls contents, fatty acid composition and thermal profiles of olive leaves (*Olea europeae* L.) as by-product. Grasas y aceites, 65 (3), e035. DOI: https://doi.org/10.3989/gya.0102141

Besnard, G., Khadari, B., de Navascués, M., Fernández-Mazuecos, M., El Bakkali, A., Arrigo, N., Baali-Cherif, D., Bronzini de Caraffa, V., Santoni, S., Vargas, P., Savolainen, V. (2013) The complex history of the olive tree: from Late Quaternary diversification of Mediterranean lineages to primary domestication in the northern Levant. Proceedings of the Royal Society B Biological Sciences, 280 (1756), 20122833. DOI: https://doi.org/10.1098/rspb.2012.2833

Botsoglou, E., Govaris, A., Christaki, E., Botsoglou, N. (2010) Effect of dietary olive leaves and/or a-tocopheryl acetate supplementation on microbial growth and lipid oxidation of turkey breast fillets during refrigerated storage. Food Chemistry, 121 (1), 17-22. DOI: https://doi.org/10.1016/j.foodchem.2009.11.083

Bouaziz, M., Sayadi, S. (2005) Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. European Journal of Lipid Science and Technology, 107, 497-504. DOI: https://doi.org/10.1002/ejlt.200501166

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28 (1), 25-30. DOI: https://doi.org/10.1016/S0023-6438(95)80008-5

Carrillo, C., Cavia, M.D.M., Alonso-Torre, S.R. (2012) Antitumor effect of oleic acid; mechanisms of action: A review. Nutricion Hospitalaria, 27, 1860-1865. DOI: https://doi.org/10.3305/nh.2012.27.6.6010

Carta, G., Murru, E., Banni, S., Manca, C. (2017) Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. Frontiers in Physiology, 8, 902. DOI: https://doi.org/10.3389/fphys.2017.00902

Casilla García, M.E., Becerra, R.A., Cotrado Cotrado, J., Casilla Rondán, J.I., Huatuco Coaquira, J.L., Bedoya Justo, E.V. (2025) Genetic Diversity of Olive (*Olea europaea* L.) Cultivars Assessed by Genotyping-by-Sequencing in Southern Peru. Agriculture, 15 (12), 1237. DOI: https://doi.org/10.3390/agriculture15121237

Castillo-Correa, M., Montalban-Hernandez, C., Navarro- Hortal, M.D., Pena-Guzman, D., Badillo-Carrasco, A., Varela-Lopez, A., Hinojosa-Nogueira, D., Romero Marquez, J.M. (2025) Exploring the Influence of Extraction Methods, Solvents, and Temperature on Total Phenolic Recovery and Antioxidant Capacity in Olive Leaf Extracts: A Systematic Review with Quantitative Synthesis. Separations, 12, 236. DOI: https://doi.org/10.3390/separations12090236

Castillo-Luna, A., Miho, H., Ledesma-Escobar, C.A., Priego-Capote, F. (2023) Comparison of Drying Techniques for Extraction of Bioactive Compounds from Olive-Tree Materials. Foods, 12 (14), 2684. DOI: https://doi.org/10.3390/foods12142684

Cavalheiro, C.V., Picoloto, R.S., Cichoski, A.J., Wagner, R., de Menezes, C.R., Zepka, L.Q., Da Croce, D.M., Barin, J.S. (2015) Olive leaves offer more than phenolic compounds – Fatty acids and mineral composition of varieties from Southern Brazil. Industrial Crops and Products, 71, 122-127. DOI: https://doi.org/10.1016/j.indcrop.2015.03.054

Connor, W.E. (2000) Importance of n-3 fatty acids in health and disease. The American journal of clinical nutrition, 71 (1 Suppl.), 171S-175S. DOI: https://doi.org/10.1093/ajcn/71.1.171s

Cör Andrejč, D., Butinar, B., Knez, Ž., Tomažič, K., Knez Marevci, M. (2022) The Effect of Drying Methods and Extraction Techniques on Oleuropein Content in Olive Leaves. Plants, 11, 865. DOI: https://doi.org/10.3390/plants11070865

Dabbou, S., Rjiba, I., Nakbi, A., Gazzah, N., Issaoui, M., Hammami, M. (2010) Compositional quality of virgin olive oils from cultivars introduced in Tunisian arid zones in comparison to *Chemlali* cultivars. Scientia Horticulturae, 124, 122-127. DOI: https://doi.org/10.1016/j.scienta.2009.12.017

de Oliveira, N.M., Lopes, L., Chéu, M.H., Soares, E., Meireles, D., Machado, J. (2023) Updated Mineral Composition and Potential Therapeutic Properties of Different Varieties of Olive Leaves from *Olea europaea*. Plants, 12 (4), 916. DOI: https://doi.org/10.3390/plants12040916

Demirer, B., Samur, G. (2025) Health Benefits of Olive Leaf: The Focus on Efficacy of Antiglycation Mechanisms. Nutrition Reviews, 83 (3), 551-561. DOI: https://doi.org/10.1093/nutrit/nuae162

Diez, C.M., Trujillo, I., Martinez-Urdiroz, N., Barranco, D., Rallo, L., Marfil, P., Gaut, B.S. (2015) Olive domestication and diversification in the Mediterranean Basin. New Phytologist, 206 (1), 436-447. DOI: https://doi.org/10.1111/nph.13181

Donner, M., Erraach, Y., López-i-Gelats, F., Manuel-i-Martin, J., Yatribi, T., Radić, I., El Hadad-Gauthier, F. (2022) Circular bioeconomy for olive oil waste and by-product valorisation: Actors' strategies and conditions in the Mediterranean area. Journal of Environmental Management, 321, 115836. DOI: https://doi.org/10.1016/j.jenvman.2022.115836

Feng, S., Zhang, C., Liu, L., Xu, Z., Chen, T., Zhou, L., Yuan, M., Li, T., Ding, C. (2021) Comparison of Phenolic Compounds in Olive Leaves by Different Drying and Storage Methods. Separations, 8, 156. DOI: https://doi.org/10.3390/separations8090156

Ferreira, D.M., de Oliveira, N.M., Chéu, M.H., Meireles, D., Lopes, L., Oliveira, M.B., Machado, J. (2023) Updated Organic Composition and Potential Therapeutic Properties of Different Varieties of Olive Leaves from *Olea europaea*. Plants, 12, 688. DOI: https://doi.org/10.3390/plants12030688

Filgueira-Garro, I., González-Ferrero, C., Mendiola, D., Marín-Arroyo, M.R. (2022) Effect of cultivar and drying methods on phenolic compounds and antioxidant capacity in olive (*Olea europaea* L.) leaves. AIMS Agriculture and Food, 7 (2), 250-264. DOI: https://doi.org/10.3934/agrfood.2022016

Galanty, A., Grudzińska, M., Paździora, W., Paśko, P. (2023) Erucic Acid-Both Sides of the Story: A Concise Review on Its Beneficial and Toxic Properties. Molecules, 28, 1924. DOI: https://doi.org/10.3390/molecules28041924

Giulitti, F., Petrungaro, S., Mandatori, S., Tomaipitinca, L., de Franchis, V., D'Amore, A., Filippini, A., Gaudio, E., Ziparo, E., Giampietri, C. (2021) Anti-tumor Effect of Oleic Acid in Hepatocellular Carcinoma Cell Lines via Autophagy Reduction. Frontiers in Cell and Developmental Biology, 9, 629182. DOI: https://doi.org/10.3389/fcell.2021.629182

Goyal, A., Dubey, N., Verma, A., Agrawal, A. (2024) Erucic Acid: A Possible Therapeutic Agent for Neurodegenerative Diseases. Current Molecular Medicine, 24 (4), 419-427. DOI: https://doi.org/10.2174/1566524023666230509123536

Hashmi, M.A., Khan, A., Hanif, M., Farooq U., Perveen, S. (2015) Traditional Uses, Phytochemistry, and Pharmacology of *Olea europaea* (Olive). Evidence-Based Complementary and Alternative Medicine, 2015 (1), 541591. DOI: https://doi.org/10.1155/2015/541591

Ibrahim, E.H., Abdelgaleel, M.A., Salama, A.A., Metwalli, S.M. (2016) Chemical and nutritional evaluation of olive leaves and selection of the optimum conditions for extraction of their phenolic compounds. Journal of Agricultural Research Kafr El Sheikh University, 42 (1), 445-459.

Japón-Luján, R., Luque de Castro, M.D. (2007) Static-dynamic superheated liquid extraction of hydroxytyrosol and other biophenols from alperujo (a semisolid residue of the olive oil industry). Journal of Agricultural and Food Chemistry, 55 (9), 3629-3634. DOI: https://doi.org/10.1021/jf0636770

Jeon, S., Choi, M. (2018) Anti-inflammatory and anti-aging effects of hydroxytyrosol on human dermal fibroblasts (HDFs). Biomedical Dermatology, 2, 21. DOI: https://doi.org/10.1186/s41702-018-0031-x

Jiang, L., Wang, W., He, Q., Wu, Y., Lu, Z., Sun, J., Liu, Z., Shao, Y., Wang, A. (2017) Oleic acid induces apoptosis and autophagy in the treatment of Tongue Squamous cell carcinomas. Scientific Reports, 7, 11277. DOI: https://doi.org/10.1038/s41598-017-11842-5

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J.M., Barba, F.J., Prieto, M.A., Simal-Gandara, J. (2020) Bioactive Compounds and Quality of Extra Virgin Olive Oil. Foods, 9 (8), 1014. DOI: https://doi.org/10.3390/foods9081014

Kamran, M., Hamlin, A., Scott, C., Obied, H. (2015) Drying at high temperature for a short time maximizes the recovery of olive leaf biophenols. Industrial Crops and Products, 78, 29-38. DOI: https://doi.org/10.1016/j.indcrop.2015.10.031

Karam, M.C., Petit, J., Zimmer, D., Djantou, E.B., Scher, J. (2016) Effects of Drying and Grinding in Production of Fruit and Vegetable Powders: A Review. Journal of Food Engineering, 188, 32-49. DOI: https://doi.org/10.1016/j.jfoodeng.2016.05.001

Knazicka, Z., Galik, B., Novotna, I., Arvay, J., Fatrcova Sramkova, K., Kacaniova, M. - Mlcek, J., Kovacikova, E., Mixtajova, E., Jurikova, T., Ivanisova, E., Kolesarova, A., Duranova, H. (2025) Enhancing Commercial Gourmet Oil Quality: The Role of Dried Cayenne Pepper Red (*Capsicum annuum* L.) as a Natural Additive. Molecules, 30, 927. DOI: https://doi.org/10.3390/molecules30040927

Kováčiková, E., Urbanová, L., Kuželová, L., Lakatošová, J., Jančo, I., Árvay, J., Žiarovská, J., Ďúranová, H. (2025) Chemical-analytical characterization and Pun1 gene expression variability analysis of ten selected *Capsicum* varieties. Food Bioscience, 65, 106038. DOI: https://doi.org/10.1016/j.fbio.2025.106038

Lafka, T.-I., Lazou, A.E., Sinanoglou, V.J., Lazos, E.S. (2013) Phenolic extracts from wild olive leaves and their potential as edible oils antioxidants. Foods, 2 (1),18- 31. DOI: https://doi.org/10.3390/foods2010018

Lamy, S., Ben Saad, A., Zgheib, A., Annabi, B. (2016) Olive oil compounds inhibit the paracrine regulation of TNF-α-induced endothelial cell migration through reduced glioblastoma cell cyclooxygenase-2 expression. Journal of Nutritional Biochemistry, 27, 136-145. DOI: https://doi.org/10.1016/j.jnutbio.2015.08.026

Lee, O.-H., Lee, H.-B., Lee, J., Son, J.-Y., Rhee, S.-K., Kim, H.-D., Kim, Y.-C., Lee, B.-Y. (2005) Chemical properties of olive and bay leaves. The Korean Society of Food Science and Nutrition, 34 (4), 503-508. DOI: http://dx.doi.org/10.3746/jkfn.2005.34.4.503

Lfitat, A., Zejli, H., Bousselham, A., El Atki, Y., Lyoussi, B., Gourch, A., Abdellaoui, A. (2020) Comparative Evaluation of *Argania spinosa* and *Olea europaea* Leaf Phenolic Compounds and their Antioxidant Activity. Botanica, 26 (1), 76-87. DOI: https://doi.org/10.2478/botlit-2020-0007

Linder, M.E., Deschenes, R.J. (2007) Palmitoylation: policing protein stability and traffic. Nature Reviews Molecular Cell Biology, 8 (1), 74-84. DOI: https://doi.org/10.1038/nrm2084

Lins, P.G., Pugine, S.M.P., Scatolini, A.M., de Melo, M.P. (2018) *In vitro* antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon, 4 (9), e00805. DOI: https://doi.org/10.1016/j.heliyon.2018.e00805

Mansour, H.M.M., Zeitoun, A.A., Abd-Rabou, H.S., El Enshasy, H.A., Dailin, D.J., Zeitoun, M.A.A., El-Sohaimy, S.A. (2023) Antioxidant and Anti-Diabetic Properties of Olive (*Olea europaea*) Leaf Extracts: *In Vitro* and *In Vivo* Evaluation. Antioxidants (Basel), 12 (6), 1275. DOI: https://doi.org/10.3390/antiox12061275

Markhali, F.S., Teixeira, J.A., Rocha, C.M.R. (2020) Olive Tree Leaves-A Source of Valuable Active Compounds. Processes, 8 (9), 1177. DOI: https://doi.org/10.3390/pr809117

Martins, F.P., Barbosa, S., Pinheiro, V., Mourão, J.L., Monteiro, D.O. (2009) The effect of olive leaves supplementation on the feed digestibility: growth performances of pigs and quality of pork meat. Meat Science, 82, 438-443. DOI: https://doi.org/10.1016/j.meatsci.2009.02.014

McCarron, D.A., Reusser, M.E. (2001) Are low intakes of calcium and potassium important causes of cardiovascular disease? American Journal of Hypertension, 14 (6 Pt 2), 206-212. DOI: https://doi.org/10.1016/s0895-7061(01)02090-8

Monteleone, J.I., Sperlinga, E., Siracusa, L., Spagna, G., Parafati, l., Todaro, A., palmeri, R. (2021) Water as a Solvent of Election for Obtaining Oleuropein-Rich Extracts from Olive (*Olea europaea*) Leaves. Agronomy, 11, 465. DOI: https://doi.org/10.3390/agronomy11030465

Moon, H.-S., Batirel, S., Mantzoros, C.S. (2014) Alpha linolenic acid and oleic acid additively down-regulate malignant potential and positively cross-regulate AMPK/S6 axis in OE19 and OE33 esophageal cancer cells. Metabolism, 63, 1447-1454. DOI: https://doi.org/10.1016/j.metabol.2014.07.009

Orak, H.H., Karamać, M., Amarowicz, R., Orak, A., Penkacik, K. (2019) Genotype-Related Differences in the Phenolic Compound Profile and Antioxidant Activity of Extracts from Olive (*Olea europaea* L.) Leaves. Molecules, 24 (6), 1130. DOI: https://doi.org/10.3390/molecules24061130

Orsavova, J., Misurcova, L., Ambrozova, J.V., Vicha, R., Mlcek, J. (2015) Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. International Journal of Molecular Sciences, 16 (6), 12871-12890. DOI: https://doi.org/10.3390/ijms160612871

Özcan, M.M., Fındık, S., AlJuhaimi, F., Ghafoor, K., Babiker, E.E., Adiamo, O.Q. (2019) The effect of harvest time and varieties on total phenolics, antioxidant activity and phenolic compounds of olive fruit and leaves. Journal of Food Science and Technology, 56 (5), 2373-2385. DOI: https://doi.org/10.1007/s13197-019-03650-8

Palmeri, R., Siracusa, L., Carrubba, M., Parafati, L., Proetto, I., Pesce, F., Fallico, B. (2022) Olive Leaves, a Promising Byproduct of Olive Oil Industry: Assessment of Metabolic Profiles and Antioxidant Capacity as a Function of Cultivar and Seasonal Change. Agronomy, 12 (9), 2007. DOI: https://doi.org/10.3390/agronomy12092007

Pasković, I., Franić, M., Chatzistathis, T., Pongrac, P., Žurga, P., Majetić Germek, V., Palčić, I., Goreta Ban, S., Zakraoui, M., Marcelić, Š., Mravlje, J., Kaliterna, J., Pasković, M. P. (2025) Optimising Olive Leaf Phenolic Compounds: Cultivar and Temporal Interactions. Plants, 14 (17), 2789. DOI: https://doi.org/10.3390/plants14172789

Pennisi, R., Ben Amor, I., Gargouri, B., Attia, H., Zaabi, R., Chira, A.B., Saoudi, M., Piperno, A., Trischitta, P., Tamburello, M. P., Sciortino, M.T. (2023) Analysis of Antioxidant and Antiviral Effects of Olive (*Olea europaea* L.) Leaf Extracts and Pure Compound Using Cancer Cell Model. Biomolecules, 13 (2), 238. DOI: https://doi.org/10.3390/biom13020238

Poudyal, H., Campbell, F., Brown, L. (2010) Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate, high fat–fed rats. The Journal of Nutrition, 110, 946-953. DOI: https://doi.org/10.3945/jn.109.117812

Rahmanian, N., Jafari, S.M., Wani, T.A. (2015) Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. Trends in Food Science & Technology, 42 (2), 150-172. DOI: https://doi.org/10.1016/j.tifs.2014.12.009

Ruiz, M.L., Castillo, D., Dobson, D., Brennan, R., Gordon, S. (2002) Genotypic variation in fatty acid content of blackcurrant seeds. Journal of Agricultural and Food Chemistry, 50, 332-335. DOI: https://doi.org/10.1021/jf010899j

Schacky, C., Harris, W.S. (2007) Cardiovascular benefits of omega-3 fatty acids. Cardiovascular Research, 73, 310-315. DOI: https://doi.org/10.1016/j.cardiores.2006.08.019

Sharma, B., Gupta, P., Biswas, L., Verma, A.K., Pasha, A.M., Thota, P., Medhi, B. (2023) Neuroprotective potential of erucic acid via inhibition of N2a cell lines and rotenone induced Parkinson's disease rat model. Indian Journal of Pharmacology, 55 (6), 376-384. DOI: https://doi.org/10.4103/ijp.ijp_314_23

Silva, B.C.e., dos Santos, H.M.C., Montanher, P.F., Boeing, J.S., Almeida, V.d.C., Visentainer, J.V. (2014) Incorporation of omega-3 fatty acids in Nile tilapia (*Oreochromis niloticus*) fed chia (*Salvia hispanica* L.) Bran. Journal of the American Oil Chemists' Society, 91, 429-437. DOI: https://doi.org/10.1007/s11746-013-2391-0

Simopoulos, A.P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56, 365-379. DOI: https://doi.org/10.1016/S0753-3322(02)00253-6

Soldo, B., Bilušić, T., Giacometti, J., Ljubenkov, I., Čikeš Čulić, V., Bratanić, A., Bošković, P., Šola, I., Ilić, K. (2024) A Comparative Study of Oleuropein Extraction from Wild Olive Leaves (*Olea europea* subsp. *oleaster*, Hoffmanns. & Link), Its Gastrointestinal Stability, and Biological Potential. Applied Sciences, 14 (2), 869. DOI: https://doi.org/10.3390/app14020869

Stevens, Y., Winkens, B., Jonkers, D., Masclee, A. (2021) The effect of olive leaf extract on cardiovascular health markers: a randomized placebo-controlled clinical trial. European Journal of Nutrition, 60, 2111-2120. DOI: https://doi.org/10.1007/s00394-020-02397-9

Talhaoui, N., Taamalli, A., Gómez-Caravaca, A.M., Fernández-Gutiérrez, A., Segura-Carretero, A. (2015) Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. Food Research International, 77 (Part 2), 92-108. DOI: https://doi.org/10.1016/j.foodres.2015.09.011

Terker, A.S., Zhang, C., McCormick, J.A., Lazelle, R.A., Zhang, C., Meermeier, N.P., Siler, D.A., Park, H.J., Fu, Y., Cohen, D.M., Weinstein, A.M., Wang, W.H., Yang, C.L., Ellison, D.H. (2015) Potassium modulates electrolyte balance and blood pressure through effects on distal cell voltage and chloride. Cell metabolism, 21 (1), 39-50. DOI: https://doi.org/10.1016/j.cmet.2014.12.006

Terwoord, J.D., Hearon, C.M.Jr., Luckasen, G.J., Richards, J.C., Joyner, M.J., Dinenno, F.A. (2018) Elevated extracellular potassium prior to muscle contraction reduces onset and steady-state exercise hyperemia in humans. Journal of Applied Physiology, 125 (2), 615-623. DOI: https://doi.org/10.1152/japplphysiol.00183.2018

Yancheva, S., Mavromatis, P., Georgieva, L. (2016) Polyphenol profile and antioxidant activity of extracts from olive leaves. Journal of Central European Agriculture, 17 (1), 154-163. DOI: https://doi.org/10.5513/JCEA01/17.1.1684

Zhang, C., Zhang, J., Xin, X., Zhu, S., Niu, E., Wu, Q., Li, T., Liu, D. (2022) Changes in Phytochemical Profiles and Biological Activity of Olive Leaves Treated by Two Drying Methods. Frontiers in Nutrition, 9, 854680. DOI: https://doi.org/10.3389/fnut.2022.854680